IUCN

# Best Practice Guidelines for Great Ape Tourism

Elizabeth J. Macfie and Elizabeth A. Williamson

with contributions from Marc Ancrenaz, Chloe Cipolletta, Debby Cox, Christina Ellis, David Greer, Chloe Hodgkinson, Anne Russon and Ian Singleton

Series Editor: E.A. Williamson

Occasional Paper of the IUCN Species Survival Commission No. 38

SSC
Species Survival Commission

**About IUCN**

IUCN, International Union for Conservation of Nature, helps the world find pragmatic solutions to our most pressing environment and development challenges.

IUCN works on biodiversity, climate change, energy, human livelihoods and greening the world economy by supporting scientific research, managing field projects all over the world, and bringing governments, NGOs, the UN and companies together to develop policy, laws and best practice.

IUCN is the world's oldest and largest global environmental organization, with more than 1,000 government and NGO members and almost 11,000 volunteer experts in some 160 countries. IUCN's work is supported by over 1,000 staff in 60 offices and hundreds of partners in public, NGO and private sectors around the world.
Web: www.iucn.org

**IUCN Species Survival Commission**

The Species Survival Commission (SSC) is the largest of IUCN's six volunteer commissions with a global membership of 8,000 experts. SSC advises IUCN and its members on the wide range of technical and scientific aspects of species conservation and is dedicated to securing a future for biodiversity. SSC has significant input into the international agreements dealing with biodiversity conservation.
Web: www.iucn.org/themes/ssc

**IUCN Species Programme**

The IUCN Species Programme supports the activities of the IUCN Species Survival Commission and individual Specialist Groups, as well as implementing global species conservation initiatives. It is an integral part of the IUCN Secretariat and is managed from IUCN's international headquarters in Gland, Switzerland. The Species Programme includes a number of technical units covering Wildlife Trade, the Red List, Freshwater Biodiversity Assessments (all located in Cambridge, UK), and the Global Biodiversity Assessment Initiative (located in Washington DC, USA).

**IUCN SSC Primate Specialist Group**

The Primate Specialist Group (PSG) is concerned with the conservation of more than 630 species and subspecies of prosimians, monkeys, and apes. Its particular tasks include carrying out conservation status assessments, the compilation of action plans, making recommendations on taxonomic issues, and publishing information on primates to inform IUCN policy as a whole. The PSG facilitates the exchange of critical information among primatologists and the professional conservation community. The PSG Chairman is Dr. Russell A. Mittermeier, the Deputy Chair is Dr. Anthony B. Rylands, and the Coordinator for the Section on Great Apes is Dr. Liz Williamson.
Web: www.primate-sg.org/

# Best Practice Guidelines for Great Ape Tourism

Elizabeth J. Macfie and Elizabeth A. Williamson

with contributions from Marc Ancrenaz, Chloe Cipolletta, Debby Cox, Christina Ellis, David Greer, Chloe Hodgkinson, Anne Russon and Ian Singleton

Series Editor: E.A. Williamson



**Published by:** IUCN, Gland, Switzerland



**Citation:** Elizabeth J. Macfie and Elizabeth A. Williamson (2010). *Best Practice Guidelines for Great Ape Tourism.* Gland, Switzerland: IUCN/SSC Primate Specialist Group (PSG). 78 pp.

**ISBN:** 978-2-8317-1156-0

**Cover photos:** [Front] Western lowland gorilla, Loango National Park, Gabon. Photo © Christopher Orbell/MPI-EVAN. [Back] Sumatran orangutan, Gunung Leuser National Park, Indonesia. Photo © Perry van Duijnhoven.

**Layout by:** Kim Meek, [e-mail] k.meek@me.com

**Available from:** http://www.primate-sg.org

**Funded by:** United States Fish and Wildlife Service (Great Ape Conservation Fund)

# Table of Contents

# Section 1: Executive Summary

Tourism is often proposed 1) as a strategy to fund conservation efforts to protect great apes[1] and their habitats, 2) as a way for local communities to participate in, and benefit from, conservation activities on behalf of great apes, or 3) as a business. A few very successful sites point to the considerable potential of conservation-based great ape tourism, but it will not be possible to replicate this success everywhere. The number of significant risks to great apes that can arise from tourism require a cautious approach. If great ape tourism is not based on sound conservation principles right from the start, the odds are that economic objectives will take precedence, the consequences of which in all likelihood would be damaging to the well-being and eventual survival of the apes, and detrimental to the continued preservation of their habitat. All great ape species and subspecies are classified as Endangered or Critically Endangered on the IUCN Red List of Threatened Species (IUCN 2010), therefore it is imperative that great ape tourism adheres to the best practice guidelines in this document.

The guiding principles of best practice in great ape tourism are:

- Tourism is not a panacea for great ape conservation or revenue generation.
- Tourism can enhance long-term support for the conservation of great apes and their habitat.
- Conservation comes first—it must be the primary goal at any great ape site and tourism can be a tool to help fund it.
- Great ape tourism should only be developed if the anticipated conservation benefits, as identified in impact studies, significantly outweigh the risks.
- Enhanced conservation investment and action at great ape tourism sites must be sustained in perpetuity.
- Great ape tourism management must be based on sound and objective science.
- Benefits and profit for communities adjacent to great ape habitat should be maximised.
- Profit to private sector partners and others who earn income associated with tourism is also important, but should not be the driving force for great ape tourism development or expansion.
- Comprehensive understanding of potential impacts must guide tourism development; positive impacts from tourism must be maximised and negative impacts must be avoided or, if inevitable, better understood and mitigated.

The ultimate success or failure of great ape tourism can lie in variables that may not be obvious to policymakers who base their decisions primarily on earning revenue for struggling conservation programmes. However, a number of biological, geographical, economic and global factors can affect a site so as to render ape tourism ill-advised or unsustainable. This can be due, for example, to the failure of the tourism market for a particular site to provide revenue sufficient to cover the development and operating costs, or it can result from failure to protect the target great apes from the large number of significant negative aspects inherent in tourism. Either of these failures will have serious consequences for the great ape population. Once apes are habituated to human observers, they are at increased risk from poaching and other forms of conflict with humans. They must be protected in perpetuity even if tourism fails or ceases for any reason. Great ape tourism should not be developed without conducting critical feasibility analyses to ensure there is sufficient potential for success. Strict attention must be paid to the design of the enterprise, its implementation and continual management capacity in a manner that avoids, or at least minimises,

1 These guidelines are relevant to great apes. We do not specifically address tourism development with lesser apes (gibbons and siamangs) or other primates. Throughout the document the term ‘ape’ refers to ‘great ape’, even though many issues covered are also relevant to lesser apes.

the negative impacts of tourism on local communities and on the apes themselves. Monitoring programmes to track costs and impacts, as well as benefits, are essential to inform management on how to optimise tourism for conservation benefits.

These guidelines have been developed for both existing and potential great ape tourism sites that wish to improve the degree to which their programme contributes to the conservation rather than the exploitation of great apes. In Sections 2–4 we summarise the history and lessons learned during three decades of great ape tourism and associated impact studies. This is followed with specific best practice guidelines in Section 5 that are based on experience and impact studies. Section 8 provides the reader with reference material, including useful literature and a set of sample tourist guidelines from several ape tourism sites. This document should be viewed as an essential part of the toolkit for any site practicing or considering great ape tourism as part of its conservation programme.

# Section 2: Introduction

## 2.1 Primate Specialist Group and the SGA

The Section on Great Apes (SGA) of the IUCN/SSC Primate Specialist Group (PSG) is a group of more than 100 experts involved in research on and conservation of the great apes. The role of the SGA is to promote conservation action on behalf of great apes based on the best scientific information available. The SGA serves as a forum for discussion and information exchange; its members establish guidelines for best practices in research and conservation, formulate action plans and advise on the effective protection of great ape populations in the wild. The SGA advises governments on effective conservation strategies based on current knowledge of the populations and distributions of the great apes and the many pressures that threaten their survival. As an integral aspect of this role, the SGA facilitates the exchange of information among primatologists and the professional conservation community.

### 2.1.1 Links to other best practice guidelines for great ape conservation

Drawing on expertise from within the IUCN network, the PSG has produced a series of best practice guidelines for conservation practitioners, field scientists, governments, donors and development organisations involved in great ape conservation. All titles in the series are available for download from the PSG website (<www.primate-sg.org/best.practices.htm>). Other documents in the series cover issues that interrelate with tourism We recommend that readers of the tourism guidelines also refer to these other guidelines, as together they represent a toolkit for best practice in great ape conservation and management. Specific interactions between the documents are summarised here and will be highlighted in relevant recommendations in this document.

**Health monitoring and disease control in great ape populations (Leendertz *et al.* in press):** The prevention of disease transmission is one of the key issues underpinning best practice in ape tourism. The disease best practice guidelines are therefore a key reference for the tourism guidelines, and will provide the reader with: guidelines for developing health monitoring and surveillance programmes; details on methodology for sampling, testing and post-mortem analysis; and contacts for the global network of health professionals and laboratories interested in great apes. They will also provide in-depth guidance on the prevention of disease transmission between humans and great apes, including employee health programmes for organisations whose staff come into close proximity with apes. Disease risk is relevant not only in the tourism context, but in any situation where humans and apes come into proximity.

**Human–Great Ape Conflict (Hockings and Humle 2009):** The conflict guidelines provide a framework for designing and implementing activities to mitigate conflict between apes and humans competing for access to critical resources such as food (natural or cultivated) and habitat (forest conversion). In cases where great apes are habituated to humans, there is a chance that the level of conflict will increase as apes lose the fear of humans that previously kept them away from human settlements and crops. Communities may resent the fact that tourism income generated

from viewing crop-raiding apes is accruing to protected area authorities. Any site conducting or planning ape habituation should refer to the conflict guidelines to better respond to situations that may arise.

**Surveys and monitoring of great ape populations (Kühl *et al.* 2008):** Any site considering the development of great ape tourism will need baseline information on the population of apes at their site and will need to carry out regular monitoring of the population during habituation and subsequent tourism operations.

**Reducing the impact of commercial logging on great apes (Morgan and Sanz 2007):** It is less likely that ape tourism programmes will be developed in logging sites than in pristine habitats. However, some timber concessions pursuing Forest Stewardship Council (FSC) certification may consider ecotourism development, and a number of great ape populations exist outside protected areas in exploited or privately-owned forests subject to mixed-management objectives, which might include tourism. There are links, therefore, between tourism and logging in these sites. Additionally, a number of recommendations in the logging guidelines may be relevant in certain tourism development contexts, such as if tourism infrastructure may require some limited tree felling.

**Re-introduction of great apes (Beck *et al.* 2007):** There are many great ape sites where re-introduction is a current or potential activity and, for specific guidelines on methods, the reader is referred to the relevant guidelines. Current expert opinion is that tourism should not be carried out with ex-captive great apes due to inherent over-habituation that can lead to a failure of rehabilitation, incurring risks of injury, disease transmission and even death to both humans and apes. In the current document, therefore, we recommend as best practice that tourism should not be developed in ex-captive sites. However, in reality a number of ex-captive sites do operate tourism and it is important that these sites are informed about tourism best practices (see 2.4.1 for more information).

## 2.2 Purpose of these guidelines

Great ape tourism is widely practiced and generally promoted as a tool to conserve great apes and their habitats. The development of tourism is often proposed by donor agencies, great ape range-state governments and conservation agencies as a priority intervention, with a view to increasing revenues and community involvement, as well as promoting financially self-sustainable forests and protected areas, and bringing economic development to a region or country. A number of sites

**Western lowland gorilla, Bai Hokou, Central African Republic. Photo © Chloe Cipolletta.**

have gained significant experience and 'lessons learned' from implementing great ape tourism since the 1970s (McNeilage 1996; Butynski 2001). From their inception, many ape tourism sites have been using basic precautions to minimise risks to the apes, and these can now be justified with the results of significant experience and scientific research. Much has been documented about the costs, risks and benefits of great ape tourism, with significant debate about its overall impacts (e.g., Williamson *et al.* 2001). Over the years research and monitoring have provided the data to support modifications to ape tourism programme design and management to minimise negative impacts (Butynski 1998; Butynski and Kalina 1998; Homsy 1999; Litchfield 1997, 2007).

The purpose of this document is to provide its target audience (defined below) with current standards of best practice in the design and implementation of ape tourism as a means of promoting great ape conservation and the preservation of their forest habitats. These guidelines will also:

- emphasise the inherent risks posed by great ape tourism;
- reinforce the message that great ape tourism is not a panacea applicable to all sites; and
- conclude that if the conservation focus of tourism with the associated control mechanisms recommended by this document cannot be sustained, then great ape tourism should not be considered and a search for an alternative means of revenue and political support for conservation and protection actions should be undertaken.

## 2.3 Target audience

The primary target audience for these guidelines is practitioners designing and implementing great ape tourism activities in the field, as well as policy makers within practitioner institutions. The guidelines will also assist 'users' of great ape tourism in private sector businesses to better inform their clients. Conservation professionals and researchers, who may not implement tourism themselves but whose field projects involve humans approaching great apes or conducting activities in ape habitat, would also likely benefit from lessons learned in the impact analyses and prevention recommendations.

**Viewing mountain gorillas in Rwanda. Photo © José Kalpers.**

**Primary target audience—practitioners and policy makers:**

The ***practitioners*** of great ape tourism who will benefit from reading these guidelines include those currently implementing or designing tourism activities as a tool to support great ape conservation, including the implementing arms of the following types of organisations:

- protected area authorities within great ape range-states;
- conservation agencies and their field projects;
- national and international non-governmental organisations within great ape range-states; and
- researchers who may implement great ape tourism alongside primary research activities.

The ***policy makers,*** whose policies we hope will be influenced by these guidelines. include all those responsible for developing or approving tourism-related policy within the following organisation types:

- great ape range-state government ministries or departments;
- protected area authorities in great ape range states;
- conservation organisations active in great ape range states; and
- donors (foundations, bi- and multi-lateral) who fund or may consider funding programmes in great ape range states that involve great ape tourism.

**Additional target audience—users and associates:**

The ***'users'*** of great ape tourism include the tens of thousands of tourists who visit great ape tourism sites annually, tourism industry professionals and tour operator associations. While it will not be possible to reach every tourist through these guidelines (and that would require a different style of product), we have written this document with a view to promoting an understanding among the higher level 'users' of tourism activities, including the tourism industry and tour operator associations. Through enhanced understanding by tourism industry professionals of the risks to great apes and the means of reducing negative impacts, we anticipate that visitors arriving at great ape tourism sites will be better prepared and more willing to comply with regulations. We encourage the production of updated briefing materials for tourists, both at individual sites as has been done for gorillas (IGCP 2004; WCS Field Veterinary Program 2008; BRD 2009), chimpanzees (JGI-Uganda 2006) and orangutans (Ancrenaz 2006), or for broader taxonomic groups and geographic areas (Litchfield 1997). We will promote the dissemination of briefing materials and best practice concepts to tourism stakeholders and lodge operators in both the private sector and community tourism enterprises. Some of the recommendations herein could be adapted to a wider context involving local communities living within or adjacent to great ape habitats.

A number of other ***associates*** working with great apes, such as researchers, will find information in this document of use to guide their activities. Great ape researchers are in effect long-term visitors with the same, or higher, potential as other visitors for negative impacts on their subjects resulting from habituation and extended close-range presence. As such, **many of the recommendations for tourism best practice can and should be applied or adapted to research situations**. A number of recommendations in this document were trialled in the research context and, in some cases, longer-term visitors are able to apply controls (such as quarantine) that are even more protective to wild apes than is possible with tourists. Researchers studying the impacts of tourism will similarly find these guidelines useful and will, we hope, be able to broaden the scope of impact assessments to provide further guidance to ape tourism management.

## 2.4 Great ape tourism scenarios covered in this document

### 2.4.1 Wild vs. ex-captive sites

This document is intended for sites practicing or considering tourism with wild great apes in their natural habitats. It is not intended to address captive situations. However, due to the increase in the number of great ape orphan sanctuaries, rescue and rehabilitation centres (many of which carry

out or are considering re-introduction), in reality a number of sites do not fit easily into the wild vs. captive categories. To complicate matters further, some of these sites allow tourists to visit ex-captive apes. To avoid confusion, site categories are presented below and assessed for the degree to which the recommendations in this document should apply

| **Type of ape population visited in the site** | **Notes** |
|---|---|
| Wild apes – no ex-captives present | • The main focus of the document. |
| Wild apes with rare or occasional ex-captives rescued from poaching events and reintroduced, or translocated, after short duration in captivity (one-off or very rare cases) | • Over-habituation to humans is a risk factor that increases with length of time in captivity and leads to increased potential for contact between humans and apes during tourism visits, with associated risk for disease transmission, injury or death.<br>• 'Wild' tourism best practice recommendations apply, as outlined in this document. |
| Fully rehabilitated ex-captives co-ranging with wild apes in natural habitat:<br>• no food provisioning<br>• no contact with any provisioned ex-captives | • The presence of potentially over-habituated ex-captives in the forest increases the risk of contact between humans and apes during tourist visits, with associated risk for disease transmission, injury, or death. Any disease transmitted via such contact can easily spread to wild apes.<br>• 'Wild' tourism best practice recommendations apply, as outlined in this document. |
| Ex-captives – free ranging with no range overlap or contact with wild apes at present<br>• not provisioned | • Other expert groups have recommended that ex-captives should not be used for tourism.* However, if tourism is carried out with these individuals, best practice for wild ape tourism as outlined in the current document should be adhered to.<br>• The presence of potentially over-habituated ex-captives will increase the risk of contact between humans and apes during tourist visits, with associated risk of disease, injury and death.<br>• Adjustment in ranging patterns may in some sites result in future range overlap with wild populations and any disease transmitted via tourist contact with ex-captives may pose a risk to wild apes. |
| Ex-captives provisioned away from tourists<br>• free-ranging<br>• provisioned, but not as part of tourist visit<br>• tourism away from feeding platforms or areas | • The presence of potentially over-habituated ex-captives will increase the risk of contact between humans and apes during tourist visits, with associated increased risk for disease, injury or death.<br>• Apes that associate humans with food will be more likely to initiate contact with humans to solicit or raid bags for food and this will increase risks for disease transmission or injury.<br>• At some sites, there is potential overlap with wild apes.<br>• See note* regarding expert opinion on tourism with ex-captives.<br>• 'Wild' tourism best practice recommendations apply, as outlined in this document. |
| Ex-captives provisioned at feeding platform with tourists present:<br>• free-ranging<br>• provisioned during tourist visits<br>• tourism at feeding station or platform | • Not the purpose of this document, especially as the animals are fed, which is contrary to the recommendations in this document.<br>• These sites have different risk factors related to disease transmission and injury at feeding sites due to food attracting humans and apes into close proximity.<br>• At some sites, there is potential overlap with wild apes.<br>• Even though expert opinion recommends that tourism should not be carried out to ex-captives (see footnote 2), if tourism is taking place, the recommendations in this document may be a useful reference for reducing risks at these sites. |
| Fully fenced sanctuary sites<br>• no potential contact with wild apes | • Not covered in the document |

* The Pan African Sanctuaries Alliance (PASA) does not endorse tourism to ex-captive great apes due to higher risk to tourists and field assistants (Carlsen *et al.* 2006). In addition, an IUCN-sponsored workshop recommended unanimously that no tourism be allowed with rehabilitant orangutans that are eligible for or have already returned to forest life (Rosen and Byers 2002). We have adopted this recommendation as best practice.

## 2.5 Introduction to great ape tourism

Tourism is often promoted as a tool for conserving apes and their habitats through the generation of revenue to fund conservation efforts, while also providing educational opportunities, and social and economic development. Tourists are increasingly desirous of adventurous activities involving travel to remote international wildlife areas where they can view endangered species in their natural habitat rather than in captivity, and many are especially drawn to activities marketed as ecotourism or sustainable tourism. Great apes figure high on the list of animals that many would like to see, and people travel great distances to visit them in the wild. Currently, there are a number of sites where people can view chimpanzees (*Pan troglodytes*), western gorillas (*Gorilla gorilla*), eastern gorillas (*Gorilla beringei*), Bornean orangutans (*Pongo pygmaeus*) and Sumatran orangutans (*Pongo abelii*). A few bonobo (*Pan paniscus*) sites are in the initial stages of tourism development. Many tourism programmes involve habituation to allow the approach of tourists to a viewing distance of 7–20 metres, which would be impossible with unhabituated apes. However, this is not the only model for tourism, as there are sites offering walks through natural habitat during which wild apes may be seen, viewing of apes from platforms or hides at forest clearings (e.g., 'bais' in Central Africa), or searching for wild unhabituated orangutans by boat (e.g., Kinabatangan in Sabah) or by vehicle (e.g., forest reserves in Sabah).

Many tourists will be satisfied with seeing only one group of apes and may choose to visit a particular species or subspecies based on its popularity or media coverage (e.g., 'Dian Fossey's' mountain gorillas), which results in a degree of competition in the market. However, others are interested in visiting a number of different sites and in fact the idea of a primate watching 'life-list' as is common for birdwatchers is being promoted (Mittermeier *et al.* 2010). This idea could apply not only to species, but also to subspecies and indeed to different populations of each subspecies, as suggested in a regional tourism plan for the Virunga Massif (Mehta and Guchu-Katee 2005).

### 2.5.1 Can we call great ape tourism 'sustainable tourism' or 'ecotourism'?

Many great ape tourism sites would like to market themselves as 'ecotourism' or 'sustainable tourism' destinations. However, there is debate as to whether the terms should apply to great ape tourism. The definitions of these tourism terms are quite precise, although their details vary slightly:

- Minimal-impact travel to relatively-undisturbed natural areas for the express purpose of experiencing these areas and their wildlife (Boo 1990).
- Responsible travel to natural areas that conserves the environment and improves the well-being of local people (TIES 2005).

In principal, great ape tourism projects should strive to attain the criteria stipulated in the definitions of ecotourism, and should also be sustainable. In practice, however, this has not always been the case. The general trend is to refer to great ape tourism as 'ecotourism', especially by those in the tourism industry and private sector and by others who seek to market the activity or destination to tourists who make choices based on their desire to be 'ecotourists'. However, Caldecott (pers. comm.) points out that great ape tourism has yet to qualify as ecotourism in that it has not been shown that the apes and their habitat remain unharmed.

Epler Wood (1996) suggested that ecotourism should: 1) avoid damaging or destroying the integrity or character of the natural or cultural environments being visited; 2) educate the traveller on the importance of conservation; 3) provide revenues for the conservation of natural areas and the management of protected areas; and 4) bring economic benefits to the local communities in the area. Most ape tourism projects do not fulfil these four criteria. Tourism involves risks to apes and it may not be possible to satisfy the 'minimal impact' (Boo 1990) criteria. While regulations are put in place to minimise the risks, as tourist numbers increase, it may become harder to apply them.

> "More and more visitors act as tourists rather than as ecotourists and eventually destroy what they came to see" (Russon, Susilo and Russell 2004)

Since great ape tourism is not without risk to the apes visited, the term 'sustainable tourism' may be more appropriate. However, if sufficient attention is paid to minimising risks, and if the development of financially-viable ape tourism can contribute to the development of associated conservation activities and risk-mitigation programmes, as recommended in this document (i.e., disease

monitoring, employee health programmes, improved law enforcement, enhanced monitoring of apes), the net benefit to great ape conservation will be positive.

In addition to risk, there are also financial issues. Great apes survive in a few highly vulnerable forest habitats, and the costs of management programmes to protect them are extremely high. If tourism provides sufficient financial resources to cover the operational costs of conservation, this may be one of the few means of sustainably funding the protection of these populations.

> "Apes desperately need allies, even if those allies are in it for the money" (Wrangham 2001)

However, financial sustainability will not be possible in all cases. The initial development costs and the associated infrastructure and service requirements can be extremely high, especially in remote forests that have little or no infrastructure (Blom 2001). In addition, the tourism market may not be robust enough to provide sufficient income to an increasing number of new great ape tourism sites. It is important to consider financial sustainability and viability of the overall programme before tourism is initiated.

Great ape tourism must result in improved conservation of the apes and their habitat, achievable only if tourism supports conservation activities in the habitat and stimulates support for conservation through changes in politics or consumer behaviour, or through benefits to local communities sufficient to offset their lost opportunities concerning resource extraction or habitat conversion (Singleton and Aprianto 2001). Monitoring programmes to measure the performance and impacts of tourism programmes should shed light on whether these goals are being achieved.

The production of these guidelines will provide an opportunity for great ape tourism sites to develop and improve their programmes in line with best practice. They should also be used for training and awareness-raising on how to avoid or minimise negative effects. In time, adherence to the *IUCN Best Practice Guidelines for Great Ape Tourism* could become a badge of honour that sites might wish to adopt for marketing purposes, or that tourism certification authorities could use when evaluating great ape tourism sites. In summary, we will refer only to 'great ape tourism'; we will not call it 'ecotourism'.

## Section 3: Global Experience with Great Ape Tourism

### 3.1 History of great ape tourism

Tourism has been developed at a number of great ape sites all over the world. Through different periods in its history and with different methods, previous experience in tourism development and management provides lessons learned to improve future tourism and to achieve conservation objectives.

**Eastern Gorillas:** Mountain gorilla tourism is amongst the world's best-known wildlife experiences. Mountain gorillas have been visited by tourists since 1955, although in the early years visits were largely unregulated and poorly managed (Butynski and Kalina 1998). Habituation specifically for tourism began with eastern lowland gorillas (*Gorilla beringei graueri*) in Kahuzi-Biega National Park, DRC, in the 1970s, and with mountain gorillas (*Gorilla beringei beringei*) in the Volcanoes National Park, Rwanda, in 1979. Programmes focused on mountain gorillas in the DRC followed in the 1980s, then in Uganda in the 1990s. Tourism was initiated to provide economic alternatives to converting large areas of forest for other uses, such as cattle pasture and agriculture (Weber and Vedder 2001).

While DRC suffered from political instability throughout the 1990s, tourism in Uganda and Rwanda has gone from strength to strength, providing persuasive financial arguments for continued preservation of gorilla habitat, with tourist demand proving surprisingly resistant to both price increases and political events. Mountain gorilla tourism provides significant revenue to the protected area authorities and governments, resulting in improved surveillance and increased protection of the gorillas (Harcourt 1986; Weber 1993; Macfie 2007a). Mountain gorilla tourism in Rwanda has achieved global recognition, informing and inspiring the global ecotourism movement, and at the same time providing financial support for the conservation of gorilla habitat, and stimulating

political will to protect gorillas in perpetuity (Williamson and Fawcett 2008), with a proven economic value exceeding that of alternative extractive land uses (Hatfield and Malleret-King 2006).

**Western Gorillas:** Tourism programmes focused on western gorillas were initiated in the 1990s and are of two different types. Five sites now offer viewing of unhabituated gorillas from fixed platforms at large swampy clearings or 'bais' (Boumba Bek, Lobéké and Nki in Cameroon, Langoué in Gabon and Mbeli Bai in the Republic of Congo), but only two sites offer tracking of habituated western gorillas (Bai Hokou in Central African Republic and Mondika in the Republic of Congo).

The slow development of western gorilla tourism may be attributed to a number of factors. Western gorillas are widely acknowledged to be difficult to habituate to human presence, thereby limiting tourism potential. This may be due to their denser habitats, infrequent vocalisations, larger home ranges and longer day ranges (Tutin and Fernandez 1991; Doran-Sheehy *et al.* 2007), exacerbated by previous exposure to hunting, and factors leading to less visible trail sign (Williamson and Fawcett 2008). A tourism programme at Lossi, in the Republic of Congo, succeeded with habituation (Aveling 1999; Bermejo 2004), but this gorilla population was decimated by the Ebola virus (Bermejo *et al.* 2006). However, habituation has been achieved at Bai Hokou and Mondika, where it is now possible for trackers to follow gorillas daily. Another factor in western gorilla tourism is that the tourist experience may be impeded by poor visibility in the dense tropical forests that make up much of their habitat. Langoué and Mbeli Bai use platforms for viewing at 'bais' as it is not possible to follow gorillas into the forest. In addition to factors related to the nature of the gorillas or their habitat, western gorilla tourism programmes have also suffered from poor infrastructure and high travel costs relative to other destinations in Africa that have political stability and a diversity of tourist attractions (Wilkie and Carpenter 1999). However, factors that have led to the slow development of western gorilla tourism have also provided opportunities to develop tourism in which apes are not the sole focus, but are one of a number of attractions. This in itself may ensure better control over tourism development and improved ape conservation.

**Chimpanzees:** Some chimpanzee research sites (notably Gombe Stream and Mahale Mountains National Parks in Tanzania) have been receiving visitors for over 30 years and since the 1990s, a number of other sites in East Africa (e.g., Kibale and Queen Elizabeth National Parks in Uganda, Nyungwe National Park in Rwanda) have offered guided nature walks during which visitors have the possibility of viewing chimpanzees feeding in fruiting trees. Over the years, tourism at these sites has expanded and the negative impacts of increasing tourist numbers and proximity to chimpanzees have been mitigated by stringent booking systems and tight controls on tourist conduct, including the wearing of surgical masks to reduce disease transmission (e.g., Purcell 2002; Hanamura *et al.* 2006; TANAPA and FZS 2007). More recently, a number of sites in both East and Central Africa have been offering visits to chimpanzee groups habituated specifically for tourism. As an example, in Nyungwe National Park habituation efforts are focused on three groups of chimpanzees and on bringing tourism management and operations in line with Rwanda's mountain gorilla tourism programme (Hurst 2007, 2008a,b). Sites in Central Africa that offer forest-walks with a chance of viewing unhabituated or semi-habituated chimpanzees include Lobéké in Cameroon, Loango in Gabon, Taï in Côte d'Ivoire and Gola in Sierra Leone.

**Bonobos:** Currently, no sites offer tourism with bonobos, which are endemic to the Democratic Republic of Congo (DRC). Bonobo tourism is planned at Lac Tumba/Malebo (WWF 2008), and two research sites in the Lomako Yokokala Faunal Reserve (Dupain 2007), which are also developing community income-earning activities associated with visiting researchers[2]. Not only is DRC emerging from over a decade of conflict, but also bonobo sites are extremely remote, so bonobo tourism will likely cater to small numbers of hardy enthusiasts or high-end (wealthy) tourists. As with any other ape research sites, we strongly recommend that bonobo researchers consult these guidelines and be aware of the potential risks they pose to apes and of possible mitigation measures.

2 Some research sites in DRC and Cameroon use the term 'scientific tourism' to describe their income-earning activities, including payments for accommodation and technical services, such as field assistants, trackers and guides (Dupain pers. comm.; Tagg pers. comm.).

**Bornean and Sumatran Orangutans:** Orangutan tourism was launched in Sepilok, Malaysia, in the 1960s, although it has focused on rehabilitant orangutans at or near rehabilitation centres. This began as a strategy to protect wild orangutan populations and reflected the difficulties of observing the least social of the great apes in the canopy.

Orangutan rehabilitation projects have used tourism to generate income to finance other conservation activities, while providing legal sanctuary for confiscated orphans and with hopes of advancing conservation education (Frey 1975; Aveling and Mitchell 1982; Rijksen 1982). Two rehabilitation centres that began operations in the 1970s (Sepilok in Sabah, Malaysia, and Bohorok in Sumatra, Indonesia) were the first to accept tourists and have remained the most involved in rehabilitant-orangutan-based tourism (although Bohorok has been closed as a rehabilitation centre and has not received any more orangutans since 1995). These sites have experienced heavy tourist influx: Bohorok reached up to 35,000 visitors in one year, although numbers dropped below 5,000 following a flash flood in 2003 that destroyed the tourism infrastructure (Rijksen and Meijaard 1999; Singleton and Aprianto 2001; Dellatore 2007). In 2006, Sepilok received 97,000 visitors, including over 55,000 foreign nationals (Ambu 2007). While annual revenues have been significant (estimated at between US$43,000 and US$240,000 by Rijksen and Meijaard 1999), the problems arising from such heavy visitation have been well documented (Cochrane 1998; Singleton and Aprianto 2001; Rosen and Byers 2002; Low 2004; Singleton *et al.* 2004; Dellatore 2007). The problems consist of the difficulty of controlling large numbers of visitors, proximity to orangutans, illegal feeding and unregulated tourism, all of which lead to reduced orangutan survival and over-development in the local area (Singleton and Aprianto 2001). These sites conduct tourism at feeding platforms near the rehabilitation centres or in the adjacent forest. Sometimes guides call orangutans to approach visitors and provide food rewards—a dangerous practice that increases disease risks and aggression, and can lead to injury of both tourists and orangutans (Russon, Susilo and Russell 2004; Dellatore 2007). Consequently, experts recommend that no tourism be allowed with rehabilitant orangutans that are eligible for or already returned to forest life (Rosen and Byers 2002). Despite the Indonesian government's involvement in regulating, if not halting, tourism at rehabilitation centres, some continue to operate tourism unofficially. A recent analysis of orangutan tourism found that 57% of tours visited rehabilitants exclusively and 97% included rehabilitants (Russon, Susilo and Russell 2004). Orangutan tourism focused on rehabilitants, especially when visited in unnatural contexts such as cages and feeding platforms and by extremely large numbers of visitors, does not meet many of the criteria that define ecotourism and as such should not be promoted as ecotourism or considered best practice.

Rehabilitant orangutans, Tanjung Puting National Park, Central Kalimantan, Indonesian Borneo. Photo © Anne Russon.

Commercial tours to visit wild orangutans have been operating since the mid-1980s, but are less common than rehabilitant tours. They tend to be more expensive and require more time in orangutan habitat (Russon, Susilo and Russell 2004). Given the remoteness of sites typically involved and the difficulties of finding, habituating and observing wild orangutans, support from researchers, wildlife or nature conservation agencies and government authorities is critical to developing these tours. The only sites that tourists visit regularly with the intention of viewing *wild* orangutans are Kinabatangan in Sabah, Malaysia (Ancrenaz 2006) and, to a lesser extent, the Danum Valley in Sabah and Tanjung Puting in Central Kalimantan, Indonesia, where a few tourists walk in the forest looking for wild orangutans but most see rehabilitants. In Kinabatangan, tourism takes the form of dawn or dusk river cruises with opportunities to view wild orangutans from a boat, or forest walks to visit habituated orangutans (Ancrenaz 2006). In 2008, the Kinabatangan programme, which is operated and owned by local community members, received US$95,000 from 249 tourists of 14 nationalities (Ancrenaz pers. comm.). The revenue and attention generated by tourism is probably one of the reasons that Kinabatangan retains its status as a conservation area, demonstrating the potential for viable tourism programmes based on a 'wilderness experience' and the *possibility* of viewing wild orangutans while exploring their habitat.

## 3.2 Lessons learned from existing great ape tourism programmes

### 3.2.1 Great ape tourism—conservation tool or conservation threat?

Ape tourism is often promoted as a tool to enhance the conservation status and protection of great apes and to serve as a primary draw to attract visitors to an area or country, thereby enhancing the protection of all species sharing their habitat (Adams and Infield 2003; Litchfield 2007). National tourism programmes centred on the opportunity to view great apes have launched a few range states, such as Rwanda and Uganda, into premier tourist destinations and have provided significant funding for conservation activities, as well as accruing tourism-associated revenue to local and national economies. However, these successes may not be replicable at other sites for a number of reasons, and the tourism market may not be able to support the number of sites currently proposing to develop great ape tourism.

Policy makers often view great ape tourism as a rich source of revenue, which may run counter to the principle of keeping tourist numbers small in line with 'ecotourism' and nature tourism definitions (Macfie 2007a). An important lesson lies in the prevalence of business interests driving policy decisions and threatening the conservation success of tourism projects globally (Kruger 2005). In the development of any great ape tourism activity, conservation principles must take precedence over profit to private sector stakeholders and other groups that earn tourism revenue. While a successful tourism programme will provide numerous opportunities for income generation, and private sector engagement in service provision is important (Maddison 2004), the prime aim of developing and operating this revenue-generating mechanism should be to support the costs of great ape conservation and to address the needs of communities living adjacent to ape habitats. If the priorities are allowed to invert, with increasing profits for the private sector becoming the driving force for great ape tourism, the programme will have gone completely off course.

A number of negative impacts of tourism affect not only the apes, but also local communities and the environment (see Section 4 for discussion of the impacts of great ape tourism). Therefore, great ape tourism *cannot* be an ideal solution to address the need for sustainable conservation funding at all sites. It must be approached cautiously and should only be instigated in areas that can develop and maintain the standards required to attract a viable segment of the market, and that have the commitment to principles of conservation to adequately control tourism and mitigate its negative impacts. Only if all these prerequisites are met can the risks associated with great ape tourism be prevented so that it does not itself become a conservation threat.

### 3.2.2 Global interest in great ape tourism as a conservation strategy

A number of global initiatives have adopted or endorsed great ape tourism as a conservation strategy, including the Great Apes Survival Partnership (GRASP), a UNEP/UNESCO initiative to save great apes from extinction. The Kinshasa Declaration, signed at the first GRASP intergovernmental meeting in 2005, promotes economic benefit from great ape ecotourism as a reason for ensuring

their survival (UNEP-GRASP 2005), and a number of great ape range states that signed up to this declaration are currently looking to develop tourism. These efforts are being actively promoted by government officials and technical advisors, who are understandably interested in sourcing sustainable income for their protected area and conservation programmes. Similarly, a wide range of conservation and development donors show interest in tourism initiatives, since they represent a model for sustainability that could allow conservation areas to be weaned off donor funding. It is unlikely that the global tourism market can support an ever-growing number of tourism sites; nevertheless, global interest by conservation groups, donors and tourists is an asset to tourism development as a conservation strategy at sites that demonstrate best practice.

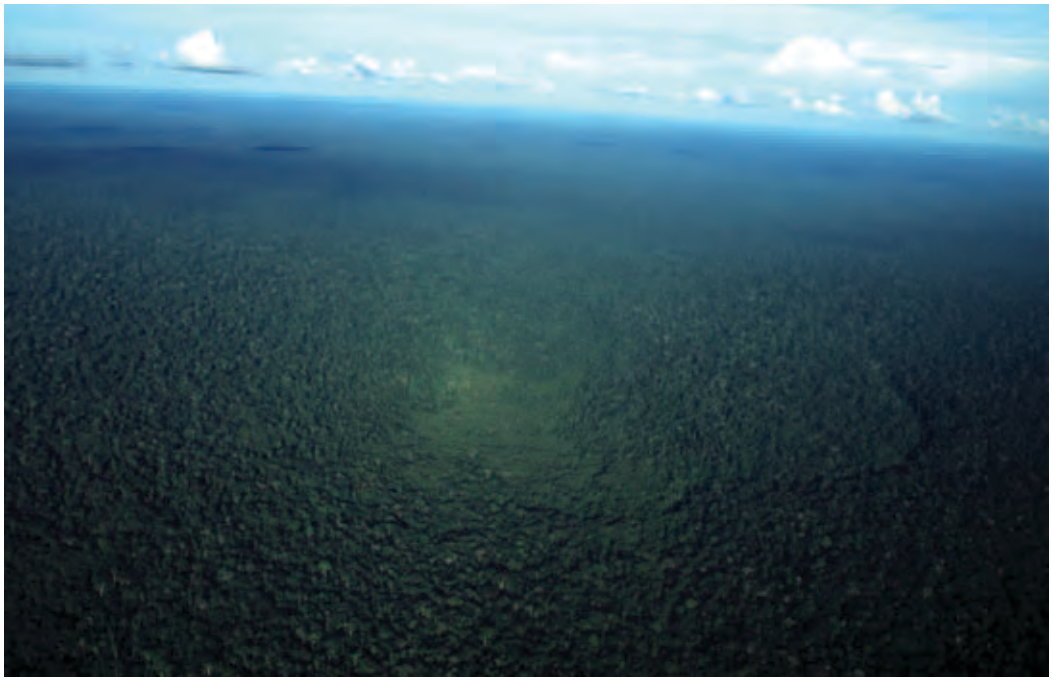
### 3.2.3 Species differences relevant to great ape tourism

There are a number of biosocial and ecological differences among the great ape taxa and socio-political differences between their range states which can affect great ape tourism as is currently practiced. It is impossible, therefore, to recommend a single model of great ape tourism as best practice. Species-specific characteristics and habitat features will greatly affect what can be achieved in a particular area. Consequently, these guidelines propose common best practices applicable to all taxa and sites together with notes on variations that would apply in specific situations (Section 5), and present examples of tourism regulations from a range of sites (Appendix I).

### 3.2.4 Great ape tourist profiles

The profile of visitors attracted to different tourism sites varies with ease of access, physical fitness requirements, types of tourism offered and infrastructure. These factors also determine how much tourists are willing to pay for the experience (Chafe 2004; Bush and Fawcett 2008), how long they stay in the area, other tourist activities they will be interested in, accommodation standards, community programmes they are willing to support, and conservation awareness programmes that the site should conduct. The profile of tourists to a particular site may also change over time (Duffus and Dearden 1990). Early visitors are typically knowledgeable and careful to have low impact, but as tourism becomes established, more visitors arrive who are less knowledgeable or concerned. Any particular site will therefore need to evaluate how it fits into the market, and design its tourism and associated programmes accordingly, paying attention to general best practice as well as guidelines specific to local factors.

It is also important that each site maintains a flexible approach to marketing, pricing and service provision, so that it may reach out to other sectors of the tourism market when unexpected situations, such as lack of security, arise, which may alter the type of tourist willing to visit the country or site (see Section 3.2.8). This will enhance the continuity of conservation funding from tourism.



Aerial view of Congo Basin forest. Photo © Liz Williamson.

### 3.2.5 Different types of great ape tourism

Existing great ape tourism sites vary in the experience they offer, ranging from essentially guaranteed viewing, when tourists are able to view habituated apes that are tracked daily, viewing unhabituated or semi-habituated apes from a platform, to forest walks or river cruises, during which unhabituated apes may or may not be encountered by chance.

### 3.2.6 Managing tourist expectations

When designing and marketing great ape tourism programmes, it is important to assess the experience to be offered to visitors. Any guarantee of viewing will heighten the tourists' expectations and put pressure on field staff to meet them, even at the risk of failing to adhere to rules and regulations. The expectations for a particular site will depend on the type of tourist, the habitat, the particular species or subspecies being visited[3] and the particular activity offered. Activities must be marketed appropriately so that visitors are not disappointed, and so that they understand they are contributing to lower-impact tourism by staying further away from the animals, viewing from a platform, and not clearing vegetation to improve their view (Greer and Cipolletta 2006). For example, most wild orangutan tours market opportunities to *look* for wild orangutans, but few promise seeing them (Russon, Susilo and Russell 2004).

### 3.2.7 Replication of success stories is not always possible or desirable

The success of mountain gorilla tourism has, over the years, stimulated a flurry of projects hoping to replicate these successes with other great apes and especially with western gorillas (e.g., Gami 1999; Lanjouw 1999a,b; Djoh and van der Wal 2001; Focken 2002). Western gorilla tourism programmes will likely be less successful for a number of reasons, and should not be promoted purely for economic benefits, due to concerns about financial viability (Wilkie and Carpenter 1999; Blom 2000, 2001, 2004; Wilkie, Carpenter and Zhang 2001; Williamson *et al.* 2002). However, if sustainable long-term financial support has been committed and significant conservation benefits are expected, then tourism could be justified (Greer and Cipolletta 2006). Experts have also debated whether Critically Endangered taxa, such as the Cross River gorilla (*Gorilla gorilla diehli*), should be habituated for *any* purpose, whether tourism or research. These guidelines are not prescriptive; if the net conservation outcome, as predicted by suitably designed and conducted feasibility and impact analyses, is beneficial to a Critically Endangered population, tourism *may* be a viable tool. Highly fragmented populations that are already under pressure may not be able to withstand the impacts of tourism, despite the aspirations of stakeholders who see tourism as a means of development.

### 3.2.8 Insecurity affects tourism markets

Many great apes live in countries that have suffered from civil war (e.g., Côte d'Ivoire, Liberia and Sierra Leone in Africa; the Aceh Province of Sumatra in Indonesia). Great ape tourism sites, especially those catering primarily to the more risk-averse luxury tourism market, will find occupancy rates plummeting following high-profile incidents in which tourists are either targets (e.g., Bwindi in 1999) or unintended victims, as in the Bali bombings in 2002 and 2005, which can result in a perception of regional insecurity. Due to the fickle nature of the luxury tourist market, it is important not to exclude average or lower-budget travellers, as these visitors will return more quickly to sites that may have acquired notoriety for insecurity or crime. However, on a more positive note, if a particular site already has a high reputation, tourism may rebound relatively quickly after negative events, as evidenced by the speed with which tourism recovered in Rwanda after the genocide, and even *during* rebel activity in the DRC.

---

3 For example, chimpanzees are more mobile than gorillas and orangutans, requiring greater physical exertion for the visitor to keep up, while photographic opportunities will be limited by the apes' location (in trees, on the ground, or in dense vegetation). Therefore managing expectations must take into account the specific conditions of the site.

### 3.2.9 Global economy affects tourism markets

While not specific to great ape tourism, global economics will affect the viability of tourism programmes. Occupancy rates can fall following economic instability, as was seen in falling bookings and increasing cancellations at many international destinations following the 2008-09 global economic crisis (UNWTO 2009). The types of tourist that tend to visit a particular site will determine that site's vulnerability to economic fluctuations. A site that relies on lower-budget backpackers and adventure tourists may be less affected as these people do not usually use their life savings to fund their trips. This highlights the value of offering services and activities that appeal to a wide variety of tourists, as the risks of market fluctuations will be buffered.

### 3.2.10 Habituation—an invariably long and risky undertaking

Great ape taxa differ widely in the effort required to habituate them: mountain gorilla groups have been habituated in as little as one year, but take on average two years; western lowland gorillas and chimpanzees will allow humans to approach to reasonable viewing distances (10–20 metres) after two to five years of consistent follows (Williamson and Feistner 2003; Greer and Cipolletta 2006). The ease of habituation depends on the species/subspecies' characteristics, the nature of their previous experience with humans and structure of their habitat (Tutin and Fernandez 1991; van Krunkelsven *et al.* 1999). Visibility in lowland forest is poor and great apes are usually obscured even within 10 metres of an observer, whilst sudden contacts are difficult to avoid in dense forest and may hinder habituation by frightening the animals or causing physical danger to apes and visitors alike (Williamson 1988). However, it is important to note experiences with mountain gorillas, where low vegetation and uneven topography provide ideal conditions for observation, occasionally from the opposite side of a ravine; or with eastern chimpanzees that can be observed across a valley with binoculars.

Habituation of orangutans is also a challenging endeavour due to their cryptic and semi-solitary nature. Wild orangutans are elusive and often difficult to locate in the forest. Habituation involves following lone individuals, requiring skilled and dedicated staff to do nest-to-nest follows. When first encountered, most orangutans display agonism by kiss-squeaking or long calls (flanged males), and breaking and throwing branches. Some orangutans hide in the canopy without moving for hours or even days, as long as people remain nearby, while others flee rapidly along the ground or from tree to tree. In Kinabatangan, habituation can take only 10–14 days (but this may be due to low natural fear of humans resulting from the absence of hunting in the area, Ancrenaz

Care should be taken to prevent access to tourist infrastructure by habituated apes! Photo © Uwe Kribus.

pers. comm.) and Sumatran orangutans have been habituated in as few as 3 days (Singleton, pers. comm.). Nevertheless, some orangutans seem resistant to habituation and these individuals should not be pursued (Ancrenaz pers. comm.).

Habituation carries a number of risks for both great apes and humans (Williamson and Feistner 2003; Goldsmith 2004, 2005a). One known negative impact is stress, which can be both inferred from behavioural reactions (e.g., orangutans staying in their nests for days to avoid humans) and confirmed with corticosteroid monitoring (Czekala and Robbins 2001; Nizeyi 2005). Stress can have many consequences, including deleterious impacts on reproductive success and on health, such as reduced immunity to illness, and may cause aberrant behaviour. Whether from stress or from other behavioural reactions to human presence, habituation may result in temporary or longer-term alterations to normal ranging patterns such as home-range use and day-range length (Goldsmith 2005b; McFarland 2007). If this pushes the animals out of protected areas and into contact with adjacent areas used by humans, the potential for increased human-great ape conflict and exposure to human diseases will rise (Macfie 2007a; Hockings and Humle 2009). If apes associate human settlements with food, this will also result in behavioural change and range alteration.

Risks to humans conducting habituation efforts can be predicted from the reactions of the apes under habituation. While habituation is designed to slowly reduce the distances at which human observers are tolerated without aggression or fearful reactions, in its early stages some individuals may attack those working to habituate them, resulting in injury and exposing both to higher risks of disease transmission. Best practices for the habituation of great apes are needed to guide ape research or tourism sites.

Nonetheless, it is important to balance the risks against the positive side effects that habituation can have on the ability of field staff to monitor and protect great apes. With tourism programmes, the fact that guides and trackers follow ape groups every day facilitates health monitoring and surveillance of illegal activities, allowing for prompt attention to any poaching or encroachment in the area, and veterinary interventions, such as snare removals.

Reports from the Virungas present the percentage of immature gorillas in the population as an indicator of reproductive health and to assess habituation impact. Long-term records show that the percentage of immature mountain gorillas has been higher in habituated vs. unhabituated gorillas (Weber and Vedder 1983; Kalpers *et al.* 2003). This may be confounded by the selection of large, reproductive groups for tourism or research, or by improved law enforcement in the habituated groups' home ranges, but as a consistent finding over 20 years of conservation efforts, at least suggests that habituation does not automatically lead to reproductive failure in a group.

### 3.2.11 Enforcement of tourism regulations is critical, but often suboptimal

Sites offering great ape tourism operate under a number of booking systems, rules and regulations designed to protect their target species from the negative impacts of tourism. However, at some sites these rules and regulations are ignored much if not all of the time (Sandbrook 2006; Sandbrook and Semple 2006; Dellatore 2007; Whittier 2009). At a number of sites with easy access and a high chance of viewing apes, tourism management that at first enforced strict adherence to tight controls has relaxed over time, suggesting that continued reinforcement of the rationale behind tourism rules and regulations is needed. Controls fail because conservation is often not the first priority of key actors, such as booking clerks, tracker-guides, or the tourists themselves, whose priorities may run counter to conservation, either through ignorance or selfishness. Problems include pressure from private sector operators on harried booking clerks, which results in overbooking; trackers and guides who relax or ignore regulations to obtain better tips, tourists who do not understand or care about the risks and put pressure on their guides to get closer, and even unscrupulous staff or community members operating additional visits to habituated apes to earn extra income without depositing the tracking fees with the appropriate institution. All of these examples increase the potential for negative impacts on the apes without providing any conservation benefits. Continuous improvement and enforcement of rules, regulations and systems that support ape tourism as a conservation-based activity are therefore critical, as is awareness-raising among tourists and tourism professionals *prior* to their arrival. Without improved enforcement of

the rules and regulations designed to protect apes from potential risk, ape tourism will not be a viable or even an acceptable component of the conservation toolkit.

### 3.2.12 Environmental Impact Assessments and feasibility studies

As with any proposed development that has the potential to impact wildlife and natural processes, feasibility and impact assessments are critical in the planning phase of any great ape tourism project. Environmental Impact Assessments (EIA) are mandated by many range-state environmental management authorities and, if tailored to the particular context, will allow stakeholders to evaluate a number of impacts. Whenever habituation is being considered, it is extremely important to conduct a full cost-benefit analysis, as there are many advantages and disadvantages to habituation, both for the great apes themselves, as well as for the institutions that will manage its outcomes. The International Gorilla Conservation Programme (IGCP) has developed a standardised tool to guide this analysis by asking all the appropriate questions, sourcing all the necessary data, and undergoing a balanced review to make informed recommendations. This process has been dubbed the 'Habituation Impact Assessment' or HIA (Macfie 2007a). A recent study in Nigeria looked at the feasibility of developing Cross River gorilla tourism (Macfie 2007b). Conducting such studies and analyses can be expensive but the investment is favourable compared to the high costs of developing tourism at a site that turns out to be unviable, and the cost in conservation terms of carrying out an activity that causes hardship to the very species it was designed to protect.

### 3.2.13 Impact studies and monitoring are critical

The non-extractive nature of viewing wild animals in their natural environment often leads to an assumption of sustainability, yet these programmes are generally established in fragile environments, opening them up to a mass market in which wildlife is repeatedly and actively sought out (Jacobson and Figueroa Lopez 1994; Tapper 2006). Little is known of the true impacts of tourism on great apes, their physical environment, or other resident wildlife, and even less is quantified. Difficulties are compounded by a lack of baseline data, problems of separating out the effects of tourism from other impacts such as natural environmental change, and the length of time for some effects to become apparent (Briassoulis 1991).

Given these constraints, impact studies conducted during 35 years of great ape tourism provide valuable data to inform the recommendations for best practice in managing great ape tourism:

Western lowland gorilla, Loango National Park, Gabon. Photo © Josephine Head/MPI-EVAN.

- Studies assessing the behavioural impacts and disease risks incurred by mountain gorilla tourism have led to more restrictive rules, such as an increase in the minimum viewing distance from 5 to 7 metres (Homsy 1999), and the importance of limiting the duration of tourist visits (Fawcett 2004; Muyambi 2005).
- Chimpanzee research and tourism projects have documented known human pathogens causing mortality in wild chimpanzees (Wallis and Lee 1999; Leendertz *et al*. 2006; Kaur and Singh 2008; Köndgen *et al.* 2008) and have proven that the wearing of surgical masks is both feasible (TANAPA and FZS 2007) and effective in disease prevention (Boesch 2008; Lukasik-Braum and Spelman 2008).
- Evaluation of three decades of orangutan tourism has provided opportunities to document and improve management practices (Russon, Susilo and Russell 2004). Recent research (Dellatore 2007) has shown that the behaviour of orangutans is significantly altered by tourism in Bukit Lawang, which includes both wild and ex-captive orangutans. The main changes recorded include restricted ranging (staying in areas of high tourism use), altered activity budgets (less foraging), increased incidence of aggression towards people, and high infant mortality. Of particular concern is the practice of feeding orangutans to either entice them to approach tourists or to appease them when they approach and attempt to steal food. This study concluded that behavioural health and reproductive success are poor and that tourism must be restructured to better manage and protect the orangutan population.
- The implementation of programmes monitoring the movements, behaviour and health status of great apes affected by tourism is vital to detect and mitigate known and emerging impacts (e.g., Kaur and Singh 2008) and to inform the design of impact mitigation measures such as employee health monitoring (Ali *et al*. 2004).
- Bio-monitoring activities contribute to more effective and safer tourism programmes. For example, part of the success of the mountain gorilla tourism programme is due to extensive knowledge of gorilla diet, daily-travel distance and ranging patterns that make it possible to predict group movements and locate the gorillas with relative ease. Predictability of daily-activity rhythms is also important for the tourism programme and visits are timed to coincide with gorillas' rest periods when possible, facilitating excellent observation conditions for the visitors (Plumptre and Williamson 2001).
- One gap in the study of great ape tourism to date is the lack of monitoring of negative impacts on the habitat, especially in cases where relatively small areas of forest are used intensively. It is also possible that protection and law enforcement efforts carried out to support tourism may result in positive impacts on forest habitat, and these should be monitored and documented.

### 3.2.14 Great ape tourism as a development tool for local communities

Benefits from great ape tourism that accrue at the local level can be considerable. Revenue-sharing schemes have been successfully established at a number of tourism sites (Ancrenaz *et al.* 2007; Archabald and Naughton-Treves 2001). Around the mountain gorilla tourism hub of Buhoma in Bwindi Impenetrable National Park (BINP) in Uganda, the value of tourism revenue reaching local people is more than four times the value of all other revenue sources combined (Sandbrook 2008; Blomley *et al.* 2010). Direct employment as a guide or tracker is a much-valued benefit in areas where formal employment opportunities are scarce: The Bai Hokou project hires over 60 BaAka pygmies on a rotational system (Hodgkinson 2009), whilst mountain gorilla conservation organisations are estimated to employ around 150 people (MGVP 2004). Indirect benefits may also be stimulated, such as locally-owned enterprises, or revenue-sharing schemes that fund infrastructure such as schools and hospitals (Sandbrook 2006). Tourism can also give residents a sense of pride and ownership—important factors which contributed to park staff remaining at their posts during periods of extreme insecurity in the Virungas (Plumptre and Williamson 2001).

Yet caution should be exercised before assuming that these benefits will both compensate programme-related costs and lead to altered behaviour towards conservation efforts. Adams and

Infield (2003) concluded that a revenue-sharing scheme around the Mgahinga Gorilla National Park in Uganda did not promote pro-conservation attitudes, a finding repeated in other studies (Hodgkinson 2009). Blomley *et al*. (2010) reported a positive relationship between community attitudes and community development programmes around the same Ugandan parks, although this impact was concentrated in the tourism hubs and was not widespread. However, the most commonly reported cause behind an observed reduction in the level of illegal activities was increased law-enforcement effort, indicating the important and complementary role that law enforcement plays in achieving conservation outcomes.

Where significant benefits are generated, serious consideration must be given to their distribution, to avoid disbursing benefits in a manner unconnected with conservation objectives, thus limiting their effectiveness in contributing to cost reparation or poverty reduction. A clear example is access to employment opportunities, usually dictated by education level, gender, age and domination by local elites (Sandbrook 2006). These challenges are exacerbated by the sheer scale of poverty and high human population densities around some great ape tourism sites. For example, while the Sabyinyo Lodge in Rwanda generated over $100,000 for local communities in its first year of operations, when viewed in light of the numbers of people living in the area, this translated to only $10 per person (Mwine pers. comm.). Blomley *et al*. (2010) report that while the Bwindi tourism programme appears to have been effective at delivering both individual and collective benefits, and making the link between these benefits and the presence of gorillas, it has failed to reach the poorest members of the community. Furthermore, benefits may not be viewed as adequate compensation if they are provided in a form which is inappropriate or that individuals fail to value.

In summary, if great ape tourism is to be effective as a development tool, there needs to be very careful consideration of both the costs and benefits being accrued, and how they are distributed among local residents, who are too often disenfranchised and living in extreme poverty. Tourism programmes should emphasise active participation of the poorest members of local communities.

### 3.2.15 Importance of economic valuations and tourism demand studies

When developing or monitoring great ape tourism it is tempting, especially for governments and the private sector, to regard the economic benefits as the *raison d'être* for these programmes. However, it is important that income from great ape tourism is not seen as the ultimate objective, but as an additional benefit of this conservation tool.

Bonobo, Lui Kotale, Salonga National Park, DRC. Photo © Caroline Deimel/MPI-EVAN.

Some countries have expanded their tourism programmes by increasing the numbers of tourists visiting each group of apes and/or increasing the number of ape groups visited by tourists, which exacerbates the risks to the apes and their habitats. However, research shows that many tourists would be willing to pay higher fees for an experience that is more exclusive and appears less intrusive, with smaller groups of tourists (Bush and Fawcett 2008).

In addition, a number of studies have pointed out the fallacy in the assumption that tourism revenues stay in-country and/or trickle down to benefit the local people who bear the costs of living near to ape habitats. While tourism revenues do fund the park authorities, the most significant revenues accrue internationally (Cochrane 1998; Moyini 2000; Hatfield and Malleret-King 2006; Sandbrook 2008). Tourism development activities should therefore address means of maximising the revenue that is retained in-country, and especially locally.

Studies of tourism economics are useful to demonstrate issues of the viability of ape tourism, which is thought to be unviable at many sites (Font, Cochrane and Tapper 2004; Wilkie and Carpenter 1999; Baboulene 2008). A case study of Dzanga-Sangha concluded that tourism was unlikely to cover management costs or to play a significant role in the long-term financing of the protected area (Blom 2000). However, tourism is a significant source of employment in that region and is increasingly important to the local economy, involving local people in sustainable economic development activities. Tourism revenue has also contributed to greater acceptance of the conservation project by local populations and subsequently has improved compliance with conservation regulations. Therefore, it is important to consider how ape tourism revenues are accrued and disbursed, and to adjust the perception that ape tourism exists primarily to generate income for range state governments and park authorities.

### 3.2.16 Importance of management evaluations of tourism staff conduct

Ape tourism sites might be well designed and strive towards best practice, with strict rules and regulations developed, disseminated and prominently displayed. Nonetheless, it is common that even after presenting the regulations directly to tourists, staff then manage a tourist visit in violation of one or more regulations, most commonly concerning the minimum distance rule (e.g., Sandbrook and Semple 2006). This may be simply due to the difficulty of managing tourists, or unpredictable movements by the apes, but in many cases it is due to the absence of supervision, monitoring and enforcement, and at times exacerbated by the desire to generate larger tips. If staff are regularly monitored and evaluated on their conduct of a tourist visit, and results are discussed openly by the evaluator, staff will improve their tourism management.

### 3.2.17 Location, location, location

Tourists seeking great ape tourism opportunities may be drawn to a particular site by its ease of access, or precisely the opposite; location is therefore key. Proximity to well-established wildlife tourism circuits, such as the savannah safaris in East Africa, may boost occupancy rates for ape tourism sites. This may help to explain why tourism in Central Africa has been slower to develop even in the better-established and relatively accessible sites, despite their abundant and charismatic wildlife. Conversely, for some tourists the opportunity to get away from the usual circuits is appealing, and they will consider the extra effort required to get to new sites in remote locations worthwhile.

### 3.2.18 Provisioning/feeding is not appropriate for habituation or tourism

In the early years of primate research a number of sites used food to facilitate habituation. Over time, a number of risk factors developed with provisioning, including behavioural alteration, aggression between group members, aggression towards observers leading to injury, reduced distance or contact that increases disease risks, and parasite contamination of feeding sites (Wrangham 1974; Wallis and Lee 1999; Bertolani and Boesch 2008). Ape research sites discontinued provisioning because of these risks, but it is continued at some ex-captive orangutan sites, where the park authorities feed orangutans at designated platforms and in some cases local guides flout the rules by feeding orangutans in other, unregulated locations where they entice orangutans to approach with food, putting both orangutans and tourists in danger (Dellatore 2007). The potential for negative impacts on the apes, or for litigation in cases of tourist injury, suggest that provisioning

should be stopped, even when carried out by government bodies. If unregulated feeding occurs, monitoring and enforcement, combined with education, are critical to halting this dangerous activity. It would also be advisable to reduce the feeding of ex-captives at platforms to the minimum necessary for their survival and monitoring, and these platforms should not be used as a tourist attraction. Once feeding is no longer a survival requirement, it should be discontinued.

### 3.2.19 Reducing disease-transmission with N95 surgical respirator masks

The wearing of surgical facemasks by people coming into proximity with apes in research and tourism projects has been much debated, since one of the biggest risks of human–ape disease transmission comes in the form of air-borne pathogens (Cranfield 2006). Respiratory disease is the most prevalent cause of mortality in some ape populations (Wallis and Lee 1999; Nutter *et al.* 2005; Hanamura *et al.* 2007; Kaur *et al.* 2008; Whittier, Nutter and Stoskopf 2009). In 1999, IGCP's assessment of the mountain gorilla tourism rules (Homsy 1999) recommended increasing the minimum-viewing distance from 5 to 7 metres, on the basis of research on distances that respiratory droplets and aerosolised particles can travel. However, due to concerns about mask management and compliance, the decision to use masks was postponed, pending further evidence of the link between disease transmission and human presence.

When reviewing mask effectiveness, it is important to remember that much of the literature on facemasks assesses protection of the wearer from infection, but in the case of tourism a potentially-infectious person is wearing the mask and our concern is to keep infectious particles *in*, not out. There are a number of pros and cons associated with the use of masks. Positive factors include that under ideal conditions masks are an effective barrier to exhaled pathogens. Although mask effectiveness lessens over time or in less than ideal conditions, the reduction in large particle aerosolisation is still far more effective than wearing nothing. Arguments against the use of masks include the fact that apes must be habituated to visitors wearing them. Tourists also must be educated to ensure compliance, especially as any discomfort associated with the mask could reduce compliance. Under cooler situations, such as at high altitude, poorly fitting masks may cause fogging of glasses and interfere with photography and binocular use[4]. The burden of ensuring mask supply is also a concern, as masks vary in effectiveness, and masks of appropriate quality are essential to the protective properties. Waste management is also an issue, as masks dropped in the forest would become fomites carrying concentrated potentially-infectious particles with significant disease risk.

A number of high-profile disease outbreaks in ape populations have been reported (Wallis and Lee 1999; Ferber 2000; Leendertz *et al.* 2004; Hanamura *et al.* 2007; Hosaka 2008; Köndgen *et al.* 2008), as well as data showing that, in the right wind conditions, contaminated droplets can travel up to three times the recommended 7 metre minimum distance (Cranfield 2006). Reports from multiple sites confirm that the rules established to protect apes from disease transmission are not enforced adequately or consistently and that safe distances are not maintained (Sandbrook and Semple 2006; Dellatore 2007; Nakamura and Nishida 2009). Consequently, there is increasing advocacy for the use of facemasks by great ape researchers, tourists and staff, in addition to other disease prevention measures. This practice is currently more common at research sites, especially those that have experienced fatal disease outbreaks in their study population (e.g., Taï National Park, Côte d'Ivoire); however, use of masks is also on the rise at tourism sites (e.g., chimpanzee tourism in Mahale Mountains National Park, Hanamura *et al.* 2006; mountain gorilla tourism in the DRC and Rwanda, Hurst 2008c; MGVP 2008, 2009).

Masks vary in quality and efficiency. The main differences between a mask and a respirator are that masks fit relatively loosely and protect the wearer from large aerosol particle transmission whereas respirators have a sealing surface and fit tightly over the nose and mouth—they are designed to prevent both small and large particle aerosol transmission (CDC 2004; CDC 2006). N95 respirators are of better quality and have a better fit and seal than basic surgical masks, thereby providing

4 MGVP (2008) tested N95 'duck-bill' shaped respirators, which provide more breathing room, and found that they are more comfortable, not as hot and do not cause eyeglasses to fog up as often.

Tourists wearing N95 surgical masks, Virunga National Park, DRC. Photo © Virunga National Park.

improved prevention of aerosolised particle transmission. The better seal of an N95 mask may provide some relief from fogging of camera lenses or binoculars, but conversely the seal may reduce comfort and compliance if tourists feel it is more difficult to breathe. Facial hair is also a problem, as the seal is no longer ensured. Guidance on fitting and wearing of masks must be presented before approaching a group of apes, when the tourists will be rushing. Masks are only effective if they are worn properly.

We recommend that multi-layered, surgical-quality N95 (or higher[5]) respirators be worn whenever tourists or staff approach apes to a distance of 10 metres or less, that these must be properly used and disposed of, and that wearing a mask must not be considered justification for weakening other disease prevention rules. If N95 masks are not available, paper surgical masks may be used. N95 respirator masks cost approximately US$0.40 each plus the cost of shipping. This is small compared to the overall cost of great ape tourism operations, although the reliability of supply chains has to be assured. Issues of compliance and effectiveness will be critical in the management of masks as part of a disease prevention programme. Compliance, comfort, tourist acceptance and mask disposal should all be monitored and the results used to inform and improve regulations and procedures. For more information on N95 respirators see Appendix II.

### 3.2.20 The problem of tourism with formerly-captive great apes

Tourism to view ex-captive great apes, while not the main focus of this document, takes place at a number of sites. Ex-captive and wild apes, especially orangutans, interact at some sites, so there may not be a clear wild vs. captive distinction (see table in Section 2.4.1). Due to the particular risks posed by overhabituation, specialists recommend that tourism be discontinued with rehabilitants eligible for release, or already released to free forest life, and in forests where rehabilitants range (Rosen and Byers 2002). Similarly the Pan African Sanctuary Alliance (PASA) does not endorse tourism with ex-captives due to the high risks to tourists and field staff (Carlsen *et al.* 2006).

5 Respirators that filter out higher percentages of aerosolised particles are also acceptable (N99 or N100), but more expensive.

Despite the Indonesian government's agreement to halt tourism with ex-captives, it still takes place at a number of orangutan sites (e.g., several sites in Tanjung Puting National Park and around Nyaru Menteng in Central Kalimantan, Bohorok in Sumatra). Tourism to ex-captive orangutans is often poorly controlled, which jeopardises both orangutan conservation and the education rationale of such visits, and reduces the likelihood of successful rehabilitation (Rijksen and Meijaard 1999; Russon, Susilo and Russell 2004). Recent analyses suggest that existing sites must prohibit the feeding of free-ranging rehabilitant orangutans by tourist guides, and enforcement must be ensured by patrols to prevent illegal feeding and enticing of orangutans onto tourist trails (Dellatore 2007). Formal education programmes targeting local tour guides, rangers, and tour operators, as well as the tourists (local, national and international) should promote awareness of the dangers of feeding free-ranging orangutans, especially ex-captives. This will serve to regulate human behaviour in the forest (Dellatore 2007).

### 3.2.21 Conclusions from lessons learned

Given the high cost of developing tourism and the associated infrastructure, along with the need to ensure protection of habituated apes in perpetuity, the establishment of new ape tourism sites should never be undertaken lightly. In addition, the management requirements to develop and effectively implement tourism are labour-intensive and need major commitments in terms of financial and human resources. Added to the equation is consideration of the multitude of impacts of great ape tourism. It is imperative, therefore, that any potential ape tourism project be subject to a full, objective analysis of its feasibility, impact and sustainability, including a multi-stakeholder review, before funding is committed and before promises are made to local communities as to the arrival of tourism and its associated development. Only sites that have a good chance of success, as judged by independent feasibility and impact analyses, and that demonstrate the commitment necessary to exert maximum control and impact mitigation in line with these best practice guidelines, should be developed.

Volcanoes National Park, Rwanda. Photo © Lynn Barrie and Frances Broussard

# Section 4: Potential Impacts of Great Ape Tourism

The large number of impacts of great ape tourism, both positive and negative, are summarised in the tables below.

## 4.1 Table of potential benefits of great ape tourism

| Benefits | Assumptions | Notes |
|---|---|---|
| **Monitoring:** Regular visitation enhances monitoring. | • Funding for monitoring programmes is secured. | • Monitoring plan must be in place before habituation begins. |
| **Veterinary surveillance and care:** Habituation and regular visits facilitate health monitoring, resulting in quicker diagnosis and rapid intervention. | • Funding for veterinary surveillance and response team is secured.<br>• Human expertise and laboratory facilities are in place and accessible. | • Finalise health monitoring, treatment and disease outbreak contingency plans before habituation begins. |
| **Law enforcement:** Known home ranges, habituation and increased observer presence improve protection of ape groups or individuals by law-enforcement teams. | • Security in the region allows law-enforcement monitoring.<br>• Finance, logistics and staff are in place to support/implement enforcement. | • Increase enforcement presence in area before habituation. |
| **Revenue generation:** Potential source of tourism revenue for the protected area, through fees for ape viewing, tracking and associated activities (e.g. nature walks, accommodation). | • Local, regional, international security situation allows tourism.<br>• Financial systems are in place to ensure sufficient revenue remains with ape habitat management to cover conservation costs.<br>• Tourists are interested and willing to visit and take up permits.<br>• Tourism is well managed. | • Financial analysis of potential revenue to be generated through great ape tourism activities is essential to impact assessment. |
| **Community benefits:** Potential source of monetary and non-monetary benefits for communities. | • Methods to ensure revenue streams to communities in place.<br>• Project designed so that communities are involved at all stages of project development. | • Develop or expand benefit-sharing systems to absorb revenue.<br>• Build capacity to ensure that communities play an active role in benefit sharing. |
| **Benefits to private sector:** Tourism revenues accruing through multiplier effects to private sector in tourism and service industries—state, national, regional, international. | • Tourists are interested and willing to visit, take up permits and visit other attractions.<br>• Private sector tourism industry well managed, with training ensured. | • Marketing to enhance revenue streams that spin-off from tourism permits. |
| **National economic benefits:** Increased government earnings from taxes, visas and other income associated with tourism. | • Effective national finance systems.<br>• Transparency. | |
| **Community participation and support:** Increased participation by and support from local communities for protected areas, forest management and ape conservation as a result of community benefit streams. | • Methods are in place to ensure community participation in tourism development and to maximise tourism benefit streams flowing to communities, through revenue sharing and other spin-offs. | • Promote and facilitate active engagement in habitat conservation and tourism by local communities.<br>• Ensure support for community capacity to run these projects.<br>• Ensure tourism benefits are understood as linked to protecting forest and apes' existence. |
| **Research and learning:** Potential for increasing knowledge base about apes. | • Research and ranger-based monitoring provide data for centralised databases and information systems. | • Research opportunities may be more limited in tourism groups. |
| **Political goodwill, local and national pride and image:** Apes and habitat valued as a means to enhance development and local and/or national image. | • Political value of tourism revenue outweighs perceived value of land conversion away from conservation. | • Decision not to habituate may result in loss of political goodwill and/or loss of support to protected area or forest. |
| **Regional cooperation:** Regional tourism initiatives can stimulate further regional collaboration on ape conservation actions. | • Political will and transboundary relations supportive of regional cooperation. | |
| **International awareness and support:** Donors interested in financial self-sustainability. Internationally-recognised programme will enhance long-term commitment by government. | • Tourism is well-managed and seen as sustainable source of revenue. | • Document tourism impact studies and distribute to international organisations.<br>• International tourists often return home as long-term supporters. |
| **Enhanced conservation of apes and their habitat as a result of all the above.** | | |

## 4.2 Table of potential costs and disadvantages of great ape tourism

| Disadvantages | Mitigation measures | Notes and Action Points |
|---|---|---|
| **Poaching:** Habituated apes are more vulnerable to poaching and conflict if not adequately protected, due to their loss of fear of humans. | • Once habituated, apes must always be protected through daily monitoring and patrols in their range.<br>• Protection for habituated or previously habituated groups by ranger surveillance patrols – in perpetuity.<br>• Assumption—management continuity and security. | • Discussion required on potential for de-habituation, if any.<br>• As orangutans are more solitary, it is not possible to monitor every habituated individual daily. Orangutan sites must strive towards a zero-poaching goal to protect habituated orangutans. |
| **Disease – 1:** Habituating makes apes more vulnerable to the introduction of disease during habituation process. | • Disease prevention activities for apes.<br>• Strict habituation-team protocols.<br>• Mitigation, if possible, to be discussed further with veterinary advisors. | • Veterinary advice on minimising stress and disease risk during habituation. |
| **Disease – 2**[a]**:** Habituation allows close approach of humans to apes, therefore increases risk of disease transmission through ongoing disease exposure. | • Strict enforcement of rules and regulations on tourist and research visits to apes.<br>• Training and continual evaluation.<br>• Regular review of protocols in light of new research.<br>• Education of tourists prior to visit. | • Design and implement visit evaluations to assess compliance.<br>• Develop veterinary response and outbreak contingency plan.<br>• Distribute and discuss disease-risk document (or synthesis) to tourism-development team and stakeholders.<br>• Continual analysis of ape morbidity and mortality data. |
| **Cost implications – 1:** Financial implications of the costs of habituation are high—timeframe of years.[b] | • Financial support for habituation process must be guaranteed before launch. | • Ensure adequate funding before habituation launch. |
| **Cost implications – 2:** Operating costs (staff, equipment and infrastructure) are high for tourism activities and for protection and monitoring of habituated groups in perpetuity. | • Tourism development stakeholders need to ensure that there is a long-term financial plan to cover costs even if there is a slump in the tourism market. | • Carry out economic and market surveys to analyse sustainability before developing tourism plan.<br>• Develop emergency support plan to cover operations in periods of unstable tourism market. |
| **Diversion of management attention:** Tourism may take resources away from core conservation focus. | • Reinforce conservation as primary goal in strategic plans and tourism development plans. | • Source tourism development funds from additional/new sources.<br>• Recruit additional personnel. |
| **In-migration:** Successful tourism development may encourage growth of human communities around ape habitat. | • Local/district development plans should limit uncontrolled growth | • EIA process should address potential for over-development and population increase. |
| **Range alteration:** Habituated apes may alter their range. This could result in groups or individuals ranging outside protected areas into areas with heightened poaching pressure, or into proximity with human infrastructure, resulting in increased risks of disease, poaching, injury and conflict with humans. | • Daily monitoring of all individuals is essential, both while under habituation and after habituation during tourism operations. This monitoring must continue in perpetuity.<br>• Law enforcement patrols in entire home range of habituated individuals/groups. | • Monitoring of groups or individuals under habituation is critical to judge the extent to which range adjustment may take place as a result of habituation process. |
| **Human-great ape conflict – 1:** Potential for increased conflict with humans and livestock if apes leave protected habitats (even if they ranged outside protected areas before habituation) or if they overlap with human activities (for example in multiple-use zones). | • Sensitisation.<br>• Revenue sharing.<br>• Human–great ape conflict mitigation programmes.<br>• Community/livestock health outreach.<br>• Assessment of home range during group choice. | • Additional research needed on whether habituation leads to increase in crop-raiding behaviour. |
| **Human-great ape conflict – 2:** Conflict heightened if tourism is conducted with apes that crop-raid on private land. | • Explore idea of 'entry' fee if tourism visits might be conducted on community land/farms. | |
| **Over-habituation:** Long-term habituation may lead to over-habituation[c], with potential for more contact with humans, injury to humans and apes, and increased disease risk through proximity. | • Research reducing over-habituation.<br>• Enforce rules!<br>• Deter approach of apes.<br>• Review guidelines for human behaviour when close to apes. | • Continued assessment and research into the effects of long-term habituation. |

| Disadvantages | Mitigation measures | Notes and Action Points |
|---|---|---|
| **Stress – 1:** Habituation is a stressful process for apes—initial stress during habituation may potentially lead to increased vulnerability to disease, as well as reduced reproductive rates. | • Develop and use 'best practices' for habituation to minimise stress.<br>• Develop and implement research protocol for stress monitoring during habituation. | • Develop best practice guidelines for great ape habituation.<br>• If new habituation undertaken, design monitoring programme to assess stress factors. |
| **Stress – 2:** Chronic stress[d] following habituation during operation of tourism. Stressful situations would include natural behaviours (e.g., fighting and interactions) and human interactions. | • Strict adherence to reviewed regulations to minimise chronic stress. | • Review tourism management to minimise stress inducers.<br>• Develop stress-monitoring plan. |
| **Behaviour change and social disruption:** research has revealed significant impacts of tourism on ape behaviour. | • Design visit/visitor regulations in light of behavioural changes observed.<br>• Strict adherence to regulations. | • Synthesise and present research results to staff and decision-makers.<br>• Tourism management review to reduce impact on behaviour.<br>• Ongoing research/monitoring of habituated groups. |
| **Reduced reproductive success:** behavioural impact, stress, disease and immunosuppression may all lead to reproductive failure, with impacts on population size over time. | | • Research on habituation impact on reproductive behaviour[e], maternal care and infant mortality. |
| **International condemnation:** Lack of support if perception is of excessive tourism. | • Carry out a feasibility study and impact review before any new tourism habituation is initiated.<br>• Circulate feasibility study report if habituation is recommended. | • Funding for feasibility/impact studies should be included in tourism development initial scoping plan. |
| **Habitat impact:** Negative impact of tracking activities on habitat—vegetation and other animal species. | • Conduct tracking with only essential cutting of trails.<br>• Limit number of tourists in a group.<br>• Limit number of groups in an area. | • Develop protocol for trackers and guides to minimise impacts on habitat. |
| **Pollution and habitat impact of tourism infrastructure and activities.** | • Conduct EIA prior to development of tourism infrastructure. | • Additional regulations to minimise waste associated with tourism. |
| **Military escorts for tourists, if required, increase all impacts** | • Develop code of conduct for military escorts to minimise impact. | |
| **Uncontrolled development:** Tourism, if not controlled with conservation objectives, may stimulate construction of unplanned, unsightly lodges and camps with negative environmental impacts. | • Zoning plans to be developed to control infrastructure in tourist area. | • Market surveys will provide potential developers with occupancy estimates to inform plans. |
| **Knock-on effect to other ape sites:** Development of ape tourism at one site will lead to requests/raised expectations for tourism to be developed at other sites. | • Manage expectations in nearby sites.<br>• Conduct market surveys to analyse potential market for ape tourism in any site under consideration. | • Failed expectations may result in backlash against conservation of apes and habitat. |
| **Negative impact on local people:** Lack of benefits compounded by rising crime and costs, social or cultural impacts, etc. | • Develop and implement plans to optimise community impacts. | • Community impacts will affect attitudes towards conservation. |
| **Negative impact on apes and habitat as a result of all the above.** | | |

[a] Note the balance between disease risk and veterinary care: Habituation allows for increased veterinary care/disease monitoring and enhanced opportunity for medical care. Leaving unhabituated groups results in reduced disease exposure but less/no opportunity for veterinary support.

[b] Habituation for some species or subspecies takes 2 years or more, and tourism development should operate on a 5-year time plan.

[c] Prolonged exposure and overhabituation may establish a hierarchy between humans and apes, resulting in a potential for injury.

[d] Acute stress vs. chronic stress—in chronic stress, even when no longer acutely stressed, research in mountain gorillas has shown that stress hormone levels remain higher than pre-exposure (Nizeyi 2005).

[e] Data from Bwindi gorillas show a slight (non-significant) reduction in growth of habituated groups vs. unhabituated groups (Robbins pers. comm.). Conversely, during repeated censuses in the Virungas, the proportion of immature mountain gorillas has been higher in habituated than unhabituated groups. Note that this may be confounded by selection of groups with more females and juveniles for tourism/research, and/or the fact that these groups are better protected.

### 4.3 Discussion of key tourism impacts

As shown in the tables above, there are a number of benefits and advantages of great ape tourism, as well as a long list of potential risks and disadvantages. Prominent amongst the benefits is the potential for some sites to earn significant revenues and to promote local, national and international goodwill, which together may provide significant support for conservation efforts in ape habitats (Harcourt 2001). However, this must be weighed against a number of costs, amongst which the potential for disease transmission, behavioural change and human-great ape conflict stand out as significant challenges to the often-voiced opinion that great ape tourism should be widely developed.

#### 4.3.1 Key positive impact—sustainable conservation funding

Great ape tourism has the potential to generate significant revenues, not only for site management authorities, but also for local communities, local and national governments and the private sector. Once the costs of developing tourism have been met through grants, loans or other investments, a *successful* ape tourism site will cover operational costs as well as the costs of conservation management of the site. Tourism can also produce enough revenue to support wider conservation efforts. Great ape tourism has the potential, therefore, to provide sustainable conservation funding.

However, when considering the economic benefits of tourism, which may be significant at some sites, it is important for planners and decision makers to factor in the high cost of developing and operating tourism programmes. The costs of developing ape tourism as a conservation activity include significant expenditures during habituation, which can take two years or longer, and during which no income can be expected. At the same time, funding must be sourced to cover the establishment of appropriate infrastructure for tourism operations, as well as staff recruitment and training. It is also essential that a contingency plan is in place to fund continued operations of key protection and monitoring activities at times when tourism levels may be low, during both predictable low seasons and in case of unforeseen events, such as security issues and global economic trends that impact tourism. Once great apes are habituated, they must be protected in perpetuity and this is expensive. Certainly not all sites will be able to meet these costs through tourism income alone, as many factors determine the ability to attract and maintain a sector of the limited global market for ape tourism. Thus there is a limit to the number of sites in any one country or region and for any one species or subspecies that will be viable; therefore national and regional planning, communication and collaboration are required to ensure that tourism is not developed at sites that ultimately prove unviable.

Sumatran orangutan, Gunung Leuser National Park, Indonesia. Photo © Perry van Duijnhoven

### 4.3.2 Key positive impact—enhanced monitoring and protection of apes

Certainly when apes are habituated and followed regularly, for either tourism or research purposes, the level of protection and law-enforcement effort in their home range is greatly enhanced, as is the potential for veterinarians to intervene to manage disease and human-caused injuries. Additionally, tourism enhances local, national and international awareness of the need to conserve great apes and the threats they face, leading to increased financial and political support for their protection.

### 4.3.3 Critical negative impact—disease transmission

Among the numerous impacts of ape tourism outlined in Tables 4.1 and 4.2, all of which require attention, two stand out not only as having potentially disastrous consequences but also because they are to a large extent preventable through strict adherence to best practice as described in this document. These are disease transmission and behaviour change.

The potential for disease transmission is another significant risk associated with tourism. Great apes are susceptible to human-borne diseases due to our close phylogenetic history and are particularly vulnerable to diseases to which they have had no previous exposure and thus have no natural resistance (Ferber 2000; Wallis *et al.* 2000; Woodford *et al.* 2002; Garber 2008). Habituation produces stress in apes and stress may increase susceptibility to diseases, including those carried by humans, whether tourists, park rangers, researchers or local residents. The diseases of greatest concern are those that are easily transmitted without direct or prolonged contact (Leendertz *et al.* in press). A number of sites have experienced disease outbreaks, some with multiple ape fatalities, that were either suspected or proven to be associated with humans (Macfie 1991; McNeilage 1996; Homsy 1999; Wallis and Lee 1999; Woodford *et al.* 2002; Kaur and Singh 2008).

The risks of disease transmission have driven caution in the design of rules and regulations controlling tourism management and the conduct of visits, including limits on tourist numbers, time spent with apes and viewing distances (Hastings *et al.* 1991; Macfie 1991, 1996; Kortlandt 1996; Wallis and Lee 1999; Mudakikwa 2001). Although a number of experts have warned of disease risks (Homsy 1999; Wallis *et al.* 2000) and provided indirect evidence of disease transmission (Lonsdorf *et al.* 2006; Hanamura *et al.* 2007; Hosaka 2008), until recently evidence of direct transmission to wild apes was limited to bacterial and parasitic infections (Graczyk *et al*. 2002; Goldberg *et al.* 2007; Rwego *et al.* 2008).

However, new research provides more convincing evidence of virus transmission between humans and wild apes (Kaur *et al.* 2008; Köndgen *et al.* 2008), adding considerable weight to the arguments for strict protocols guiding the use of apes for tourism and research. While disease may be introduced into the habitat by adjacent communities, refugees, military and so on, tourists and researchers present a particular concern due to their close, relatively prolonged contact with great apes, and moral responsibility. Tourists also represent the greatest number of new contacts for a group of apes, ranging from six new visitors per day to many more at sites not yet implementing strict limits. Field staff and researchers must adhere to best practice and follow strict employee health monitoring protocols. International tourists come from diverse and often distant countries, have usually been in close confines with other travellers (e.g., on aeroplanes and other transport), and the resulting exposure to pathogens may be exacerbated by the stress of travel (Wilson 1995; Ostroff and Kozarsky 1998; Adams *et al.* 2001). As tourism can result in persistent psychological stress and increased susceptibility to disease in great apes (Hudson 1992; Hofer and East 1994; Meder 1994), disease transmission risks will be exacerbated by close contacts with infected tourists (Sandbrook and Semple 2006). However, few tourists can be given systematic health checks, therefore, it is with good management that we have an opportunity to minimise risks. Most great ape tourism sites request that tourists self-report any clinical signs of illness and defer their visit, nonetheless tourists manifesting symptoms have been known to visit habituated apes (Ostroff and Kozarsky 1998; Adams and Infield 2003; Sandbrook 2006; Muehlenbein *et al.*, 2008), thereby carrying disease pathogens into the apes' environment.

Disease processes affecting apes, but not originating with tourists, can also affect tourism. A tragic example of this is the devastating impact of Ebola, which killed 95% of known individual gorillas in outbreaks in Gabon and Republic of Congo (Walsh *et al.* 2003; Caillaud *et al.* 2006), including two groups at Lossi that had been habituated for tourism (Bermejo *et al.* 2006). Ebola has also killed

habituated chimpanzees in the Taï National Park (Formenty *et al.* 1999). Ebola is among a number of diseases that are transmitted from apes to humans, although most are not as deadly. This reinforces, however, the point that disease can move in both directions and tourists visiting great apes have a vested interest in following disease-prevention protocols.

Health experts can provide advice on disease patterns and outbreaks, to inform ape tourism management. For example, investigation into a recent case of Marburg virus in Uganda (a haemorrhagic disease similar to Ebola, thought to be carried by bats and highly lethal to great apes) concluded that a bat cave was the likely source of infection of a Dutch tourist (Timen *et al.* 2009). Seven days later she viewed mountain gorillas from a distance of a few metres. This gives cause for concern about any ape tours that include bat caves—cave visits should be scheduled *after* viewing great apes or avoided altogether in countries with a history of Marburg, due to the public health risk (Timen *et al.* 2009*)*.

Disease risks underpin many of the rules and regulations controlling great ape tourism and indeed are considered one of the three greatest threats to the long-term survival of great apes (along with poaching and habitat loss). Attention to disease control is critical to any tourism programme and, as a key companion to this document, the reader is strongly encouraged to read the *IUCN Best Practice Guidelines for Health Monitoring and Disease Control in Great Apes* (Leendertz *et al.* in press).

### 4.3.4 Critical negative impact—behavioural change

Habituation to humans is known to affect great ape behaviour and be stressful, and can result in displays of aggression towards humans, altered activity budgets and changes in ranging patterns (Grieser Johns 1996; Cipolletta 2003; Williamson and Feistner 2003; Blom *et al.* 2004; Nizeyi 2005; Goldsmith *et al.* 2006; Doran-Sheehy *et al.* 2007; Bertolani and Boesch 2008; Klailova *et al.* 2010). Aberrant behaviour is another potential side effect of stress. Only a handful of studies have assessed behavioural change in the presence of tourists: western gorillas show higher rates of aggression, with dominant males spending significantly less time sleeping and resting (Hodgkinson and Cipolletta 2009), while mountain gorillas display altered activity patterns, including more time spent moving and increased monitoring (Fawcett 2004; Muyambi 2005). Orangutans in Bukit Lawang spend less time foraging, travelling and socialising in the presence of tourists (Dellatore 2007), although these changes could be caused by guides attracting orangutans with food—a practice judged inappropriate in these best practice guidelines. Tourism can also have an

**Mountain gorillas, Virunga National Park, DRC. Photo © Russ Mittermeier/CI.**

indirect negative impact on social interactions, as habituated apes may have reduced opportunities to interact with unhabituated individuals (Ancrenaz pers. comm.; Williamson pers. obs.).

There is clearly a need to minimise impacts on behaviour, not only for the primary reasons of preserving the health and welfare of the apes, but also because tourists are paying to observe natural behaviour and this should not be influenced by tourism itself. The long-term implications of these impacts are not yet known. The precautionary principle suggests, however, that even in the absence of direct proof of negative behavioural impact we should enhance tourism control and adaptively manage tourism activities to avoid behavioural change. The fact that stress contributes to range alteration will inevitably affect tourism logistics, as has been observed with transboundary mountain gorilla groups, and should be an added incentive to ensure enforcement of rules designed to minimise such impacts.

#### 4.3.5 Critical negative impact—vulnerability to poaching

Once great apes have been habituated for tourism or research they are more vulnerable to approach by humans in general, who may get close before triggering a flight response. This exposes habituated apes to increased risks of capture, injury or death, deliberate or accidental, at the hands of poachers or soldiers. The apes' vulnerability during periods of insecurity was demonstrated by the slaughter of habituated gorillas in Kahuzi-Biega National Park (Yamagiwa 1999) and Virunga National Park (Kalpers *et al*. 2003), including the high profile gorilla 'executions' in 2007 (Williamson and Fawcett 2008). Consequently habituated apes must be monitored every day, and protected by teams conducting law-enforcement patrols. Governments and NGOs must fulfil their responsibility to protect habituated groups and their habitat by implementing well-structured law enforcement and monitoring programmes, although such activities may be compromised during periods of insecurity. The presence of law-enforcement teams not only deters illegal activities, but also enables management and veterinary teams to respond immediately should any illegal activities take place. Commitment to daily monitoring is an essential requirement for any and all habituated apes and must be carried out in perpetuity, as de-habituation may not be achievable.

### 4.4 Conclusions on tourism impacts

To address the large number of negative impacts of tourism, especially those highlighted above, it is imperative that great ape tourism management and associated rules and regulations are designed with impact mitigation in mind, and that they can withstand the pressure of growing demands for increased revenue and increased development of tourist 'opportunities'. It is also essential that training of tourism field personnel, enforcement of regulations, and dissemination of the content and rationale for these recommendations, are given highest priority by organisations developing and operating great ape tourism. Key audiences are not just the tourists and the staff of the tourism enterprises, but also decision-makers in the protected area authorities and relevant ministries. In addition, services that protect habituated apes from illegal activities and disease must be funded and implemented. This document should provide a useful resource, laying out the key concepts for mitigating negative impacts while at the same time optimising the positive impacts of great ape tourism.

## Section 5: Guidelines for Best Practice in Great Ape Tourism

At this point in the document, the reader will be aware of the lessons learned through global experience with great ape tourism (Section 3) and the large number of potential impacts of great ape tourism (Section 4). This information should foster an understanding and willingness to accept and implement the guidelines formulated here in Section 5, which represent best practice in the design and management of tourism. These recommendations are based on the guiding principle that great ape tourism must benefit great ape conservation. All potential impacts, both positive and negative, must be understood, evaluated, and considered in the planning and management of tourism initiatives such that positive impacts are exploited and maximised to their highest level, while negative impacts are minimised or, better still, avoided altogether.

# GENERAL GUIDELINES FOR ALL GREAT APE SPECIES

## 5.1 Guiding principles for using tourism as a great ape conservation tool

### 5.1.1 Tourism is not a panacea for great ape conservation or revenue generation

Tourism can contribute to great ape conservation but will not be viable at all sites. Sites must meet the criteria listed in Sections 5.2 and 5.3, or they are not appropriate for great ape tourism. Sites that fail to generate the revenue anticipated may suffer a backlash against the conservation effort, so care should be taken to avoid raising false expectations among politicians, managers and local communities.

### 5.1.2 Tourism can enhance long-term support for conservation

Great ape tourism may enhance the financial, aesthetic and cultural value of apes and their habitats as perceived by local communities, policy-makers and political leaders in the great ape range states, thereby promoting long-term support for conservation of apes and their habitats (Harcourt 2001).

### 5.1.3 Conservation must be the primary goal of great ape tourism

Conservation must be given priority over economic and political concerns at all great ape tourism sites. Any site that undertakes great ape tourism must place continued and enhanced emphasis on protection, law enforcement, environmental awareness-raising and other conservation activities. The effort and resources required to develop and operate tourism should not divert resources and attention away from the conservation focus.


Gorilla model at headquarters of Volcanoes National Park, Rwanda. Photo © Martha Robbins/MPI-EVAN.

### 5.1.4 Conservation benefits must significantly outweigh risks

Great ape tourism development proposals should undergo full feasibility and impact assessments, and should not be implemented unless the benefits anticipated outweigh the potential risks. Tourism and its associated impact mitigation measures must significantly improve the conservation outcome compared to a no-tourism scenario. Only programmes that will enhance conservation efforts and improve protection of the ape population should go ahead. While this is a general guideline for all great apes, it is crucial for Critically Endangered and small populations due to their precarious conservation status.

### 5.1.5 Conservation investment and action must be assured in perpetuity

Anti-poaching activities must be launched in parallel with habituation efforts, especially in Central Africa where poaching of great apes for food is at its highest levels. Once habituated, great apes and their home ranges must be protected and monitored daily by law enforcement teams with on-call veterinary expertise. These activities are necessary not only for conservation, but also to support tourism development and management, and must be continued in perpetuity. Financial contingency plans for periods of low tourism should be in place before tourism is developed.

#### 5.1.6 Great ape tourism must be based on sound objective science

Great ape tourism can be controversial, and not all conservationists agree that it is an acceptable activity. To defend great ape tourism as a sustainable component of a conservation strategy, conservation must take priority over economic and political interests (Section 5.1.3), decisions affecting tourism must be results-led and based on sound and objective science, and regulations governing visits must be scientifically-formulated and rigorously enforced (Butynski and Kalina 1998).

#### 5.1.7 Benefits and profit for local communities should be maximised

For great ape tourism to properly meet the criteria for *sustainable* tourism, it must maximise both direct and indirect benefits to adjacent communities that bear the costs of conservation, including opportunity costs (Grosspietsch 2007). While conservation must take priority over other interests, tourism should strive to contribute to poverty reduction wherever possible and, at the very least, should do no harm to local communities (SGLCP 2009). Direct benefits include local recruitment of tourism staff and sharing a percentage of tourism revenue with adjacent communities. Indirect benefits include marketing and support for services that earn additional income for communities (such as tourism infrastructure which is partially or wholly community-owned and operated). Care should be taken to ensure that benefits are not focused on a small section of a community but are accessible to the majority. Full consultations should be conducted to ensure that benefits are provided in a manner both recognised and valued by local residents. Guidance on involving communities in tourism activities is available (e.g., Gutierrez *et al.* 2005; Ancrenaz *et al.* 2007; Rajaratnam *et al.* 2008), as are lessons learned through the development and implementation of revenue-sharing and other community programmes centred on great ape tourism (Archabald and Naughton-Treves 2001; Adams and Infield 2003; Blomley *et al.* 2010).

#### 5.1.8 Profit to private sector partners must not be a driving force

In the development of any great ape tourism activity, conservation principles must take precedence over profit generation for private sector stakeholders. While a successful tourism programme will provide opportunities for income to accrue at various levels, the primary aim of developing and operating this revenue-generating mechanism is to support the cost of conservation efforts. The needs of communities living in or adjacent to ape habitats must also be addressed; however, if the priorities become inverted, with profit to the private sector becoming the driving force behind great ape tourism, then stakeholders must analyse how the priorities could have gone astray and how to rebalance them.

#### 5.1.9 Comprehensive understanding of impacts must guide tourism development

Great ape tourism has a number of advantages and disadvantages, all of which must be clearly understood by everyone involved in the planning and implementation. These issues should be kept in mind at all stages of the design, development and management of great ape tourism. The guidelines in this document are founded on the principle of optimising impacts for conservation. Any site that cannot sustain impact-optimising activities, financially or institutionally, should not initiate a great ape tourism programme.

### 5.2 Assessment phase

All proposed great ape tourism activities must be evaluated as to their suitability, feasibility and impacts. Only if a site is judged appropriate at this stage should planning go ahead.

#### 5.2.1 Stakeholder awareness of costs and benefits

Prior to developing a tourism site, all stakeholders in the decision-making and design phases should be guided through a discussion that allows for consideration of full spectrum of advantages and disadvantages to make sure that their decisions are well informed. This will help to ensure that if tourism development goes ahead, there is support for, and commitment to, the time and funding required to implement activities, and that controls are in place to maximise benefits and mitigate negative impacts, as covered in Sections 3 and 4.

### 5.2.2 Criteria for great ape tourism sites

The following criteria must be met for great ape tourism to be considered as a conservation strategy:

a. Presence of a sufficient number of apes[6], with ranging patterns that will allow for reasonable year-round or predictable seasonal viewing. In the absence of site-specific research to inform this criterion, surveys should be carried out to assess the density and distribution of apes present. [7]

b. Funding already committed to cover tourism development along with the required impact-optimising activities and long-term obligations (including the costs of great ape health monitoring, treatment of disease, and employee health programmes).

c. Both site and programme conform to national legal and regulatory requirements (e.g., EIA, zoning) for all activities and associated infrastructure.

d. Tourism market for this ape taxon, country, location and so on, is sufficient to support the recurrent costs of conservation activities and tourism operations, as analysed through a business plan incorporating financial models of income and expenditure.

e. Preliminary analysis suggests that the addition of this site fits within the tourism carrying capacity for the particular taxon or region.

f. Physical habitat (forest/vegetation structure, topography, waterways) allow for low-impact and safe access to view apes, either on foot or from boats, as appropriate to the site.

g. Research suggests that habituation to the appropriate viewing distance will be possible (not less than 7–10 metres, with or without masks respectively).

h. Awareness of key conservation issues or threats that pose a risk to habituated apes and that tourism could help to address (e.g., poaching, human-great ape conflict).

i. Ability of the site's management to absorb the added responsibility of operating and maintaining a tourism initiative (additional staffing, infrastructure, law enforcement, and control measures to optimise booking systems and prevent unauthorised tourism).

j. Credible indications that effective management will be put in place to maintain conservation priorities over the long term, to address and mitigate all recognised negative impacts, and that acceptable education and economic benefits will be delivered to local communities.

k. Presence of, or ability to develop through capacity-building programmes, sufficient human resources in terms of skilled guides, wardens and impact-monitoring staff.

l. Understanding of whether and how tourism could affect existing levels of human-great ape conflict, either positively or negatively.[8]

m. Awareness of disease in both humans and livestock that might be transmitted to apes through the activities of staff and/or tourists.[9]

n. Knowledge of socioeconomic and political context that might either support or pose a risk to great ape tourism (e.g., Plumptre *et al*. 2004).

o. Ability to provide appropriate infrastructure required for tourists to access and stay at or near the site, including road, river or air transport, hotels, lodges and campgrounds.

---

6 A 'sufficient' number of apes would be determined by factors specfic to the taxon and site under review.

7 See *Best Practice Guidelines for Surveys and Monitoring of Great Ape Populations* (Kühl *et al.* 2008).

8 See *Best Practice Guidelines for the Prevention and Mitigation of Conflict Between Humans and Great Apes,* (Hockings and Humle 2009).

9 See *Best Practice Guidelines for Health Monitoring and Disease Control* (Leendertz *et al*. in press).

p. Ability to control the development of tourism-related infrastructure in the area through zoning or other regulation, to prevent over-development in or adjacent to great ape habitat.

q. Willingness of national authorities and institutions to develop and improve services that would support and stimulate tourism programmes, including immigration, security, tour operator networks, marketing and tourist information, and infrastructure (e.g., airports, domestic flights, roads and hotels).

r. Knowledge of existing or potential ape re-introduction programmes, and awareness of how these would affect tourism development[10]. Note that we endorse the recommendation of other expert groups that tourism programmes should not be developed with ex-captive apes because of the potential dangers to both apes and tourists.

McNeely (1992) included 'guaranteed wildlife viewing' as a general criterion for nature tourism; however, in this document we recommend that great ape tourism sites do *not* offer viewing guarantees due to the difficulty of observing wild apes and the possibility of increased behavioural impact and disease risks if distance and other protective measures are violated to satisfy a guarantee.

### 5.2.3 Feasibility studies and impact analysis of potential sites

The optimum method of deciding whether ape tourism is an acceptable and appropriate conservation strategy, and meets all criteria in Section 5.2.2, is to subject the proposed site and programme to a full feasibility study and impact (cost/benefit) analysis. Great apes should not be habituated or exposed to the risks associated with tourism at a site that has been judged unviable, unsustainable, or inappropriate for any reason. A feasibility and impact study should follow EIA models, examining biological, physical, social, political, behavioural, disease, economic, market, infrastructure, policy and institutional factors relevant to the proposed site and tourism activities (Section 3.2.12). Impact assessments must take into account the results of previous impact studies and ongoing research, and require stakeholder commitment to abide by the conclusions of the study, even if the programme or site is ultimately found to be inappropriate or unviable for great ape tourism. Funding for this type of analysis should be built into programme design budgets.

### 5.2.4 Further assessments required for decisions on tourism expansion

Once a great ape tourism site has been established and is operating successfully, there will be a growing awareness or perception—either real or inflated—of the financial benefits accruing to institutions, businesses and individuals. As a result, ape tourism sites, even those not at optimum or maximum occupancy, will eventually come under pressure from various sources to expand the number of tourists allowed per visit or visits per day. The demand may be for an increase in the maximum number of people allowed to view already habituated groups, or it may request habituation of additional groups in the same area, or in new areas, or in some cases may involve allowing tourists to view groups studied by researchers.

Any decisions to expand operations should be made with caution, as many of the negative impacts on the apes increase with every additional visitor (Homsy 1999; Macfie 2005). The option of exposing additional apes to habituation and tourism should be subject to a rigorous impact and feasibility analysis, similar to the feasibility study required for a new site. The intention of such analyses is to reduce the impacts on the apes and habitat, to suggest mitigation measures, and to guide choice of group if a decision is made to proceed. The motivation for expansion should be analysed to judge whether alternative actions, such as enhanced booking systems, might address stakeholder requirements without increasing tourist numbers or the number of apes visited. Additionally, the tourism programme at its current level should be evaluated for signs of weakness, for example, suboptimal tourism management and control. It would be unwise to expand and subject additional apes to the risks of poor management before addressing the current system by improving booking

10 See *Best Practice Guidelines for the Re-introduction of Great Apes* (Beck *et al.* 2007).

and tourism control measures. A methodology for this type of analysis has been developed to guide tourism and research habituation decisions for mountain gorillas in the Virunga/Bwindi landscape (Macfie 2007a). The Habituation Impact Assessment (HIA) includes processes and decision trees that are relevant, or could be adapted, to other sites and great ape taxa.

## 5.3 Planning phase

Once a site is judged appropriate for great ape tourism, the following recommendations will ensure best practice in programme design.

### 5.3.1 Impact optimisation as a core component of programme design

Beyond building awareness of tourism impacts, as discussed above, it is essential that activities and controls to maximise the conservation benefits of tourism and minimise negative impacts are built into the programme from the outset. A cost-benefit analysis must consider the financial implications of operating all the required impact-optimising activities proposed in this document (such as enhanced law-enforcement monitoring, disease surveillance and treatment, and employee health programmes). Impact optimisation must be planned and funded, to set the stage such that a tourism programme can be viable and rooted in preservation, not exploitation, of the apes.

### 5.3.2 Habituation Impact Assessment (HIA)

As a component of an impact analysis and feasibility study, an analysis of factors specifically associated with habituation of a particular group of apes should be conducted. An Habituation Impact Assessment should analyse the potential impacts of habituating a group of apes, suggest possible alternatives, recommend specific sites for tourism development, and provide guidance on the impact-mitigation measures to put in place alongside tourism activities (Macfie 2007a).

### 5.3.3 Criteria for choice of site or group

Following a feasibility study and/or an HIA, if great ape habituation and tourism development are to proceed, it is vital that appropriate choices are made concerning which individuals, groups or communities of great apes will be viewed by tourists. The most important criteria to consider in choosing a group or community are the following:

a. **For African apes – size and composition of group or community:**
   - **Minimum size of group or community:** For tourism operations where visitors approach groups of habituated chimpanzees, bonobos or gorillas to distances of 7–10 metres (with or without masks respectively), the total number of people

Chimpanzee, Kibale National Park, Uganda. Photo © Alain Houle.

including guides and trackers, should not be greater than the number of apes >1 year old in the group. During their vulnerable first year, infant apes are not counted in group-size criteria. For a tourism programme designed for 4 tourists + 2 staff (see Section 5.5.6), a target group of apes should comprise at least 6 individuals aged >1 year.

- **Maximum size of group or community:** At sites with multiple ape groups or communities to choose between, the largest groups and those with high growth rates should not be exposed to tourism. These groups represent a larger percentage of the population and therefore present a greater risk if a serious or fatal disease were introduced. At sites with few groups to choose from, decisions must be based on factors related to conservation impact.
- **Composition of group or community:** 'Ideal' group composition will be determined by species-specific behavioural and demographic factors, such as typical immigration/emigration patterns, and intra-group aggression and cohesiveness. A group that appears likely to disintegrate should be ruled out as a candidate for habituation. However, once a group has been habituated, it (and any splinter groups) must be protected in perpetuity, even if tourism is discontinued.

b. **For semi-solitary Asian great apes – behavioural and demographic criteria:**
   - **Group size:** Orangutan tourism operations are generally based on viewing individuals in trees from the ground or from boats, therefore group size guidelines do not apply.
   - **Social structure:** Orangutan social structure should be considered when choosing sites: Orangutan individuals are members of loosely-organised communities; females and their dependent offspring are members of 'kin clusters' with overlapping home ranges (Singleton *et al*. 2009).
   - **Gender and age:** Adult male orangutans travel long distances and may leave their core range for months at a time, during which they will be 'lost' to tourism. Adult females have smaller home ranges, are therefore easier to find and, make more appropriate candidates for habituation. Stress, however, may affect breeding success and the decision to habituate breeding females should be made with caution. Females with young infants who show distress should not be followed.
   - **Individual sensitivity to habituation and viewing activities:** Orangutans show strong individual differences in their reactions to being followed by humans. Some habituate relatively easily while others do not. Individuals showing obvious signs of stress (hiding behaviour, fleeing, kiss-squeaking) after 10 days of regular contact should not be pursued further.

c. **Percent of population exposed to tourism:** Expert advice will dictate the maximum percent of a given population to be subject to the risks of tourism; some groups or individuals should be left undisturbed. Some stakeholders have proposed an absolute maximum of 50% groups and individuals in small populations (e.g., Bwindi), where the protective effects of tourism may balance the risks. However, 50% of a large population could not be supported by the tourism market. Given wide variations in great ape population size, precise recommendations will be site-specific.

d. **Trends in group size:** A group that is growing in size is likely to be a better choice for tourism than one that is shrinking for any reason. The financial implications of halting tourism if an habituated group becomes too small include not only the costs of tourism development but also the costs of protecting the group indefinitely. The continuation of tourism might be justified if the associated law-enforcement and monitoring activities could reverse a downward trend.

e. **Home-range location and ranging patterns:** The location and size of an individual's, group's or community's home range is critical to the feasibility of tourism for the reasons given below:
   - **Accessibility:** Depending on how the tourism programme will operate (daily return hike or boat trip vs. a mobile camping/tracking experience), the ability to reach and observe a target group within the duration of a standard visit will affect the choice of group.
   - **Access to and from tourism infrastructure:** Factors such as proximity to existing or planned tourism infrastructure (trails, booking offices, visitor centre, accommodation) should enter into group selection.
   - **Seasonal and annual or supra-annual reliability:** Seasonal and annual variations in ranging patterns will affect how tourism is managed, such that departure points and accommodation requirements may vary through the year.
   - **Risks of human-great ape conflict:** Habituating apes that range into community areas would exacerbate existing conflicts with humans, and these would be heightened if income were generated by crop-raiding apes. Therefore groups known to have such tendencies should not be habituated.
   - **Ranging in areas subject to illegal activities:** If a group ranges into an area that experiences high levels of illegal activities, the enhanced monitoring and law enforcement that come with tourism may diminish the risks of poaching or injury. However, if hunting is a known threat, habituation to humans will put the apes at greater risk; in such cases, habituation should proceed only if effective protection can be assured.
   - **Beneficiaries:** Group choice may be influenced by factors relating to who will benefit—from local employment or provision of tourism services, to revenue-sharing mechanisms. The distribution of benefits over a wide area, or to a new location, should be considered.
   - **Zoning and other policy issues:** Policy issues may dictate or prevent tourism in certain areas, thereby ruling out groups that range there.
   - **International boundaries:** Unless regional agreements are in place, apes that range across international or other significant geo-political boundaries should not be chosen for tourism, due to the risks of 'losing' them, or other administrative complications.

f. **Home-range overlap and ape density:** A group or community whose range has less overlap with adjacent groups, or is in an area of relatively low density, would be at lower risk from some negative impacts of tourism, such as the introduction of infectious disease.

   *N.B.* When viewing from hides or platforms (western lowland gorillas) or from boats or vehicles (orangutans), many of the above factors are not relevant.

### 5.3.4 Developing and refining habituation protocols

Habituation is defined as the acceptance by wild animals of a human observer as a neutral element in their environment. The process of habituation depends on the species under consideration, its social organisation, density, previous experience with humans, and structure of the habitat (Williamson and Feistner 2003). While habituation of orangutans typically takes from a few weeks to a several months, habituation of African apes generally requires 2 to 5 years.

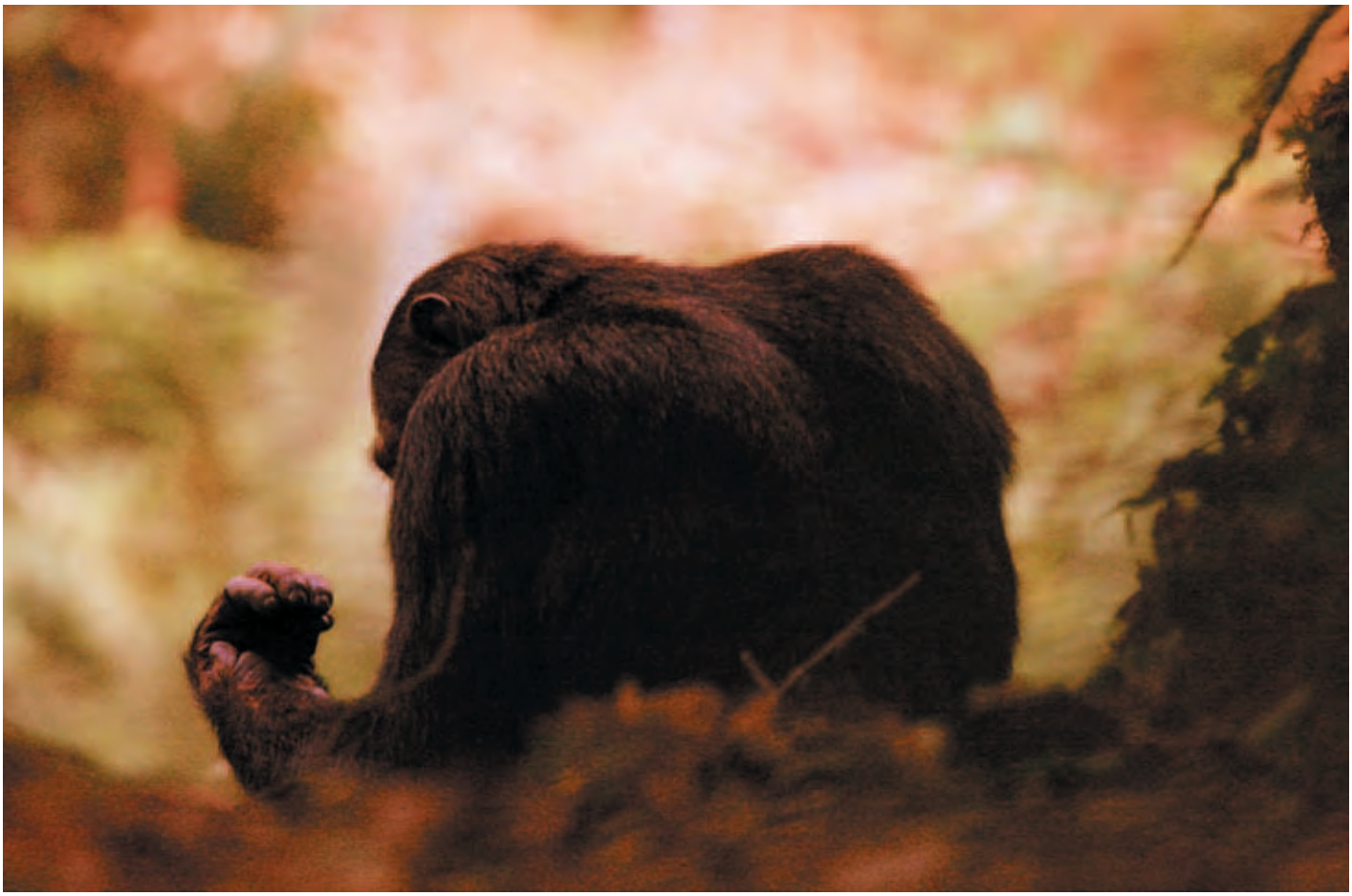
Most great ape taxa have been successfully habituated for research or tourism, resulting in a significant accumulation of knowledge and experience. Those leading new habituation efforts should familiarise themselves with lessons learned, and tailor their techniques to the target population or site. Habituation protocols should address technical and logistical issues to enhance habituation while minimising impacts on behaviour, health and habitat. Protocols should provide advice on the size, composition and conduct of habituation teams, and the team's approach should be guided

by knowledge of the apes' feeding ecology and ranging patterns. Proximity, posture and behaviour of habituators should be modified in response to alarm and display behaviours. In general the preferred approach is to aim for a distance at which the apes are aware of the team's presence without pushing them into flight mode. Any flight or increased frequency of alarm or aggressive behaviours should cause the team to retreat, and maintain a greater distance until these behaviours reduce in frequency. This distance should be maintained for a pre-determined length of time each day, with incremental attempts on successive days, weeks and months to gradually reduce the distance without inducing a flight response or triggering aggression and alarm behaviours. As best practice is designed to minimise behavioural impacts and disease risks, habituation should never proceed to distances closer than the minimum distance approved for tourism (see Section 5.5.13), and physical contact should never be instigated by an habituator.

How a group is approached is one of the most important elements of successful habituation. Certain behaviours should be avoided, such as making loud noises, sudden gestures or surreptitious movements. Typical reactions to observer presence include flight, avoidance, curiosity, display and ignore, and occasionally attack. The key to habituation is to maximise regular positive interactions, when the animals' first reaction is neither fear nor alarm. Systematic records are necessary to assess progress towards habituation and should include information on duration of contact, distance, reactions and activity budgets (Williamson and Feistner 2003; Ancrenaz pers. comm.).

### 5.3.5 Tourism development plans for sites judged appropriate and feasible

Once a site has passed through all the assessments detailed above and been judged suitable for great ape tourism, a full development plan should be prepared, documenting the actions needed to implement tourism. Plans should summarise all site and impact assessment recommendations, addressing each to ensure compliance, and address the development and implementation guidelines detailed on page 38 (see Sections 5.4 and 5.5).



**Chimpanzee, Nyungwe National Park, Rwanda. Photo © Julian Easton.**

**Contents of a Typical Tourism Development Plan:**

a. Objectives

b. Guiding principles and policies

c. Site assessment and impact study results

d. Site description

e. Habituation protocols

f. Ape tourism limits
- Number of groups/individuals
- Percentage of population

g. Site access
- Road and trail access
- Boat and air access if feasible

h. Infrastructure plans
- Local zoning plans
- Accommodation plans
  - Accommodation policies
  - Lodge/hotel/tented camps
  - Huts, chalets, campsites
- Trails
- Offices
- Visitor education centre
- Gates and ranger posts

i. Staffing requirements
- Management staff
  - Wardens
  - Finance staff
  - Booking staff
- Field staff
  - Trackers
  - Tourist guides
  - Hospitality staff
  - Visitor information staff
- Recruitment plans
- Training plans
- Policies on external staff (e.g., external guides)

j. Equipment
- Communications
- Field equipment
- First Aid

k. Ape monitoring and health protocols

l. Booking systems and pricing structure

m. Guides and guide services

n. Visitor information

o. Publicity, marketing, etc.

p. Transport, emergencies

q. Visitor regulations

r. Veterinary cover

s. Diversification of tourist activities

t. Community conservation programme
- Revenue sharing to benefit local communities
- Other benefit-sharing programmes
- Awareness and outreach
- Community Impact monitoring plan

u. Regional cooperation (if applicable)

v. Impact mitigation plan

w. Finances:
- Budget and funding plan for tourism development costs
- Operations budget
- Tourism income models
- Community income models
- Income models for other stakeholders

x. Emergency / Contingency Plans:
- Security plan
- Disease outbreak response plan
- Funding plan for tourism closure
- Human-ape conflict mitigation

Tourist lodge, Bwindi Impenetrable National Park, Uganda. Photo © Liz Macfie.

## 5.4 Development phase

*Guidelines during Habituation:*

### 5.4.1 No provisioning

In the past, provisioning with food was used to kick-start habituation at a few chimpanzee research sites. Feeding is still practiced to draw orangutans to tourist accessible areas with the approval of conservation authorities and, although not authorised, is occasionally used to entice orangutans to approach tourists. Lessons learned from these sites suggest that this practice heightens aggression both between apes and towards observers, and such close contact or injury increases the risks of disease transmission (Wallis and Lee 1999). Disease risks also increase with provisioning as food items can act as vehicles ('fomites') for infectious agents to enter the ape population. In addition, provisioning facilitates parasite contamination, if apes are repeatedly fed in the same areas. Therefore, provisioning is no longer practiced at great ape research sites and should not be used in great ape tourism. Tourism sites where feeding has occurred in the past should halt this activity and step up enforcement, together with risk-awareness training for any staff, tourist guides and tourists who think that feeding apes is acceptable.

### 5.4.2 Adherence to habituation protocols

As described in Section 5.3.4, habituation of great apes should follow protocols founded on experience. This will be an iterative learning process—lessons learned should be incorporated into protocol revisions and made available to other projects.

### 5.4.3 Habituation target distances

The habituation target distance for apes that will be viewed by tourists on foot should be 10 metres. If observers will be provided with N95 masks, then the target distance may be reduced to 7 metres.

### 5.4.4 Habituation to observers wearing surgical masks

Since we recommend as best practice that observers (tourists, staff, researchers) who are likely to approach apes to less than 10 metres should be wearing N95 surgical respirator masks, habituation teams should do the same to allow apes to become accustomed to the masks. In addition, habituators themselves pose disease risks if the apes lack prior exposure to human pathogens, so wearing masks would be an added precaution.

Chimpanzee, Nouabalé-Ndoki National Park, Republic of Congo. Photo © Ian Nichols.

### 5.4.5 Avoidance of overhabituation

Excessive habituation is indicated by unacceptably close proximity, physical contact and aggression towards humans, with increased risks of injury, disease and even death. Overhabituation can result in apes approaching tourists, initiating contact and in some cases attempting to obtain food, all of which can be dangerous for both humans and apes. Mountain gorilla and orangutan tourists often report being approached or touched by apes, and staff must try to prevent these interactions. Extreme loss of fear of humans can lead to apes ranging and even nesting in community areas, and to increased crop-raiding. In a few cases, local people have been physically attacked by wild great apes (Hockings and Humle 2009), and tourists have been attacked by rehabilitant orangutans (Singleton and Aprianto 2001; Dellatore 2007). In summary, overhabituation must be prevented at all costs, feeding should not be allowed, and habituation efforts should never go beyond predetermined levels specified in the tourism development plan. Any attempts by apes to approach closer than the minimum distance or to touch human observers should be discouraged with means appropriate to the context, and the habituation team must move away to maintain their distance.

Young eastern lowland gorilla, Kahuzi-Biega National Park, DRC. Photo © John Martin/CI.

*Impact Mitigation:*

### 5.4.6 Health monitoring and veterinary response

All great ape tourism sites should participate in, and benefit from, long-term health monitoring programmes. A wealth of reference material on conservation medicine and treatment protocols is available (e.g., Cranfield, Gaffikin and Cameron 2001; Deem, Karesh and Weisman 2001; Krief *et al.* 2005; Cranfield 2008) and is summarised in Leendertz *et al.* (in press).

Ape tourism operations should include veterinary response teams, either on-site or available to respond to emergencies. These teams should have clearly defined roles and responsibilities, including diagnostic and treatment protocols. It is important to establish guidelines on the degree of intervention appropriate for different situations: to treat diseases and injuries that are proven or suspected to be human-caused, but perhaps not those considered to be natural (unless there is a risk to the population, or when treatment is judged appropriate for humane reasons, Decision Tree Writing Group 2006).

### 5.4.7 Employee health programmes

Great ape tourism projects should provide health screening and treatment for all field staff, especially staff that are likely to come into close proximity with habituated apes. Provision of health care helps to address a basic need of local staff, while at the same time enabling screening, prevention and treatment of common diseases that pose a risk to great apes. The Mountain Gorilla Veterinary Programme (MGVP) operates employee health programmes in three countries and serves as a resource for others wishing to develop similar services (Nutter and Whittier 2001; MGVP 2002; Ali *et al.* 2004; Employee Health Group 2004). When designing such programmes, it is essential to assess staff living conditions and to consider extending the programme to cover immediate household members, although this would increase costs. Common components include vaccination against preventable diseases, diagnostic tests, routine chest x-rays or tuberculosis tests, first aid provision and training, and health education.

### 5.4.8 Community health programmes

Health outreach to monitor disease and improve hygiene in local villages is an important adjunct at great ape projects. Field staff and tourists often spend time in community areas before they enter ape habitat (Guerrera *et al*. 2003). Therefore, devoting attention to community health will provide additional protection to the apes, while at the same time providing a needed service to neighbouring communities.

### 5.4.9 Community outreach and involvement in great ape tourism activities

In locations where apes live in close proximity to human communities, it is important to find ways to involve local people in tourism activities. This will be a means of gaining their support, which is key to the long-term success of tourism (Ancrenaz *et al.* 2007; Rajaratnam *et al.* 2008).

**Environmental Education:**

The success of ape tourism will be greatly enhanced by well-designed environmental education and awareness activities, both to promote understanding and acceptance of the conservation programme and its associated tourism, as well as to stimulate the development of value-added community income generation linked to tourism. The design of education programmes will not be detailed here, as there is a wealth of reference material available. Suffice it to say that education should not stop with simply relaying facts, but go further, to explore the complexities of conservation and to explain the value of wildlife and their habitats. Awareness programmes should be developed by professional educators in partnership with community members to identify appropriate campaign messages (Wallis and Lonsdorf 2010), and should themselves undergo cost-benefit assessment as they must not compromise great ape conservation through excessive visitation (Singleton and Aprianto 2001).

**Revenue Sharing:**

One excellent means of stimulating community support for conservation is via a system for sharing a proportion of tourism revenue with the adjacent communities that carry most of the burden of living close to ape habitat. Revenue sharing encourages sustainable conservation by contributing to the improvement of the living conditions of neighbouring communities. This can be achieved through:

- **Conservation impacts:** to reduce illegal activities; to ensure sustainable conservation; and to increase community responsibility for conservation
- **Livelihoods impacts:** to improve livelihoods by supporting projects that contribute to poverty alleviation; to compensate for loss of access to ape habitat and/or crop damage; to provide alternatives to resources in ape habitat; and to encourage community-based tourism
- **Relationship impacts** (between tourism project and local population): to build trust; to increase ownership; to reduce conflicts; to increase participation; and to empower communities

The positive effects of revenue sharing can be increased by ensuring the following:

- Programme identity—funds must be seen to be linked to continued conservation of ape habitat.
- Partnerships with local government—the key player in local development and poverty alleviation.
- Community participation in the design, implementation and monitoring of revenue sharing.
- Revenues shared complement and supplement, rather than substitute for, other funding.
- Transparency and accountability.

Adhering to these guiding principles will lead to specific programme components, including the amounts to be shared (typically a percentage of gross revenue) and the beneficiary target area (typically the communities that have an impact on ape habitat and/or areas in which crop-raiding or other human-wildlife conflicts occur). Above all, revenue-sharing programmes should provide benefits to groups (entire communities if possible) rather than individuals, and should target sectors representing the 'poorest of the poor' and other disadvantaged groups, as they are priorities for poverty alleviation, as well as being the most likely to exploit natural resources in ape habitat, whether legally or illegally.

**Supporting Community-Owned and Operated Tourist Services and Products:**

The feasibility of supporting locally-owned companies or associations that will become involved with, or take charge of, great ape tourism or associated services must be assessed and given priority. Indeed, if local communities bear the costs of living close to protected areas and wildlife, it seems logical to give them a sense of ownership when economic incentives can ensue from great ape tourism. Community involvement might be in the provision of guiding services, transport, accommodation and food, or the sale of local products to tourists. Examples of successful community-owned enterprises include *Red Ape Encounters*, a company which offers orangutan viewing in the Kinabatangan (Rajaratnam *et al.* 2008), and the Nkuringo Conservation and Development Foundation, which co-owns an area of mountain gorilla habitat at the BINP boundary in Uganda on which a community-owned luxury tourist lodge is co-managed with a private-sector partner. Lessons learned underscore that care must be taken to foster good relations with private sector operators to avoid the perception of a monopoly beneficiary. While a protected area authority may already view the community as a priority, it must also promote awareness of this principle among the private sector, which might otherwise exercise political or financial clout that could jeopardise the community's benefits (Kazooba 2008; Tentena 2010).

**Other Community Conservation and Benefit-Sharing Programmes:**

A number of other community programmes can be mutually beneficial to great ape tourism. As conservation and poverty alleviation can be complementary goals, a comprehensive programme that involves and benefits adjacent communities will have a greater chance of success. This may include targeted local recruitment, participation in business enterprises linked to tourism, agricultural extension, micro-credit schemes, and controlled access to forest resources (if local regulations allow).

**A Conservation Basis for All Community-Development Programmes:**

As with all community-development programmes linked to conservation, managers should aim to maximise benefits to neighbouring communities without encouraging immigration, which would exacerbate development issues and have negative consequences for conservation.

### *Management Systems:*

#### 5.4.10 Tourism booking systems

Great ape tourism booking systems should adhere to the following principles to maximise benefits to conservation and to stakeholders:

- **Robust and foolproof:** As best practice in great ape tourism requires strict application of rules and regulations, booking systems must be robust enough to prevent over-booking, which could lead to conflict at departure points and pressure on staff to break the rules. Systems for bookings held with an initial deposit until a deadline for full payment, or loss of deposit if not confirmed, should be clearly spelled out so that all visitors, whether booking directly or through a tourism agency, can access a fair and equitable system for obtaining permits.
- **Internet-based bookings:** Internet-based systems will foster improved bookings and occupancy rates as long as they are professionally designed and managed, and allow tourists a safe and secure method to reserve and pay for permits. Small projects, or those just entering the market, may not have the capacity to maintain an electronic booking system, but as their operations grow there will be advantages to moving away from traditional means (post, telephone, radio) towards an electronic system that prevents over-booking.
- **Tourist diversity:** Booking systems should be developed to accommodate the spectrum of tourists, from high-end clients booking through tour operators who handle permits, accommodation, transport and guiding, to low-budget tourists organising their own logistics. Low-budget tourism tends to benefit local enterprises and to be more reliable during times of insecurity or other market depressors, whereas high-end tourism expenditures are often higher, but accrue at national/international levels rather than locally. In addition, local citizens should be encouraged to experience their own heritage through a favourable pricing structure.
- **Local and national tourism providers:** While there are often expectations that great ape tourism will make everyone rich, these are unlikely to be fulfilled. Tourism businesses with strong regional or international linkages have an unfair advantage in the tourism market. Therefore booking systems should allow smaller operators to acquire a share of permits if they wish to tap into the market for linked services, such as accommodation, transport and transfers.
- **Informative:** Communications with those wishing to book ape tourism permits must clearly explain the rationale behind rules and regulations, especially those that restrict bookings such as limits on visitor numbers and the minimum visitor age of 15 years.
- **Seasonality:** Programme design should include evaluation of seasonal marketing or low-season rates (e.g., Nishida and Mwinuka 2005) to alleviate pressure during high seasons that might lead to violation of tourism rules. However, it is also important to consider that low seasons can allow for rest or reduced-exposure of habituated apes to the stressors and risks of tourism.

- **Stand-by systems:** At sites with multiple groups of apes available for tourism, booking systems that allow permits for one group to be held back as 'stand-by' only (not booked in advance) can resolve the problem of a group being unavailable on a particular day (having ranged too far or a veterinary intervention required), or accidental overbooking.

### 5.4.11 Pricing structures

Appropriate pricing is vital to maximising revenue and should follow the guiding principle that conservation is the primary goal of great ape tourism. When establishing a pricing structure, it is important to consider the following:

- **Unique experience:** Fees charged for great ape tourism must reflect the exclusive nature of ape viewing and should not be under-valued. Market surveys show that people are willing to pay large fees for this privilege (e.g., $500 to track mountain gorillas, Bush and Fawcett 2008).
- **Conservation impact:** The overall tourism cost-benefit ratio is greatest when small numbers of tourists pay high prices. Low prices could lead to excessive visitor demand that would ultimately jeopardise conservation objectives.
- **Type of tourism:** Fees should also reflect the nature of tourism on offer (tracking with essentially guaranteed viewing at close proximity vs. observation at a bai vs. forest walk with a chance to see apes vs. river excursion). In addition, sites or countries trying to recover from a tourism slump could consider a temporary reduction in charges.
- **Tiered pricing structures:** Pricing should provide incentives to local visitors, as well as citizens and residents of range states. These visitors will improve occupancy rates, especially in low seasons or tourism market slumps, and will enhance local and national awareness of ape conservation issues.
- **Pricing structures guided by occupancy rates:** As an ape tourism site grows in popularity, it may become fully booked at certain times of year. This could result in pressure from tourists, tour operators, and even conservation authorities and government ministries, to increase visitor numbers, either by allowing more tourists per group or per day, or through additional habituation efforts. However, the first course of action should be to raise the permit price so that additional conservation funding is sourced without increasing the risks caused by expanding tourism.
- **Market studies and visitor surveys:** It is important to price activities appropriately, particularly at new sites, and decisions should be informed by market surveys targeting sectors of the tourist market that a site hopes to attract. As operations grow, visitor surveys and additional evaluations should guide pricing reviews.

### 5.4.12 Marketing efforts

Once a tourism site has been established and habituation (if appropriate) is underway, the process of marketing should begin.

- **Identify key players in the tourism market:** Market surveys will help to identify stakeholders and means of attracting appropriate sectors of the tourism market.
- **Prepare and distribute marketing materials stressing conservation principles:** Materials designed to attract tour operators and tourists to a site and to inform them of what to expect must emphasise that conservation is the priority goal of tourism. This will sensitise tourists by demonstrating that activities will be managed to minimise risks to the apes, and will better prepare tour operators to inform their clients of the rules and regulations intended to protect the apes from tourism impacts.
- **Marketing must moderate tourist expectations:** Many people consider great ape tourism to be a once-in-a-lifetime opportunity. Marketing must generate realistic expectations so that tourists understand and appreciate the typical tourist experience in a given site. The pressure to guarantee observations of wild apes should be

resisted, as it raises expectations significantly, and it is impossible to guarantee a 100% chance of observing wild animals, even if they are habituated. It is preferable to market tracking rather than viewing, stipulating that staff will follow tracks and attempt to locate the apes, but cannot guarantee they will be visible. Alternative activities should be in place and offered to visitors if the apes are not located (e.g., if a group has moved too far away).

- **Marketing must manage tour operator and other partner expectations:** Great ape tourism is viewed by many private sector partners as an opportunity to sell lucrative tourism packages. Marketing efforts must address the tendencies of tour operators to regard ape tourism as a 'product' rather than a conservation opportunity, as the former attitude may lead to disregard of regulations, abuse of visiting privileges and pressure to expand operations.
- **Marketing should promote broad tourism circuits:** Great ape tourism often operates within constraints of uncertain sightings (or poor quality viewing), in remote locations with basic visitor facilities, all of which may reduce tourist interest, occupancy and satisfaction. While striving to improve visitor facilities (along best practice guidelines), it is important to build ape tourism into circuits that highlight a region's wildlife and natural habitats, as well as specialist interests, such as bird-watching or cultural tours, to encourage longer stays in the region or country.

### 5.4.13 Staffing issues

Tourism management requires professional, competent and efficient staff, who are well paid, well trained and well equipped. The following are issues to incorporate into recruitment plans for great ape tourism.

- **Local recruitment:** To maximise benefits to communities adjacent to great ape habitat, it is important to provide local employment opportunities. Knowledge of the forest environment is usually advanced in local people who use the forest and its resources. Many have skills that are essential for tracking great apes, and are familiar with local community culture and traditions, which can enhance the visitors' experience. Formal training (see next page) to develop skills that local staff do not have will require funding and time commitments.
- **Importing skilled staff as trainers:** Only when particular skills cannot be sourced or developed locally should staff be recruited further afield. This might be the case for functions such as hospitality, management and accounting, or positions requiring an ability in a particular foreign language. Skilled staff should then provide training to local recruits.
- **Staff affiliation:** Ideally all staff guiding tourists will be hired directly by a protected area management authority, or officially recognised by that authority. If staff are employees, their strict adherence to regulations will be easier to enforce.
- **Remuneration:** Ape tourism has the potential to attract high fees, and must be adequately controlled to protect the apes from the negative impacts of strong monetary incentives. This will require loyalty to the conservation goals of a tourism programme, and staff must not be tempted to deviate from established rules for personal gain. One of the best ways to avoid corruption is to pay satisfactory salaries. In many countries, the legally-mandated minimum wage is not enough to guarantee an appropriate standard of living; thus tourism projects should assess the cost of living and provide a 'living wage' sufficient to maintain a staff member with an average-sized family. (See also tipping policies in Section 5.5.16).
- **Equipment and uniforms:** Field staff must be provided with appropriate field and communications equipment and attired in professional uniforms that clearly identify them as tourism staff. Disease transmission should be minimised by assigning specific staff members to particular groups, with an adequate supply of clean uniforms and appropriate boot washing facilities (Whittier 2009).

Tourism staff training, Budongo Forest Reserve, Uganda. Photo © Debby Cox.

### 5.4.14 Staff training

For great ape tourism to be effectively managed with conservation as its main purpose, it must be run by skilled and knowledgeable staff who understand the risks involved, that conservation is the primary objective, and who have the authority to enforce regulations in the face of pressures from both tourists and tour operators. The following issues must be taken into account when designing and financing staff training programmes:

- **Great ape behaviour and forest ecology:** Staff should be knowledgeable about the ecosystem in which they will guide visitors. Many tourists are keen to learn while hiking and tracking, and staff should be capable of answering questions about great ape biology and behaviour, and the ecology of their habitat. Tourism staff could improve their knowledge by participating in research activities.
- **Language skills:** Staff must be able to explain the rationale behind regulations, to control tourists and to communicate effectively during an emergency. They therefore need to be competent in speaking the most common language of a site's tourists.
- **Empowerment:** As well as enforcing protected area regulations and national laws, staff must have the ability to control tourists without concern for any perceived differential in social status, and they must not give priority to tourist satisfaction over ape protection. Staff training should include techniques for dealing with 'problem' tourists who resist their authority and who may aggressively push for rules to be broken.
- **First aid:** Training and equipment should prepare staff to respond appropriately in cases of accident or injury, to treat and transport tourists to safety.

### 5.4.15 Emergency contingency plans

All tourism sites must develop plans to respond to emergencies that may affect the viability of their programmes:

- **Funding contingency plans:** While successful tourism will be a good source of funding, it may not be reliable, given the fickle nature of the industry and that trends are difficult to predict. Slumps in visitation will result in lower revenues for conservation and law enforcement, but these activities *must* continue even in the absence of tourism. Financial contingency plans can include emergency support from donors, endowment funds or revenue set-asides to cover core conservation operations during low-tourism periods.

- **Disease outbreak contingency planning:** Great apes are vulnerable to disease transmitted by tourists, field staff, local communities, livestock and other wildlife. Therefore veterinary support programmes should work with site authorities to put in place disease surveillance and response plans so that quick action can be taken to prevent spread or outbreaks.[11]
- **Human–great ape conflict response plans:** Tourism can exacerbate conflicts with local people if, for example, habituation increases the incidence of crop-raiding and income is not seen to be fairly distributed. Plans to avoid or mitigate such conflicts must be in place (see Hockings and Humle 2009).
- **Security or natural disaster planning:** Any area that is prone to natural disasters, cross-border conflict, civil war, crime or terrorist attacks should not selected for tourism development; however, unforeseen events can affect any site. Thus it is important that evacuation plans and security protocols are in place to protect tourists, staff and great apes during any such event.

## 5.5 Implementation phase—regulations

Great ape tourism sites should develop detailed regulations incorporating lessons learned from other sites, and should monitor, reinforce and improve these regulations throughout the lifespan of their programme. Site-specific regulations can be developed in consultation with medical, veterinary, travel and ecotourism practitioners (Muehlenbein and Ancrenaz 2009). However, good plans are meaningless without effective enforcement, and poor enforcement has been a perennial problem for great ape tourism. Therefore, it is critical that conservation managers have the authority to institute tourism regulations, to exercise authority once tourism is underway, and to maintain that authority over the long-term. This will help to foster compliance by both staff and tourists. The general regulations given below are relevant to most great ape tourism sites.

### *Regulations – Pre-Visit*

#### 5.5.1 Dissemination of regulations via tour operators and booking agents

Prior to their arrival at a great ape tourism site, visitors should be presented with the rationale behind measures intended to minimise disease risks and other negative impacts of tourism. Printed regulations should be sent to tour operators, marketing or booking agents and, if possible, posted on a website.

#### 5.5.2 Immunisation

Many great ape sites require that tourists present proof of vaccination, or a current negative test, for a number of diseases. Vaccination requirements may include polio, tetanus, measles[12], mumps, rubella, hepatitis A and B, yellow fever, meningococcal meningitis, typhoid and tuberculosis (or proof of negative skin test within the last six months). This regulation has a number of advantages: besides preventing the spread of these particular diseases, it reinforces the visitor's perception that tourism poses a risk to the apes. This should stimulate any responsible tourist's willingness to adhere to guidelines for their visit. Relying on proof of vaccination or a negative test alone will not control all infections of concern, such as the common cold and influenza, for which there is either no vaccine or a vaccine for certain strains only.

There can be problems with vaccinations: Vaccinated tourists may develop a false sense of security and feel that they can violate other regulations because they are immunised. In addition, lead-times for vaccination mean that vaccination requirements may not be easy to administer (e.g., vaccinating only one day before a visit is generally not protective, and a modified live vaccine may

11 Disease contingency plans are available for mountain gorillas (UWA and IGCP 2000; MGVP 2004). In addition, simple procedures such as preventing staff from visiting multiple groups will prevent disease spread (Whittier 2009).

12 Laboratory tests show that immunity to measles can substitute for proof of vaccination (Budongo Forest Project 2006).

infect other contacts, apes included). To avoid disappointment, vaccination and health regulations should be provided at the time of booking so that tourists are able to organise any immunisations or tests required and obtain the necessary documentation. See Leendertz *et al.* (in press) for more information.

### *Regulations – On Arrival*

#### 5.5.3 Presentation of tourism impacts and safety issues

Appropriate information on the various impacts of tourism on great apes should be given to tourists on arrival. Presentation should be thorough and consist of both active discussion of the regulations that minimise risks and passive information transfer (such as written materials in accommodation facilities, displays and signage in check-in areas). This can be reinforced with demonstrations of the required safe distance and role-play with guides showing how they would manage an approaching ape to prepare tourists on how they should respond. If acted out, tourists will be more likely to remember what they have been told. Safety precautions should also be explained at this time and, if required, visitor liability waivers signed.

#### 5.5.4 Guided health evaluation prior to departure

During final check-in for a tourist visit, staff should inspect vaccination certificates rather than rely on self-reporting (Muehlenbein *et al*. 2008). Tourists should then be guided through a self-evaluation designed to highlight whether they might be infectious or otherwise unable to participate in the visit. This should include a checklist of symptoms such as sneezing, coughing, fever or diarrhoea within the previous 48 hours, and exposure to any significant risks (e.g., disease, bat caves). *N.B.* Self-evaluation is not enough to ensure compliance because some tourists will try to conceal symptoms; however, the process will identify those willing to decline a visit on health grounds, and facilitate the process of refunding tourists who self-report illness.

#### 5.5.5 Professional health evaluation

A health professional on-site could perform routine health checks, such as measuring body temperature, heart rate and respiratory rate. This will not be possible at all sites, but large tourism programmes should consider having a nurse or doctor on staff, in conjunction with an employee health programme. Health professionals will also be able to advise on local and global disease patterns and propose additional precautions as needed. Guides should also be trained to recognise tourists who are unwell, and given authority to exclude them from great ape tourism activities.

### *Regulations – During Visit*

Unfortunately, tourists who have travelled long distances (usually at great expense) may try to hide illness, while others could be infectious without knowing it. Consequently everyone who approaches great apes poses a disease risk and must act accordingly. Strict regulations are also important to minimise the behavioural impacts of tourist visits. Any site claiming that they adhere to best practice in great ape tourism must implement the following:

#### 5.5.6 Maximum number of tourists per group

To minimise behavioural disturbance and disease risk, strict limits on the number of tourists allowed to visit each day must be set and adhered to. In dense forest where visibility is poor, any sudden noise or movement could cause alarm and unpredictable reactions. In addition, finding a good viewing spot for each tourist can be challenging. Tourists must stay together and avoid encircling the apes being viewed. To facilitate the control of visitors, minimise danger and enhance visitor satisfaction, the number of people per party should be no more than 4 tourists accompanied by 2 guides/trackers. This should achieve a reasonable balance between apes and humans, and reduce stress and its knock-on effects. Small numbers also favour high permit prices, as tourists tend to value being part of a small and exclusive group of visitors.

This general guideline should be implemented by all new sites. However, note that species-specific recommendations on tourist numbers are discussed in Section 5.7. A number of sites operate with fewer than 4 tourists, including the sites offering viewing of habituated western lowland gorillas and

some orangutan sites—the continued viability of these sites suggests that numbers can remain low. Mountain gorilla sites and some chimpanzee sites currently operate with more than 4 tourists, and these sites should assess whether reducing tourist numbers *towards* this recommended maximum could be feasible in future, and any new ape groups opened for tourism should have a smaller number of tourists.

### 5.5.7 One tourist visit per day

- There should be no more than one visit per day to each group of apes (or individual/party/forest area in the case of chimpanzee and orangutan tourism).
- Any site that currently operates more than one visit per day should try to reduce the schedule to one visit a day per group or individual. This can be done by closing second-visit bookings over time, or by habituating a new group (guided by a full impact assessment).
- Tourism accommodation located in or near ape habitat must limit visitor movements away from the facility to prevent uncontrolled ape viewing.

### 5.5.8 No visits by people who are sick

- People who are unwell will not be allowed to visit the apes, and this must be made very clear at the time of booking. It is critical that tourists are encouraged to self-report their illnesses and be given incentives to refrain from visiting if necessary. This should not be a postponed visit (it is probable that the person would continue to be infectious for a few days), but could be a refund on-site or vouchers for other tourism services (e.g., accommodation, hiking).
- Similarly, staff members who are ill must not participate in ape visits, and must be given incentives to remain away from apes, such as guaranteed 'sick days' and a policy of non-discrimination if they cannot work because of illness.

### 5.5.9 N95 respirator masks

- All tourists and staff who are likely to approach habituated apes to within 10 metres should wear a surgical quality N95 respirator mask for the duration of their one-hour visit. Respirators that filter out higher percentages of aerosolised particles are also acceptable (i.e., N99 or N100).
- Masks should be carried by tracker/guides in appropriate waterproof containers so that they are not damaged and rendered less effective during transport. They should be distributed to tourists just before they begin actually viewing the apes.
- Masks are disposable and should not be re-used. They should be collected by the trackers/guides immediately after the visit and disposed of appropriately after the visit, as they pose a disease risk to apes and other wildlife if accidentally dropped in the forest.
- Masks must be burned upon return to tourism administration or accommodation facilities, away from areas where apes range.
- Masks that become damp or wet are less effective at blocking pathogens and should be exchanged for a new one.
- Staff must receive training in mask management, including proper fit-testing, wear, use and disposal.
- Appropriate use of masks (including fitting, handling and disposal) should be demonstrated in full to tourists at the departure point, with a review before they reach the 10-metre distance, so that masks are not put on incorrectly in a rush to see the apes.
- A surgical mask should not give the wearer a false sense of security—all other regulations (concerning hygiene, distance from the apes, time spent with them) must be enforced alongside mask provision. Appropriate education must be given to staff and tourists alike.

- Tourists feeling the urge to sneeze or cough while in proximity to the apes should turn their head away even when wearing a surgical mask, but should not remove the mask, although staff should offer a replacement mask if necessary.
- Mask management should be monitored as part of a broader tourism monitoring programme, and results used to inform and improve procedures.
- Tourist compliance and feedback should also be taken into consideration when reviewing mask management procedures.
- Procurement systems must ensure a reliable supply of appropriate masks on site.
- If N95 respirator masks are not available, surgical quality multi-layer masks may be used while N95 respirators are procured, as surgical masks provide a barrier to large-droplets. Their use should only be temporary, as surgical masks are not as effective as N95 respirators. Further information on surgical masks and N95 respirators can be found in Appendix II.

#### 5.5.10 Children younger than 15 years old prohibited from visiting

- Children below 15 years old must not be allowed to visit great apes. While parents may argue against this regulation on the basis that their child is capable of the hike or mature enough to control their fear, this safeguard is primarily for health reasons. Young people are more likely to be infected with common childhood diseases, even when properly vaccinated, and therefore pose a much greater health risk to habituated apes.

#### 5.5.11 Non-essential personnel to remain at a distance from apes

- Non-essential personnel such as military escorts or porters must stay as far away as feasible, out of sight *and earshot* during the tourist visit.
- Non-essential personnel should remain in contact with guides via walkie-talkie radios, so that they can be instructed to move if the apes head in their direction.

#### 5.5.12 Prevent contamination of the habitat with food waste

- Eating is not allowed during a visit. Food and drink must not be visible while observing great apes, but should be left with porters or other personnel who remain out of sensory range of the apes.
- Food must not be consumed within 500 metres of apes. This will minimise the accidental contaminated waste and prevent the apes from developing an association between humans and food.
- Food waste and all other rubbish must be stowed in backpacks and carried out of the forest to prevent deposition of infectious waste in the habitat.
- Food must never be used to attract apes towards tourists.

#### 5.5.13 Minimum distance to habituated great apes

- For visitors wearing N95 surgical masks, the minimum distance permitted is 7 metres (22 feet)
- For visitors not wearing N95 masks, the minimum distance permitted is 10 metres (33 feet)

#### 5.5.14 One-hour time limit

- Tourists must spend no more than one hour near habituated apes.
- This limit combined with restriction of one visit per day means that no ape should be visited by tourists for more than one hour on any day.
- If apes are not easily visible when first approached, staff should escort tourists away to a distance of 200 metres to await a time when the apes are resting or have moved into more open vegetation, and then begin the permitted hour.

Scaled-model of the minimum 7-metre distance allowed between tourists and mountain gorillas, Volcanoes National Park, Rwanda. Photo © Maryke Gray.

### 5.5.15 Hand-washing and hygiene

- Basin facilities and soap should be provided at departure points, and tourists encouraged to wash their hands before departure.
- Latrines must be provided at departure points, and tourists encouraged to use them before departure. Latrines should be constructed at appropriate distances from watercourses (at least 30 metres).
- If tourists or staff have to urinate or defecate while in the forest, faeces must be buried in a 30-centimetre hole. This should be at least 500 metres from apes' location and away from watercourses.
- Guides should carry hand disinfectant spray (such as chlorhexidine), gel, or wipes for all visitors and staff to use before approaching apes.
- Smoking is prohibited in ape habitat due to the risk of fire, and of disease transmission via contaminated cigarette butts. The smell of smoke will also scare wildlife.
- Spitting and nose blowing/clearing on the ground are forbidden—staff and tourists should use handkerchiefs as needed, and these activities should not take place near the apes.
- The same boots and clothing should not be worn to visit a different group unless it has been washed and dried between visits.

### 5.5.16 Tipping policies and staff salaries

- Tourists must be informed that tips cannot be used to encourage staff to break regulations, and staff must not view tips as justification to ignore regulations; this would also reduce the professionalism of the operation.
- Tourists dislike having rules presented to them and then seeing them broken—this reduces respect for both staff and regulations. This message must be communicated to staff through education, training and monitoring, to enhance their compliance.
- Tipping policies should be clearly displayed so that tourists are aware of the issues before starting their activity.
- Tourism staff should be paid satisfactory salaries (at least a 'living wage' and preferably higher) to minimise temptations to violate regulations for higher tips.
- Regular monitoring and staff supervision should be used to reinforce tipping issues.

- All tourism staff, from check-in clerks to trackers and guides, should benefit from tips via a shared tip box with tips distributed equally among all staff each day.
- Policies specifying that pooled tips will be divided among all tourism staff will help prevent irregularities and should be posted where they are visible to visitors.
- Tourists appreciate guidance on tipping, and appropriate amounts can be suggested.
- A no-tipping policy should be considered if tips are judged to be a prime factor in staff relaxing regulations.

#### 5.5.17 Monitoring and enforcement of rules

- It is imperative that all staff understand the rules, can explain their rationale to visitors and enforce them.
- Tourism staff should be regularly monitored and evaluated on their conduct, and results should be discussed openly between evaluators and staff.
- A post-visit checklist provided to tourists and staff could help to reinforce staff compliance, and specific cases where staff had problems enforcing rules could be used in staff training exercises.
- Regular refresher courses will reinforce staff understanding and adherence to tourism regulations, and should include training on enforcement techniques.

### *Regulations – Site Management*

#### 5.5.18 Infrastructure designed to minimise impact on apes and habitat

- EIAs should be carried out for all tourism-related infrastructure developments, in keeping with national environmental legislation.
- Tourism infrastructure, such as lodges, campsites and visitor centres, should be constructed in areas where impacts on apes and their habitats are minimal.
- If possible, tourism infrastructure should be located outside or on the edge of ape habitat, and any disruption to native vegetation, especially forest, should be kept to a minimum.
- Tourism infrastructure should not be built in areas frequented by apes, due to risks of encountering people, food preparation areas, waste disposal, or sanitation facilities, and risk of injury from electrical cables or other hazards.

A viewing platform, Mbeli Bai, Republic of Congo. Photo © Fiona Maisels.

- Tourism infrastructure must not introduce additional disease risks to ape populations. Attention to appropriate sanitation, hygiene and waste disposal is critical in this regard.
- Tourism infrastructure should not include installations that could attract apes, such as the planting of crops or fruit trees.
- If infrastructure on any scale is necessary in ape habitat, attention should be paid to reducing the impact of tree felling on the apes' feeding and ranging requirements (see Morgan and Sanz 2007).

#### 5.5.19 Staff housing and administrative infrastructure

- Staff and administrative buildings should be sited to maximise the oversight and control of tourism programmes. Managers and law enforcement teams should be posted on-site so that monitoring and protection activities can be carried out routinely.
- Staff and administration buildings must be located and designed to minimise impacts on apes and their habitat from noise and other hazards (e.g., fuel, power lines, toxins).

#### 5.5.20 Tourism accommodation should benefit local communities

- Accommodation in lodges or campsites should be managed to maximise community benefits through community-ownership, employment opportunities, or revenue-sharing schemes that provide income to members of the community or funding for social services.
- Tourist accommodation that benefits local communities should be protected from external competition. This can be achieved through zoning so that only a viable number of facilities are allowed to operate at the preferred locations.

### 5.6 Monitoring and evaluation phase

#### 5.6.1 Applied research

Tourism programmes should be supported by independent impact-assessments to inform and improve tourism policy and management systems. Formal mechanisms of review and incorporation of research results into management and policy will ensure that conservation impacts are optimised. Research programmes should include:

- **Disease monitoring:** Disease is the most serious risk associated with great ape tourism. Health monitoring records will show patterns of disease, and allow management to design prevention measures (e.g., quarantine, tourist vaccination regulations, community health projects) and to respond to disease outbreaks. Routine observations by trained personnel and non-invasive screening should be supplemented by opportunistic sampling of immobilised animals (see Leendertz *et al.* in press).
- **Behavioural monitoring:** Tourism can also have serious negative impacts on the behaviour, physiology and social dynamics of habituated apes. Independent research will highlight potential or incipient problems before they become severe and will allow adaptive management (see Fawcett 2004; Muyambi 2004; Hodgkinson and Cipolletta 2009).
- **Ecological monitoring:** Heavy tourist traffic may cause soil compaction, erosion, trampling and damage to vegetation. Controls to minimise degradation of the habitat should include prohibition of the cutting or removal of seedlings and vegetation, walking off trails, and fire.
- **Population monitoring:** Population monitoring is an essential adjunct to tourism management. Tourism should stimulate the development of research projects to meet tourism-impact monitoring and applied research requirements.

- **Law–enforcement monitoring:** The development and operation of tourism must not divert attention and resources away from the central goal of protecting great apes and their habitat. It is, therefore, important to monitor trends in illegal activities, and assess the performance and results of law-enforcement activities. Law-enforcement monitoring will highlight areas for improvement or the need for increased surveillance, and can inform management when apes are ranging into areas of illegal activity, so that prevention and response to those activities can be enhanced.
- **Conflict monitoring:** Human-great ape conflicts can be alleviated through the provision of tourism benefits to local communities, or exacerbated by tourism altering the apes' ranging behaviour and bringing them into conflict situations more frequently. It is important that conflicts are systematically monitored and the success of mitigation efforts measured.
- **Economic assessments:** The motivation for initiating great ape tourism is often the economic benefits anticipated by various institutional, local and national stakeholders, in both the public and private sectors. However, as has been stated throughout this document, conservation must be the ultimate goal of great ape tourism, and should be given priority over other interests. Therefore, it is important to monitor the economic impacts of great ape tourism to better justify its existence and to inform management decisions, such as pricing structures and booking systems. Methodology can be adapted from previous studies (e.g., Wilkie and Carpenter 1999; Hatfield and Malleret-King 2006; Bush and Fawcett 2008; WCS Gabon 2008).

### 5.6.2 Staff monitoring

Staff working in great ape tourism must be fully supported in their role as the prime defenders of great apes against the negative impacts of tourism. They need to be, and feel, able to discuss and enforce tourism rules and regulations. Their roles must be evaluated regularly to assess effectiveness and modify management, as needed. This can be achieved by regular supervision, including evaluation in the field, evaluation during tourism impact research, and feedback from tourists.

### 5.6.3 Programme monitoring and evaluation

- **Financial monitoring and transparency:** As a tool to provide funding for conservation, it is crucial that systems are in place to monitor revenue generation. Financial controllers must be able to demonstrate that income is supporting protected area management and operations, community projects and revenue-sharing programmes. Transparency will go a long way to reassuring critics of great ape tourism that this is an appropriate conservation measure.
- **Programme reporting:** Progress reports and the results of tourism impact monitoring and applied research should be produced at regular intervals (preferably quarterly, but at least annually) to stimulate internal review and timely identification of issues to be addressed.
- **Programme evaluation:** Regular medium-term (every two years) internal assessments of the performance, management and impacts of great ape tourism programmes must be carried out to accurately monitor progress and to allow for programme review and improvement. The results of management-related research (Section 5.6.1) should be used to guide improvement and adaptation in tourism programme management. In the longer-term, external evaluations should take place every 5 years to ensure appropriate implementation and to foster learning and exchange with other great ape tourism sites.

## 5.7 Species-specific guidelines

In addition to the general guidelines in Section 5.5, the following are specific to each taxon and tailored to their socio-ecology, habitat, and/or the type of tourism operating where they occur.

### 5.7.1 Eastern Gorillas

Lessons learned from over 30 years of experience with eastern gorilla tourism form the foundation of the general guidelines above and few variations are proposed for this species. Mountain gorilla socio-ecology makes them particularly amenable to tourism, which is further facilitated by features of their high altitude habitat (e.g., Williamson and Fawcett 2008). These characteristics make it possible for slightly larger tourist groups to visit in safety. Mountain gorilla tourism began with groups of 6 tourists; however, at some sites tourist group size was increased against expert advice. We maintain that the smaller number of visitors is better for both gorillas and tourists, and recommend that tourist group size be reduced from 8 to 6, and that any new groups opened for tourism should receive no more than 6 tourists. The 'gold standard' recommendations for eastern gorilla tourism (MGVP 2009) are presented in Appendix I–A.

### 5.7.2 Western Gorillas

The high profile and revenues generated by mountain gorilla tourism have inspired ambitions to replicate this success elsewhere. However, the western gorillas' socio-ecology, habitat, history and the threats they face differ significantly from eastern gorillas, and a number of factors warrant special mention. The two sites currently offering viewing of habituated western lowland gorillas, Mondika and Bai Hokou, have limited visitor group size to 2 and 3 tourists respectively (see also Appendix I–B).

- **Tailored marketing:** Western gorilla tourism will not meet expectations that have been raised by the mountain gorilla experience, so marketing must emphasise the differences and keep visitor expectations to a realistic level. It is advisable to promote western gorilla 'tracking' rather than 'viewing', as encountering a dispersed group of gorillas obscured by thick ground vegetation or high in trees might disappoint those expecting clear observations and photo opportunities.
- **Tracking expertise:** Tracking western gorillas, which have long day ranges, large home ranges and leave little trail, requires a level of expertise that often exists only among historically hunter-gatherer groups. Where possible, trackers should

**Western lowland gorillas, Mbeli Bai, Republic of Congo. Photo © Vicki Fishlock.**

be sourced from these ethnic groups, to maximise the success of habituation and tourism programmes.

- **Gorilla population density:** An unusually high gorilla density may impede habituation efforts as trackers could follow more than one group by mistake when trails cross in the overlapping ranges of different groups. However, if their density is very low, gorillas will be harder to find.
- **Multiple groups:** Sudden change in the typically smaller groups of western lowland gorillas, such as the death of the dominant 'silverback' male, can lead to group disintegration and the abrupt termination of habituation or tourism efforts. Therefore, tourism programmes should identify and commit to working with at least two groups from the outset.
- **Tourism outside of protected areas:** Most western gorillas live outside protected areas and tourism can improve the protection of some populations. In such cases, tourism must operate under clear, legally-binding agreements with local stakeholders, which define each partner's roles and responsibilities towards the long-term conservation effort, as well as to tourism development and operations. Sustainable funding must be secured not only to cover tourism development costs, but also long-term protection and conservation activities, particularly as it is more difficult to ensure funding for conservation projects outside protected areas.
- **Bai visits:** See Appendix I–C for an example of regulations for viewing from a platform.
- **Tracking unhabituated gorillas:** See Appendix I–D for an example of regulations for forest walks.

### 5.7.3 Chimpanzees

Chimpanzee parties tend to be less cohesive than gorilla groups. Although it is difficult to oversee a group of people when the chimpanzees are dispersed, staff must keep control of tourists at all times. It is critical to prevent tourists becoming separated and at risk, especially from displaying adult males. See Appendix I–E for sample regulations, but please note: Sites currently allowing groups with more than 4 tourists to visit are advised to revise this policy.

- **No provisioning:** Although this is a general guideline recommended for all species, it is emphasised here as most relevant to chimpanzee sites where provisioning has been practiced in the past, and where there were indications that provisioning resulted in increased aggression.
- **Prevention of attacks on human infants:** Chimpanzees have been known to attack human babies as an extension of their normal predatory behaviour. The minimum age of a tourist is 15 years, so small children will never be allowed to visit great apes. However, where local people are permitted to walk on designated trails, they must be forewarned of the dangers. A chimpanzee community that ranges into areas used by local people should not be habituated for tourism.

### 5.7.4 Bonobos

Bonobo tourism is under development at a few sites in the DRC, but to date there are no lessons learned specific to bonobos.

### 5.7.5 Orangutans (Sumatran and Bornean)

Participants of the 2002 Orangutan Conservation and Re-introduction Workshop (Rosen and Byers 2002) recommended against additional tourism development in wild orangutan habitat in Indonesia. This was due to concerns over security and illegal logging, combined with the remote nature of most orangutan sites and how this affects competition in the Southeast Asian regional tourism market. Civil war in Aceh ended in 2005, and tourism could again be used as a conservation and development tool (Singleton, pers. comm.). The 2002 workshop encouraged the promotion of community-based tourism initiatives only in areas that are not priorities for orangutan conservation and thus are not

candidates for immediate protection and/or incentives. Appendix I–F presents guidelines from one such project. In addition to the general guidelines, the following are specific to orangutans:

- **Minimise impacts on social interactions between habituated and unhabituated orangutans:** Although tourist visits are limited to one hour, human presence may reduce opportunities for habituated orangutans to interact with non-habituated individuals that are scared of people. This impact on orangutan sociality should be minimised by implementing the following guidelines:
    - Individual orangutans should not be visited by tourists for more than 10 days per month.
    - Tourism to individual orangutans should be suspended for at least 3 months per year. Note that if all habituated orangutans at a particular site use the same area of forest, periodic closure of the site is recommended.
    - Consort pairs should not be followed. Male orangutans are more aggressive when in consortship with a female, therefore, consort pairs should be left alone to minimise stress and risk of injury, and to avoid disruption of their reproductive behaviour.
- **Minimise impacts on vegetation:** If tourism is regularly conducted with the same individual orangutans, trampling of vegetation and trail cutting will be concentrated. This can be addressed by:
    - Limiting visitation to 10 days per month per individual (as above).
    - Suspending tourism to an individual or area for 3 months per year (as above).
    - Spreading the impact by rotating the focus of tourism activities to orangutans in different parts of the forest. When certain individuals or areas are closed to tourism (20 days per month plus 3 months per year), tourism is moved to different areas and individuals, giving the ecosystem a chance to recover, thereby increasing the long-term sustainability of tourism. This strategy exposes a greater proportion of the orangutan community and a greater area of forest to the impacts of tourism, so a balance must be achieved.
- **Zero-poaching in habituated orangutan home ranges:** The general guidelines state that all habituated great apes must be monitored daily and in perpetuity, to protect them from poaching. Due to the orangutans' semi-solitary and arboreal nature, it is impossible to monitor each individual every day. Accordingly, managers must strive towards a goal of zero poaching throughout the areas in which they range.

Tourists wearing masks viewing chimpanzees, Mahale Mountains National Park, Tanzania. Photo © Toshisada Nishida.

- **Viewing from boats or vehicles:** A few sites in Sabah, Malaysia, offer wildlife viewing excursions by boat or vehicle, and Gunung Leuser National Park in Sumatra, Indonesia, offers trekking on elephants. When orangutans are seen, they are usually at distances of 20 metres and above so the risks of disease transmission are lowered and the number of tourists per visit can be increased to 12 per boat or vehicle. However, large numbers of tourists can be noisy and intrusive, so tourist behaviour must be controlled, particularly when viewing unhabituated animals. Boat size, number of boats operating, and other site-specific factors will determine upper limits, but in general there should be no more than three boats or vehicles in proximity to an orangutan at any one time.
- **Tourists must remain in vehicle or boat at all times:** It is essential that distance maintained and tourist numbers controlled to enhance wildlife viewing and reduce impacts on the wildlife. Tourists should never be allowed to leave their vehicle or boat to pursue orangutans on foot.
- **Enforcement of no-feeding regulations:** While no provisioning is a general recommendation for all taxa, feeding is still practiced at some orangutan sites. Tourism managers should impose rules to stop the feeding of free-ranging orangutans by both tourists and guides, and indeed prohibit the carrying of any food into the forest.
- **Ex-captives:** No tourism should be allowed with reintroducable orangutans in rehabilitation centres, or in forests where rehabilitants range (Rosen and Byers 2002; Russon, Susilo and Russell 2004). Given that such tourism is currently in operation, we include regulations from Bukit Lawang as Appendix I–G.

## 5.8 Special considerations for small and Critically Endangered populations

Particular caution is required before developing or expanding tourism with Critically Endangered taxa. This classification is given to three of the four gorilla subspecies (mountain, western lowland and Cross River) and the Sumatran orangutan as (IUCN 2010). Although the three subspecies of Bornean Orangutan are listed as Endangered, the northwestern and the East Kalimantan populations of the eastern subspecies also merit special consideration because their small remaining populations are similar in size to those of the Sumatran orangutan (Soehartono *et al*. 2007).

### 5.8.1 Risk-management programmes

We recommend that a number of impact-management measures accompany all great ape tourism programmes. In the case of small or Critically Endangered populations, funding for risk management must be guaranteed before any tourism activities are launched, to ensure that negative impacts are identified and immediately addressed.

### 5.8.2 Optimise before expanding

A number of sites with Critically Endangered great apes are already conducting tourism. In some of them, tourism has made a positive contribution, generating income for comprehensive conservation programmes in and around their habitat. Income to national treasuries and a range of stakeholders has resulted in enhanced perceptions of great apes, and stimulated long-term support for conservation. While keeping these successes in mind, it is also important to step back and evaluate the future of tourism at these sites, to protect the programmes from complacency, and to prevent them sliding towards over-exploitation of the apes. There has been a general tendency to expand tourism by habituating additional animals, but for conservation to remain the primary objective, it is important to resist temptation to expand for economic gain. Economic benefits can be achieved in ways that do not involve subjecting the apes to additional tourists or exposing more animals to tourism. The recommendations below should be followed at all sites operating tourism with Critically Endangered apes:

- **Income generation that does not involve tourism expansion:** Governments and conservation authorities should encourage alternative means of stimulating earnings by authorities, the private sector and local economies, such as investment in

national enterprise development, micro-credit schemes for local enterprises, and support for other business developments.

- **No increase in the number of groups habituated for tourism:** Sites with Critically Endangered apes should avoid expanding the number of habituated groups. It is important to maintain a balance of exposed and unexposed groups to better mitigate negative impacts of tourism.
- **No increase in the number of individual apes habituated for tourism:** Habituation decisions should not be based on habituating the largest groups of apes, or the greatest number of individuals, for tourism. The larger the proportion of a population that is exposed to tourism, the greater the risk that disease could result in drastic reduction of the population.
- **Maximise revenue per tourism permit:** If there is pressure to increase revenues from great ape tourism, the first measure taken should be to increase permit prices. Revenue per permit should also be maximised by diversifying tourism activities at each site, and building ape tourism into national tourism circuits. Extending the average length of in-country stay of great ape tourists would increase the earnings associated with each permit at local, regional and national levels.

## Section 6: Conclusions

This document has provided a review of the history of great ape tourism and covered in detail the multiple costs and benefits to the conservation of great apes and their habitats. While not appropriate at every site, great ape tourism can serve as a tool to fund great ape conservation efforts. Sites that intend to develop and operate great ape tourism should use the general and specific guidelines given in Section 5 to design and implement tourism activities that are rooted in conservation, not the exploitation of great apes.

In closing, readers should review the guiding principles of best practice in great ape tourism, keeping the following in mind at all stages of planning, developing, implementing, and monitoring great ape tourism:

- Tourism is not a panacea for great ape conservation or revenue generation.
- Tourism can enhance long-term support for the conservation of great apes and their habitat.
- Conservation must be the primary goal at any great ape site and tourism can help to fund it.
- Great ape tourism should be developed only if the anticipated conservation benefits, as identified through impact studies, significantly outweigh the risks.
- Conservation investment and action at great ape tourism sites must be sustained in perpetuity.
- Great ape tourism must be based on sound and objective science.
- Tourism benefits and profit for communities adjacent to great ape habitat should be maximised.
- Profit to private sector partners and others who may derive income from tourism must not be the driving force for great ape tourism development or expansion.
- Tourism development must be guided by a comprehensive understanding of potential impacts, and managed to maximise the positive impacts and mitigate the negative impacts.

# Section 7: Acknowledgements

Sincere appreciation goes to all who contributed throughout the process of developing these guidelines. Significant input was provided by Louise Hurst, John Oates, Anthony Rylands, Chris Sandbrook, Janette Wallis and Chris Whittier. We are also very grateful to the following who generously lent their time and expertise to improving this document: Mike Cranfield, Dave Dellatore, Maryke Gray, Annette Lanjouw, Magdalena Lukasik-Braum, Angela Meder, Michael Muehlenbein, Ian Redmond, Johannes Refisch, Lucy Spelman and Angelique Todd. The final draft benefited from the deft editorial hand and guidance of Anthony Rylands. Thanks are also extended to Kim Meek for graphic design and to Lynn Barrie, Frances Broussard, Caroline Deimel, Julian Easton, Vicki Fishlock, Maryke Gray, Josephine Head, Alain Houle, José Kalpers, Uwe Kribus, Annette Lanjouw, Fiona Maisels, John Martin, Russ Mittermeier, MPI-EVAN, Ian Nichols, Toshisada Nishida, Christopher Orbell, Martha Robbins, Perry van Duijnhoven and Virunga National Park for kindly allowing the use of their photographs. This publication was sponsored by a grant to the IUCN/SSC Primate Specialist Group and Conservation International from the United States Fish and Wildlife Service (Great Ape Conservation Fund).

# Section 8: Bibliography

## 8.1 Literature cited

Adams, H.R., Sleeman, J.M., Rwego, I. and New, J.C. 2001. Self-reported medical history survey of humans as a measure of health risk to the chimpanzees (*Pan troglodytes schweinfurthii*) of Kibale National Park, Uganda. *Oryx* **35**:308–312.

Adams, W.M. and Infield, M. 2003. Who is on the gorillas' payroll? Claims on tourist revenue from a Ugandan national park. *World Development* **31**:177–190.

Ali, R., Cranfield, M., Gaffikin, L., Mudakikwa, T., Ngeruka, L. and Whittier, C. 2004. Occupational health and gorilla conservation in Rwanda. *International Journal of Occupational Environmental Health* **10**:319–325.

Ambu, L. 2007. *Strategy of the Sabah Wildlife Department for Wildlife Conservation in Sabah.* First International Conservation Conference in Sabah: the Quest for Gold Standards. Sabah Wildlife Department, Kota Kinabulu, Malaysia.

Ancrenaz, M. 2006. *Kinabatangan—Guidelines for Tourists Visiting the Red Ape Encounter Habituated Orang-Utans*. Kinabatangan Project, Sabah, Malaysia.

Ancrenaz, M., Dabek, L. and O'Neil, S. 2007. The costs of exclusion: recognizing a role for local communities in biodiversity conservation. *PLoS Biology* **5**:e289.

Archabald, K. and Naughton-Treves, L. 2001. Tourism revenue-sharing around national parks in Western Uganda: early efforts to identify and reward local communities. *Environmental Conservation* **28**:135–149.

Aveling, C. 1999. Lowland gorilla tourism in Central Africa. *Gorilla Journal* **18**:18–20.

Aveling, R.J. and Mitchell, A. 1982. Is rehabilitating orangutans worthwhile? *Oryx* **16**:263–271.

Baboulene, L. 2008. *Etude marketing et écotouristique du programme de préservation des écosystèmes du bassin du Congo*. IUCN, West and Central Africa Regional Office, Ougadougou.

Beck, B., Walkup, K., Rodrigues, M., Unwin, S., Travis, D. and Stoinski, T.S. 2007. *Best Practice Guidelines for the Re-introduction of Great Apes*. IUCN/SSC Primate Specialist Group, Gland, Switzerland.

Bermejo, M. 2004. Home-range use and intergroup encounters in western gorillas (*Gorilla g. gorilla*) at Lossi Forest, North Congo. *American Journal of Primatology* **64**:223–232.

Bermejo, M., Rodríguez-Teijeiro, J.D., Illera, G., Barroso, A., Vilà, C. and Walsh, P.D. 2006. Ebola outbreak kills 5000 gorillas. *Science* **314**:1564.

Bertolani, P. and Boesch, C. 2008. Habituation of wild chimpanzees *(Pan troglodytes)* of the South Group at Taï Forest, Côte d'Ivoire: empirical measure of progress. *Folia Primatologica* **79**:162–171.

Blom, A. 2000. The monetary impact of tourism on protected area management and the local economy in Dzanga-Sangha (Central African Republic). *Journal of Sustainable Tourism* **8**:175–189.

Blom, A. 2001. Potentials and pitfalls of tourism in Dzanga-Sangha. *Gorilla Journal* **22**:40–41.

Blom, A. 2004. A critical analysis of three approaches to tropical forest conservation based on experiences in the Sangha region. *Yale Forestry and ES Bulletin* **102**:208–215.

Blom, A., Cipolletta, C., Brunsting, A.M. and Prins, H.H. 2004. Behavioral responses of gorillas to habituation in the Dzanga-Ndoki National Park, Central African Republic. *International Journal of Primatology* **25**:179–196.

Blomley, T., Namara, A., McNeilage, A., Franks, P., Rainer, H., Donaldson, A., Malpas, R., Olupot, W., Baker, J., Sandbrook, C., Bitariho, R. and Infield, M. 2010. *Development* AND *Gorillas*? *Assessing Fifteen Years of Integrated Conservation and Development in South-western Uganda. Natural Resource Series No. 23.* International Institute for Environment and Development (IIED), London and Edinburgh, UK.

Boesch, C. 2008. Why do Chimpanzees die in the forest? The challenges of understanding and controlling for wild ape health. *American Journal of Primatology* **70**:722–726.

Boo, E. 1990. *Ecotourism: The Potentials and Pitfalls*. World Wildlife Fund, Washington, DC.

BRD. 2009. *Gorilla tourist rules pamphlet*. Berggorilla and Regenwald Direkthilfe (BRD), Muehlheim, Germany.

Briassoulis, H. 1991. Methodological issues: tourism input-output analysis. *Annnals of Tourism Research* **18:**485–495.

Budongo Forest Project 2006. *Terms of Agreement and Guidelines for Visiting Researchers Working with The Budongo Forest Project*.

Bush, G. and Fawcett, K. 2008. *An Economic Study of Mountain Gorilla Tourism in the Virunga Volcanoes Conservation Area*. Unpublished report, US Fish and Wildlife Service (USFWS), Dian Fossey Gorilla Fund International. Ruhengeri, Rwanda.

Butynski, T. 1998. Is gorilla tourism sustainable? *Gorilla Journal* **16**:15–19.

Butynski, T. 2001. Africa's great apes. In: B.B. Beck, T.S. Stoinski, M. Hutchins, T.L. Maple, B. Norton, A. Rowan, E.F. Stephens and A. Arluke (eds.), *Great Apes and Humans: The Ethics of Coexistence,* pp.3–56. Smithsonian Institution Press, Washington, DC.

Butynski, T.M. and Kalina, J. 1998. Gorilla tourism: a critical look. In: E. J. Milner-Gulland and R. Mace (eds.), *Conservation of Biological Resources*, pp.294–313. Blackwell Science, Oxford, UK.

Caillaud, D., Levréro, F., Cristescu, R., Gatti, S., Dewas, M., Douadi, M., Gautier-Hion, A., Raymond, M. and Ménard, N. 2006. Gorilla susceptibility to Ebola virus: the cost of sociality. *Current Biology* **16**:489–491.

Carlsen, F., Cress, D., Rosen, N. and Byers, O. 2006. *African Primate Reintroduction Workshop Final Report*. IUCN/SSC Conservation Breeding Specialist Group (CBSG), Apple Valley, MN.

CDC. 2004. *Guidance for the Selection and Use of Personal Protective Equipment (PPE) in Healthcare Settings*. Centers for Disease Control and Prevention (CDC), Atlanta, GA. <www.cdc.gov/ncidod/dhqp/ppe.html>.

CDC. 2006. *Interim Guidance on Planning for the Use of Surgical Masks and Respirators in Health Care Settings during an Influenza Pandemic*. Centers for Disease Control and Prevention (CDC), Atlanta, GA. <www.pandemicflu.gov/plan/healthcare/maskguidancehc.html>.

Chafe, Z. 2004. *Consumer Demand and Operator Support for Socially and Environmentally Responsible Tourism*. Center on Ecotourism and Sustainable Development and the International Ecotourism Society. Washington, DC.

Cipolletta, C. 2003. Ranging patterns of a western gorilla group during habituation to humans in the Dzanga-Ndoki National Park, Central African Republic. *International Journal of Primatology* **24**:1207–1226.

Cochrane, J. 1998. *Organization of Ecotourism in the Leuser Ecosystem*. Leuser Management Unit, Medan.

Collins, A. 2003. Health guidelines for visiting researchers in Gombe National Park to minimise risk of disease transmission among primates. *Pan Africa News* **10**:1–3.

Cranfield, M. 2006. *MGVP thoughts on Surgical Masks and Ecotourism*. Unpublished report to the IUCN/SSC Primate Specialist Group.

Cranfield, M. 2008. Mountain gorilla research: the risk of disease transmission relative to the benefit from the perspective of ecosystem health. *American Journal of Primatology* **70:**751–754.

Cranfield, M., Gaffikin, L. and Cameron, K. 2001. Conservation medicine as it applies to the Mountain Gorilla (*Gorilla gorilla beringei*). In: G. Rabb (ed.), *The Apes: Challenges for the 21st Century Conference Proceedings,* pp.238–240. Chicago Zoological Society. Brookfield, IL

Czekala, N. and Robbins, M.M. 2001. Assessment of reproduction and stress through hormone analysis in gorillas. In: M.M. Robbins, P. Sicotte and K.J. Stewart (eds.), *Mountain Gorillas: Three Decades of Research at Karisoke,* pp.317–340. Cambridge University Press, Cambridge, UK.

Decision Tree Writing Group. 2006. Clinical response decision tree for the mountain gorilla *(Gorilla beringei)* as a model for great apes. *American Journal of Primatology* **68**:909–927.

Deem, S.L., Karesh, W.B. and Weisman, W. 2001. Putting Theory into Practice: Wildlife Health in Conservation. *Conservation Biology* **15**:1224–1233.

Dellatore, D.F. 2007. Behavioural Health of Reintroduced Orangutans (*Pongo abelii*) in Bukit Lawang, Sumatra, Indonesia. MSc thesis in Primate Conservation, Oxford Brookes University, Oxford, UK.

Djoh, E. and van der Wal, M. 2001. Gorilla-based tourism: a realistic source of community income in Cameroon? Case study of the villages of Koungoulou and Karagoua. *Rural Development Forestry Network Papers* **25e:**31–37.

Doran-Sheehy, D.M., Derby, A.M., Greer, D. and Mongo, P. 2007. Habituation of Western Gorillas: the process and factors that influence it. *American Journal of Primatology* **69:**1–16.

Dreller, S., Jatzwauk, L., Nassauer, A., Pasckiewics, P., Tobys, H.-U. and Ruden, H. 2006. [Investigations on suitable respiratory protection against airborne pathogens]. *Gefahrstoffe - Reinhalten der Luft*, **66**:14–24. [In German]

Duffus, D.A. and Dearden, P. 1990. Non-consumptive wildlife-oriented recreation: a conceptual framework. *Biological Conservation* **53**:213–231.

Dupain, J. 2007. *AWF Congo Heartland Report, 3rd Quarter, FY 2007*. Unpublished report, African Wildlife Foundation, Nairobi, Kenya.

Epler Wood, M. 1996. *The Evolution of Ecotourism as a Sustainable Development Tool.* Paper presented at The Sixth International Symposium on Society and Natural Resource Management, Pennsylvania State University, 18-23 May 1996.

Fawcett, K. 2004. *The Impact of Tourism on Gorilla Behaviour—Preliminary Results from Rwanda*. Unpublished report, Karisoke Research Centre, Ruhengeri, Rwanda.

FDA. 2009. *Masks and N95 Respirators*. US Food and Drug Administration. Silver Spring, MD. <http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/GeneralHospitalDevicesandSupplies/PersonalProtectiveEquipment/ucm055977.htm>

Ferber, D. 2000. Human disease threat to great apes. *Science* **289**:1277–1278.

Focken, K. 2002. *Taï National Park Ivory Coast – PACPNT.* Paper presented at the International Workshop on Market Incentives for Biodiversity Conservation and Sustainable Use, Dakar, 25–27 June 2002.

Font, X., Cochrane, J. and Tapper, R. 2004. *Pay Per Nature View. Tourism for Protected Area Financing: Understanding Tourism Revenues for Effective Management Plans*. Leeds Metropolitan University and WWF, Leeds, UK.

Formenty, P., Boesch, C., Wyers, M., Steiner, C., Donati, F., Dind, F., Walker, F. and Le, G.B. 1999. Ebola virus outbreak among wild chimpanzees living in a rain forest of Cote d'Ivoire. *Journal of Infectious Diseases* **179(**Suppl.1**)**:120–126.

Frey, R. 1975. Sumatra's red apes return to the wild. *Wildlife* **17**:356–363.

Gami, N. 1999. Les gorilles de plaine pourquoi pas eux? *Canopée* **13**:3.

Garber, P.A. 2008. Disease transmission from humans to wild apes: perspectives on the costs and benefits of research and conservation. *American Journal of Primatology* **70**:715.

Goldberg, T.L., Gillespie, T.R., Rwego, I.B., Wheeler, E., Estoff, E.L. and Chapman, C.A. 2007. Patterns of gastrointestinal bacterial exchange between chimpanzees and humans involved in research and tourism in western Uganda. *Biological Conservation* **135**:527–533.

Goldsmith, M. 2004. *Impact of Habituation for Ecotourism on Bwindi Gorilla Behavioral Ecology—Summary of Findings and Recommendations for UWA - 2004*. Unpublished report to Uganda Wildlife Authority, Kampala, Uganda.

Goldsmith, M. 2005a. Habituating primates for field study—ethical considerations for African great apes. In: T. R. Turner (ed.), *Biological Anthropology and Ethics: From Repatriation to Genetic Identity,* pp.49–64. State University of New York Press. Albany, New York.

Goldsmith, M. 2005b. Impacts of habituation for ecotourism on the gorillas of Nkuringo. *Gorilla Journal* **30**:11–14.

Goldsmith, M.L., Glick, J. and Ngabirano, E. 2006. Gorillas living on the edge: literally and figuratively. In: N. E. Newton-Fisher, H. Notman, J. D. Paterson and V. Reynolds (eds.), *Primates of Western Uganda,* pp.405–422. Springer Verlag, New York.

Gombe Stream Research Centre and Wilson, M.L. 2006. *Health Protocol for Longer-Term Visitors to Gombe Stream Research Centre*. Gombe Stream Research Centre, Kigoma, Tanzania.

Graczyk, T.K, Bosco-Nizeyi, J., Ssebide, B., Thompson, R.C., Read, C., Cranfield, M.R. 2002. Anthropozoonotic *Giardia duodenalis* genotype (assemblage) a infections in habitats of free-ranging human-habituated gorillas, Uganda. *Journal of Parasitology* 88:905–909.

Greer, D. and Cipolletta, C. 2006. Western gorilla tourism: lessons learned from Dzanga-Sangha. *Gorilla Journal* **33**:16–19.

Grieser Johns, B.D. 1996. Responses of chimpanzees to habituation and tourism in the Kibale Forest, Uganda. *Biological Conservation* **78**:257–262.

Grosspietsch, M. 2007. Maximizing Tourism's Contribution to Poverty Reduction in Rwanda. Doctoral dissertation, Westphalian Wilhelms-University, Münster, Germany.

Guerrera, W., Sleeman, J.M., Ssebide, B.J., Pace, L.B., Ichinose, T.Y. and Reif, J.S. 2003. Medical survey of the local human population to determine possible health risks to the mountain gorillas of Bwindi Impenetrable Forest National Park, Uganda. *International Journal of Primatology* **24**:197–207.

Gutierrez, E., Lamoreux, K., Matus, S. and Sebunya, K. 2005. *Linking Communities, Tourism and Conservation: A Tourism Assessment Process*. Conservation International and The George Washington University, Washington, DC.

Hanamura, S., Kiyono, M., Nakamura, M., Sakamaki, T., Itoh, N., Zamma, K., Kitopeni, R., Matumula, M. and Nishida, T. 2006. A New Code of Observation Employed at Mahale: Prevention against a Flu-like Disease. *Pan Africa News* **13**:13–16.

Hanamura, S., Kiyono, M., Lukasik-Braum, M., Mlengeya, T., Fujimoto, M., Nakamura, M. and Nishida, T. 2007. Chimpanzee deaths at Mahale caused by a flu-like disease. *Primates* **49**:77–80.

Harcourt, A. H. 1986. Gorilla conservation: Anatomy of a campaign. In: K. Benirschke (ed.) *Primates: The Road to Self-Sustaining Populations,* pp.31–46. Springer-Verlag, New York, USA.

Harcourt, A. 2001. The benefits of mountain gorilla tourism. *Gorilla Journal* **22**:36–37.

Hastings, B.E., Kenny, D., Lowenstine, L.J. and Foster, J.W. 1991. Mountain gorillas and measles: ontogeny of a wildlife vaccination program. *Proceedings of the American Association of Zoo Veterinarians* 1991:198–205.

Hatfield, R. and Malleret-King, D. 2006. *The Economic Value of the Mountain Gorilla Protected Forests (The Virungas and Bwindi Impenetrable National Park)*. International Gorilla Conservation Programme (IGCP), Nairobi, Kenya.

Hockings, K. and Humle, T. 2009. *Best Practice Guidelines for the Prevention and Mitigation of Conflict Between Humans and Great Apes*. IUCN/SSC Primate Specialist Group, Gland, Switzerland.

Hodgkinson, C. 2009. Tourists, gorillas and guns: integrating conservation and development in the Central African Republic. Doctoral thesis, UCL (University College London) http://eprints.ucl.ac.uk/15848/

Hodgkinson, C. and Cipolletta, C. 2009. Western lowland gorilla tourism: impact on gorilla behaviour. *Gorilla Journal* 38:29–32.

Hofer, H. and East, M.L. 1994. Biological conservation and stress. *Advances in the Study of Behavior* **27:**405–525.

**Mountain gorilla with a bamboo shoot, Volcanoes National Park, Rwanda. Photo © Annette Lanjouw.**

Homsy, J. 1999. *Ape Tourism and Human Diseases: How Close Should We Get? A Critical Review of Rules and Regulations Governing Park Management and Tourism for the Wild Mountain Gorilla,* Gorilla gorilla beringei. International Gorilla Conservation Programme (IGCP), Nairobi. <www.igcp.org/wp-content/themes/igcp/docs/pdf/homsy_rev.pdf>.

Hosaka, K. 2008. A single flu epidemic killed at least 11 chimps. *Pan Africa News* **2**:3–4.

Hudson, H.R. 1992. The relationship between stress and disease in orphan gorillas and its significance for gorilla tourism. *Gorilla Conservation News* **6**:8–10.

Hurst, L. 2007. *Preliminary Assessment of Chimpanzee and Primate Tourism Management Issues in Nyungwe National Park*. Unpublished report, Wildlife Conservation Society (WCS) and The Rwanda Environment Management Authority, Kigali, Rwanda.

Hurst, L. 2008a. *Recommendations and Rationale for Eastern Chimpanzee (*Pan troglodytes schweinfurthii*) Tourism Regulations in Nyungwe National Park*. Unpublished report, Wildlife Conservation Society (WCS) and the Rwandan Office of Tourism and National Parks (ORTPN), Kigali, Rwanda.

Hurst, L. 2008b. *Chimpanzee Habituation Review and Recommendations for Nyungwe National Park*. Unpublished report, Wildlife Conservation Society (WCS) and the Rwandan Office of Tourism and National Parks (ORTPN), Kigali, Rwanda.

Hurst, L. 2008c. *Mountain Gorilla (*Gorilla beringei beringei*) Visitation Regulations Review Workshop Report*. Unpublished report, Wildlife Conservation Society (WCS) and the Rwandan Office of Tourism and National Parks (ORTPN), Kigali, Rwanda.

IGCP 2004. *Gorilla Rules*. Pamphlet, International Gorilla Conservation Programme (IGCP), Kigali, Rwanda.

IUCN 2010. *IUCN Red List of Threatened Species*. Version 2010.1. International Union for Conservation of Nature and Natural Resources (IUCN), Species Survival Commission (SSC), Gland Switzerland and Cambridge, UK.

Jacobson, S.K. and Figueroa Lopez, A. 1994. Biological impacts of ecotourism; tourists and nesting turtles in Tortugero National Park, Costa Rica. *Wildlife Society Bulletin* **22**:414–419.

JGI-Uganda 2006. *Chimpanzee Ecotourism Health Protocol Manual—Budongo Forest*. Jane Goodall Institute (JGI), Washington, DC.

Kalpers, J., Williamson, E.A., Robbins, M.M., McNeilage, A., Nzamurambaho, A., Lola, N. and Muguri, G. 2003. Gorillas in the crossfire: population dynamics of the Virunga mountain gorillas over the past three decades. *Oryx* **37**:326–337.

Kaur, T. and Singh, J. 2008. Up close and personal with Mahale chimpanzees—a path forward. *American Journal of Primatology* **70**:729–733.

Kaur, T., Singh, J., Tong, S., Humphrey, C., Cleverger, D., Tan, W., Szekely, B., Wang, Y., Li, Y., Muse, E.A., Kiyono, M., Hanamura, S., Inoue, E., Nakamura, M., Huffman, M.A., Jiang, B. and Nishida, T. 2008. Descriptive epidemiology of fatal respiratory outbreaks and detection of a human-related metapneumovirus in wild chimpanzees (*Pan troglodytes*) at Mahale Mountains National Park, western Tanzania. *American Journal of Primatology* **70**:755–765.

Kazooba, C. 2008. MPs want to end Uganda Safari's gorilla monopoly. *The East African* 28 September 2008. <www.theeastafrican.co.ke/news/-/2558/475356/-/s1ua7iz/-/index.html>

Klailova, M., Hodgkinson, C. and Lee, P.C. 2010. Behavioral responses of one western lowland gorilla (*Gorilla gorilla gorilla*) group at Bai Hokou, Central African Republic, to tourists, researchers and trackers. *American Journal of Primatology* **71**. DOI: 10.1002/ajp.20829

Köndgen, S., Kühl, H., N'Goran, P.K., Walsh, P.D., Schenk, S., Ernst, N., Biek, R., Formenty, P., Mätz–Rensing, K., Schweiger, B., Junglen, S., Ellerbrok, H., Nitsche, A., Briese, T., Lipkin, W.I., Pauli, G., Boesch, C. and Leendertz, F.H. 2008. Pandemic human viruses cause decline of endangered great apes. *Current Biology* **18**:1–5.

Kortlandt, A. 1996. An epidemic of limb paresis (Polio?) among the chimpanzee population at Beni (Zaire) in 1964, possibly transmitted by humans. *Pan Africa News* **3**:9.

Krief, S., Huffman, M.A., Sévenet, T., Guillot, J., Bories, C., Hladik, C.M. and Wrangham, R.W. 2005. Noninvasive monitoring of the health of *Pan troglodytes schweinfurthii* in the Kibale National Park, Uganda. *International Journal of Primatology* **26**:467–490.

Kruger, O. 2005. The role of ecotourism in conservation: panacea or Pandora's box? *Biodiversity and Conservation* **14**:579–600.

Kühl, H., Maisels, F., Ancrenaz, M. and Williamson, E.A. 2008. *Best Practice Guidelines for Surveys and Monitoring of Great Ape Populations.* IUCN/SSC Primate Specialist Group, Gland, Switzerland.

Lanjouw, A. 1999a. Mountain gorilla tourism in central Africa. *Mountain Forum Bulletin* **3**:7–8.

Lanjouw, A. 1999b. Tourisme aux gorilles en Afrique centrale: Plaidoyer pour la réalité. *Canopée* **13**:3.

Leendertz, F.H., Ellerbrok, H., Boesch, C., Couacy-Hymann, E., MatzRensing, K., Hakenbeck, R., Bergmann, C., Abaza, P., Junglen, S. and Moebius, Y. 2004. Anthrax kills wild chimpanzees in a tropical rainforest. *Nature* **430**:451–452.

Leendertz, F.H., Pauli, G., Maetz-Rensing, K., Boardman, W., Nunn, C., Ellerbrok, H., Aina Jensen, S., Junglen, S. and Boesch, C. 2006. Pathogens as drivers of population declines: The importance of systematic monitoring in great apes and other threatened mammals. *Biological Conservation* **131**:325–337.

Leendertz, F., Cameron, K., Cranfield, M., Gaffikin, L., Gillespie, T., Lonsdorf, E., Minnis, R., Nizeyi, J-B., Nutter, F., Reed, P., Rwego, I., Travers, D. and Whittier, C. In press. *Best Practice Guidelines for Health Monitoring and Disease Control in Great Ape Populations.* IUCN/SSC Primate Specialist Group, Gland, Switzerland.

Litchfield, C. 1997. *Treading Lightly: Responsible Tourism with the African Great Apes.* Pamphlet, Travellers' Medical and Vaccination Centre Group, Adelaide.

Litchfield, C. 2007. Responsible Tourism: a conservation tool or conservation threat? In: T.S. Stoinski, H.D. Steklis and P.T. Mehlman (eds.) *Conservation in the 21st Century—Gorillas as a Case Study,* pp.107–127. Springer Verlag, New York.

Lonsdorf, E.V., Travis, D., Pusey, A.E. and Gilby, I.C. 2006. Causes and consequences of chimpanzee *(Pan troglodytes schweinfurthii)* illness: a retrospective analysis of factors correlated to chimpanzee health at Gombe National Park. Proceedings of the XXI International Primatological Society Conference held in Entebbe, Uganda. August, 2006. *International Journal of Primatology* **27 (**Suppl.1**)**: Abst #547. Abstract.

Low, T.W. 2004. Can Ecotourism Help Protect Orang-utans? BSc thesis in Sustainable Tourism Development, Anglia Polytechnic University, Cambridge and Chelmsford, UK.

Lukasik-Braum, M. and Spelman, L. 2008. Chimpanzee respiratory disease and visitation rules at Mahale and Gombe National Parks in Tanzania. *American Journal of Primatology* **70:**734–737.

Macfie, E. 1991. The Volcano Veterinary Centre update. *Gorilla Conservation News* **5**:20.

Macfie, E. 1996. Case Report on Scabies Infection in Bwindi Gorillas. *Gorilla Journal* **13**:4–5.

Macfie, E.J. 2005. *Gorilla Tourism Numbers in Bwindi Impenetrable National Park—Position Statement. International Gorilla Conservation Programme*, Uganda Wildlife Authority, Kampala, Uganda.

Macfie, E.J. 2007a. *Habituation Impact Assessment: A Tool for the Analysis of Costs and Benefits Related to the Potential Habituation of a Gorilla Group for Either Tourism or Research*. Virunga Bwindi Gorilla Management Technical Advisory Committee, International Gorilla Conservation Programme.

Macfie, E.J. 2007b. *Studying the Potential of Gorilla-Based Tourism as a Possible Tool for the Long-Term Conservation and Management of the Afi Mountain Wildlife Sanctuary Cross River Gorilla Population, Cross River State, Nigeria*. Report to the Afi Mountain Wildlife Sanctuary Partnership, Fauna and Flora International, Cambridge, UK.

Maddison, N. 2004. Assessing Ape Based Tourism in Africa: Identification of Key Success Factors for Private Sector Engagement in Pro-Poor Tourism. MBA thesis. University of the West of England (Bristol Business School), Bristol, UK.

McFarland, K.L. 2007. Ecology of Cross River Gorillas (*Gorilla gorilla diehli*) on Afi Mountain, Cross River State, Nigeria. Doctoral dissertation, City University of New York, New York.

McNeilage, A. 1996. Ecotourism and mountain gorillas in the Virunga Volcanoes. In: V.J. Taylor and N. Dunstone (eds.), *The Exploitation of Mammal Populations,* pp.334–344. Chapman and Hall, London, UK.

Meder, A. 1994. Causes of death and diseases of gorillas in the wild. *Gorilla Journal* **9**:3–4.

Mehta, H. and Guchu-Katee, C. 2005. *Virunga Massif Sustainable Tourism Development Plan: D.R. Congo, Rwanda and Uganda*. International Gorilla Conservation Programme (IGCP), Nairobi. Kenya.

MGVP 2002 Employee Health Group. 2004. Risk of disease transmission between conservation personnel and the mountain gorillas. *EcoHealth* **1**:351–361.

MGVP. 2004. *Mountain Gorilla Disease Contingency Plan—Decision Tree*. Mountain Gorilla Veterinary Program (MGVP), Ruhengeri, Rwanda.

MGVP. 2008. *Gorilla and Chimpanzee Visitation Guidelines for Tourists, Researchers and Park Staff—MGVP Recommendations updated April 2008*. Mountain Gorilla Veterinary Program (MGVP) Inc., Ruhengeri, Rwanda.

MGVP. 2009. *MGVP Ecotourism Recommendations to ICCN – Comments to Accompany April 2008 MGVP Document on Tourism Rules*. Mountain Gorilla Veterinary Program (MGVP) Inc., Ruhengeri, Rwanda.

Mittermeier, R.A., Louis Jr., E.E., Richardson, M., Schwitzer, C., Langrand, O., Rylands, A.B., Hawkins, F., Rajaobelina, S. Ratsimbazafy, J. Rasoloarison, R., Roos, C., Kappeler, P.M. and MacKinnon, J. 2010. *Lemurs of Madagascar.* 3rd edition. Tropical Field Guide Series, Conservation International, Arlington, VA.

Morgan, D. and Sanz, C. 2007. *Best Practice Guidelines for Reducing the Impact of Commercial Logging on Great Apes in Western Equatorial Africa*. IUCN/SSC Primate Specialist Group, Gland, Switzerland.

Moyini, Y. 2000. *Analysis of the Economic Significance of Gorilla Tourism in Uganda*. Report. International Gorilla Conservation Programme (IGCP), Kampala, Uganda.

Mudakikwa, A. 2001. An outbreak of mange hits the Bwindi gorillas. *Gorilla Journal* **22**:24.

Muehlenbein, M.P. and Ancrenaz, M. 2009. Minimizing pathogen transmission at primate ecotourism destinations: the need for input from travel medicine. *Journal of Travel Medicine* **16**:229–232.

Muehlenbein, M.P., Martinez, L.A., Lemke, A.A., Ambu, L., Nathan, S., Alsistom S., Andau, P. and Saking, R. 2008. Perceived vaccination status in ecotourists and risks of anthropozoonoses. *EcoHealth* **5**:371–378.

Muyambi, F. 2004. *Bwindi Impenetrable National Park Gorilla Tourism Impact study.* Presentation for the September 2004 Virunga-Bwindi Conservation Area Regional Meeting, International Gorilla Conservation Programme, Goma, DRC.

Muyambi, F. 2005. The impact of tourism on the behaviour of mountain gorillas. *Gorilla Journal* **30**:14–15.

Nakamura, M. and Nishida, T. 2009. Chimpanzee tourism in relation to the viewing regulations at the Mahale Mountains National Park, Tanzania. *Primate Conservation* **24**:85-90.

Nishida, T. and Mwinuka, C. 2005. Introduction of seasonal park fee system to Mahale Mountains National Park: a proposal. *Pan Africa News* **12**:17–19.

Nizeyi, J. B. 2005. Noninvasive Monitoring of Adrenocortical Activity in Free-ranging Mountain Gorillas of Bwindi Impenetrable National Park in South-western Uganda*.* Doctoral Dissertation, Faculty of Veterinary Medicine, Makerere University, Kampala, Uganda.

Nutter, F. and Whittier, C. 2001. Occupational health programs for primate field researchers: improving human health care benefits nonhuman primates. In: G. Rabb (ed.), *The Apes: Challenges for the 21st Century Conference Proceedings,* pp.244–249. Chicago Zoological Society, Brookfield, IL.

Nutter, F., Whittier, C., Cranfield, M. and Lowenstine, L.J. 2005. Causes of death for mountain gorillas (*Gorilla beringei beringei* and *g. b.* undecided) from 1968-2004: an aid to conservation programs. In *Proceedings of the Wildlife Disease Association International Conference. June 26-July 1, 2005, Cairns, Australia*, pp.200–201.

Ostroff, S.M. and Kozarsky, P. 1998. Emerging infectious diseases and travel medicine. *Infectious Disease Clinics of North America* **12**:231–241.

Plumptre, A. and Williamson, E.A. 2001. Conservation oriented research in the Virunga region. In: M.M. Robbins, P. Sicotte and K.J. Stewart (eds.), *Mountain Gorilla: Three Decades of Research at Karisoke,* pp.361–390. Cambridge University Press, Cambridge, UK.

Plumptre, A., Kayitare, A., Rainer, H., Gray, M., Munanura, L., Barakabuye, N., Sivha, M., Asuma, S. and Namara, A. 2004. *The Socio-economic Status of People Living Near Protected Areas in the Central Albertine Rift*. Wildlife Conservation Society (WCS), New York.

Purcell, Z. 2002. Chimpanzee viewing and regulation: Mahale Mountains National Park. *Pan Africa News* **9**:17–19.

Rajaratnam, R., Pang, C. and Lackman-Ancrenaz, I. 2008. Ecotourism and indigenous communities: the Lower Kinabatangan experience. In: J. Connell and B. Rugendyke (eds.), *Tourism at the Grassroots: Villagers and Visitors in the Asia Pacific, pp.236–255.* Routledge, London, UK.

Rijksen, H.D. 1982. How to save the mysterious 'man of the forest'? In: L.E.M. de Boer (ed.), *The Orang Utan: Its Biology and Conservation*, pp.317–341. Dr. W. Junk Publishers, The Hague.

Rijksen, H. and Meijaard, E. 1999. *Our Vanishing Relative: the Status of Wild Orang-utans at the Close of the Twentieth-Century*. Kluwer Academic Publications, London.

Rosen, N. and Byers, O. 2002. *Orangutan Conservation and Reintroduction Workshop: Final Report*. IUCN/SSC Conservation Breeding Specialist Group (CBSG). Apple Valley, MN.

Russon, A.E., Susilo, A. and Russell, C. 2004. Orangutan-focused ecotourism: Evaluating 30 years' experience. Paper presented at XXth Congress of the International Primatological Society, 23-28 August 2004, Turin, Italy.

Rwego, I.B., Isabirye-Basuta, G., Gillespie, T.R. and Goldberg, T.L. 2008. Gastrointestinal bacterial transmission among humans, mountain gorillas and livestock in Bwindi Impenetrable National Park, Uganda. *Conservation Biology* **22**:1600–1607.

Sandbrook, C.G. 2006. Tourism, Conservation and Livelihoods: The Impacts of Gorilla Tracking at Bwindi Impenetrable National Park, Uganda. Doctoral dissertation, University College London, London.

Sandbrook, C.G. 2008. Putting leakage in its place: the significance of retained tourism revenue in the local context in Rural Uganda. *Journal of International Development*. Published Online: 1 Oct, 2008. DOI: 10.1002/jid.1507.

Sandbrook, C.G. and Semple, S. 2006. The rules and the reality of mountain gorilla (*Gorilla beringei beringei*) tracking: how close do tourists get? *Oryx* **40**:428–433.

SGLCP. 2009. *Response to Notification: Updating or Revision of the Convention after 2010*. Convention on Biological Diversity (CBD) Steering Group on Linking Conservation and Poverty (SGLCP). <www.cbd.int/2010-target/notifications.shtml>.

Singleton, I. and Aprianto, S. 2001. *The Semi-Wild Orangutan Population at Bukit Lawang; A Valuable 'Ekowisata' Resource and Their Requirements*. Unpublished paper presented at the workshop 'Eco-tourism development at Bukit Lawang' workshop, Medan, Indonesia, April 2001. PanEco and Yayasan Ekosistem Lestari, Medan, Indonesia.

Singleton, I., Knott, C.D., Morrogh-Bernard, H.C., Wich, S.A. and van Schaik, C.P. 2009. Ranging behavior of orangutan females and social organization. In: *Orangutans: Geographic Variation in Behavioral Ecology and Conservation,* pp.205–213. Oxford University Press, New York.

Singleton, I., Wich, S., Husson, S., Stephens, S., Utami Atmoko, S., Leighton, M., Rosen, N., Traylor-Holzer, K., Lacy, R. and Byers, O. 2004. *Orangutan Population and Habitat Viability Assessment: Final Report*. IUCN/SSC Conservation Breeding Specialist Group (CBSG). Apple Valley, MN.

Soehartono, T., Susilo, H. D., Andayani, N., Atmoko, S. S., Sihiti, J., Saleh, C. and Sutrisno, A. 2007. *Orangutan Indonesia: Conservation Strategies and Action Plan 2007–2107.* Directorate General of Forest Protection and Nature Conservation, Ministry of Forestry of the Republic of Indonesia, Jakarta, Indonesia.

SOS. 2008. *Sumatran Orangutan Health Protocols and Guidelines for Visitors to the Bukit Lawang Eco-tourism Site*. Sumatran Orangutan Society (SOS), Oxford, UK.

TANAPA and FZS. 2007. *Mahale Mountains National Park—Chimp Viewing Code of Conduct*. Tanzania National Parks (TANAPA), Dar es Salaam and Frankfurt Zoological Society (FZS), Frankfurt.

Tapper, R. 2006. *Wildlife Watching and Tourism—A Study on the Benefits and Risks of a Fast Growing Tourism Activity and Its Impacts on Species*. United Nations Environment Programme (UNEP)/Convention on Migratory Species (CMS), Bonn, Germany.

Tentena, P. 2010. IGG cancels gorilla park contracts. *New Vision* 23 May 2010 <http://allafrica.com/stories/201005240404.html>

TIES. 2005. *Ecotourism Fact Sheet*. The International Ecotourism Society (TIES), Washington, DC.

Timen, A., Koopmans, M.P.G., Vosson, A.C.T.M., van Doornum, G.J.J., Günther, S., van den Berkmortel, F., Verduin, K.M., Dittrich, S., Emmerich, P., Osterhaus, A.D.M.E., van Dissel, J.T. and Coutinho, R.A. 2009. Response to imported case of Marburg hemorrhagic fever, the Netherlands. *Emerging Infectious Diseases* **15**:1171-1175.

Tutin, C.E.G. and Fernandez, M. 1991. Responses of wild chimpanzees and gorillas to the arrival of primatologists: behavior observed during habituation. In: H. O. Box (ed.), *Primate Responses to Environmental Change*, pp.187–197. Chapman and Hall, London, UK.

UNEP-GRASP 2005. *Kinshasa Declaration on Great Apes*. United Nations Environment Programme (UNEP), Great Ape Survival Project (GRASP), Nairobi, Kenya.

UNWTO 2009. Testing times for international tourism. *UNWTO World Tourism Barometer* **7**:1.

Uganda Wildlife Authority and IGCP. 2000. *Regional Contingency Planning for Disease Outbreak in the Mountain Gorilla Population—Report from a Workshop held 21st June–22nd June 2000, Kisoro, Uganda.* International Gorilla Conservation Programme (IGCP), Nairobi, Kenya.

van Krunkelsven, E., Dupain, J., van Elsacker, L. and Verheyen, R. 1999. Habituation of bonobos (*Pan paniscus*): first reaction to the presence of observers and evolution of response over time. *Folia Primatologica* **70**:365-368.

Wallis, J. and Lee, D.R. 1999. Primate conservation: the prevention of disease transmission. *International Journal of Primatology* **20**:803–826.

Wallis, J. and Lonsdorf, E.V. 2010. Summary of recommendations for primate conservation education programs. *American Journal of Primatology* **72**:441-444.

Wallis, J., Woodford, M., Karesh, W., Sheeran, L., Whittier, C., Nutter, F. and Taylor, S. 2000. ASP policy statement on protecting primate health in the wild. *ASP Bulletin* **24**:9.

Walsh, P.D. *et al*. 2003. Catastrophic ape decline in western equatorial Africa. *Nature* **422**:611–614.

WCS Field Veterinary Program 2008. *Health and Safety Protocols—Great Ape Ecotourism and Research—Nouabalé-Ndoki National Park, Republic of Congo*. WCS Field Veterinary Program, Brazzaville, Republic of Congo.

WCS Gabon 2006. *Langoué Bai: Information for Visitors*. WCS Gabon. Libreville, Gabon.

WCS Gabon. 2008. *Langoué Bai, Ivindo National Park: Review of the Pilot Tourism project 2001–June 2008*. Wildlife Conservation Society, Libreville, Gabon. <http://en.calameo.com/books/00000278504447bd38612>

Weber, A.W. 1993. Primate conservation and eco-tourism in Africa. In: C. S. Potter, J. I. Cohen and D. Janczewski (eds.), *Perspectives on Biodiversity: Case Studies of Genetic Resource Conservation and Development,* pp.129–150. American Association for the Advancement of Science Press. Washington, DC.

Weber, A.W. and Vedder, A. 1983. Population Dynamics of the Virunga Gorillas: 1959–1978. *Biological Conservation* **26**:341–366.

Weber, B. and Vedder, A. 2001. *In the Kingdom of Gorillas: Fragile Species in a Dangerous Land.* Simon and Schuster, New York.

Whittier, C. 2009. Diagnostics and Epidemiology of Infectious Agents in Mountain Gorillas. Doctoral dissertation, Comparative Biomedical Sciences, North Carolina Statue University, Raleigh, NC.

Whittier, C., Nutter, F. and Stoskopf, M. 2009. Zoonotic disease concerns in primate field settings. In: G. Rabb (ed.), *The Apes: Challenges for the 21st Century Conference Proceedings,* pp.232–237. Chicago Zoological Society, Brookfield, IL.

Wilkie, D.S. and Carpenter, J.F. 1999. Can tourism finance protected areas in the Congo Basin. *Oryx* **33:**332–338.

Wilkie, D.S., Carpenter, J.F. and Zhang, Q. 2001. The under-financing of protected areas in the Congo Basin: so many parks and so little willingness-to-pay. *Biodiversity and Conservation* **10**:691–709.

Williamson, E.A. 1988. Behavioural Ecology of Western Lowland Gorillas in Gabon. PhD thesis, University of Stirling, Stirling, UK.

Williamson, E.A. and Fawcett, K.A. 2008. Long-term research and conservation of the Virunga Mountain Gorillas. In: R. Wrangham and E. Ross (eds.), *Science and Conservation in African Forests: The Benefits of Long-term Research,* pp.213–229*.* Cambridge University Press, Cambridge, UK.

Williamson, E.A. and Feistner, A.T.C. 2003. Habituating primates: processes, techniques, variables and ethics. In: J.M. Setchell and D.J. Curtis (eds.), *Field and Laboratory Methods in Primatology: A Practical Guide,* pp.25–39. Cambridge University Press, Cambridge, UK.

Williamson, E.A., Harcourt, A., Nkurunungi, J.B., Wallis, J., Litchfield, C., Blom, A. and Russell, C.L. 2001. Gorilla and eco-tourism. A series of articles in *Gorilla Journal* **22**:35–41.

Williamson, E.A., Blom, A., Bermejo, M., Cipolletta, C., Klein, K., McFarland, K., Nishihara, T. and Todd, A. 2002. Recommendations from the Tourism Working Group at the Western Gorilla Workshop, Leipzig, 2002. Unpublished manuscript.

Wilson, M.E. 1995. Travel and the Emergence of Infectious Disease. *Emerging Infectious Diseases* **1**:39–46.

Woodford, M.H., Butynski, T.M. and Karesh, W. 2002. Habituating the great apes: the disease risks. *Oryx* **36**:153–160.

Wrangham, R.W. 1974. Artificial feeding of chimpanzees and baboons in their natural habitat. *Animal Behaviour* **22**:83–93.

Wrangham, R.W. 2001. Moral decisions about wild chimpanzees. In: B.B. Beck, T.S. Stoinski, M. Hutchins, T.L, Maple, B. Norton, A. Rowan, E.F. Stephens and A. Arluke (eds.), *Great Apes and Humans: the Ethics of Coexistence,* pp.230–244. Smithsonian Institution Press, Washington, DC.

WWF. 2008. *Fact Sheet: Lac Télé—Lac Tumba Landscape*. World Wildlife Fund (WWF), Washington, DC.

Yamagiwa, J. 1999. Slaughter of gorillas in the Kahuzi-Biega Park. *Gorilla Journal* 19:4–6.

ZSL. 2009. *Guidelines for Health and Safety in Tourism Activities at Mikongo Conservation Centre*, Zoological Society of London (ZSL), London, UK.

## 8.2 Bibliography – other relevant literature

### Planning tools

Brown, M., Bonis-Charancle, J.M., Mogba, Z., Sundararajan, R. and Warne, R. 2004. *Linking the Community Options, Assessment and Investment Tool (COAIT), Consensys™ and Payment for Environmental Services (PES): A Model to Promote Gorilla Conservation in Africa*. Innovative Resources Management, Washington, DC.

Eagles, P., McCool, S. and Haynes, C. 2002. *Sustainable Tourism in Protected Areas: Guidelines for Planning and Management.* World Commission on Protected Areas (WCPA) / IUCN, Gland, Switzerland.

Lindberg, K. and Hawkins, D. 1993. *Ecotourism: a Guide for Planners and Managers.* The International Ecotourism Society, North Bennington, VT.

Steck, B., Strasdas, W. and Gustedt, E. 1999. *Tourism in Technical Co-operation: A Guide to the Conception, Planning and Implementation of Project-accompanying Measures in Regional Rural Development and Nature Conservation*. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ), Tropical Ecology Support Programme. Eschborn.

Secretariat of the Convention on Biological Diversity. 2004. *Guidelines on Biodiversity and Tourism Development: International Guidelines for Activities Related to Sustainable Tourism Development in Vulnerable Terrestrial, Marine and Coastal Ecosystems and Habitats of Major Importance for Biological Diversity and Protected Areas, Including Fragile Riparian and Mountain Ecosystems.* Secretariat of the Convention on Biological Diversity, Montreal, Canada.

Travis, D.A., Hungerford, L., Engel, G.A. and Jones-Engel, L. 2007. Disease risk analysis: a tool for primate conservation planning and decision making. *American Journal of Primatology* **68**:855–867.

USAID. 2003. *ENCAP Guidelines for Ecotourism*. United States Agency for International Development (USAID), Washington, DC.

### Additional information on surgical masks and respirators

Belkin, N.L. 1997. The evolution of the surgical mask: filtering efficiency versus effectiveness. *Infection Control and Hospital Epidemiology* **18**:49–57.

Greene, V.W. and Vesley, D. 1962. Method for evaluating effectiveness of surgical masks. *Journal of Bacteriology* **83**:663–667.

Lipp, A. 2003. The effectiveness of surgical face masks: what the literature shows. *Nursing Times* **99**:22–24.

Philips, B.J., Fergusson, S., Armstrong, P., Anderson, F.M. and Wildsmith, J.A. 1992. Surgical face masks are effective in reducing bacterial contamination caused by dispersal from the upper airway. *British Journal of Anaesthesia* **69**:407–408.

**A fully equipped tourist guide, Budongo Forest Reserve, Uganda. Photo © Debby Cox.**

# Appendix I – Sample Tourist Regulations

## A. Eastern Gorillas

*Note: The rules listed below are considered by the Mountain Gorilla Veterinary Project (MGVP) to be minimum guidelines for tourists, researchers and park staff visiting mountain gorillas in Rwanda, Uganda and the Democratic Republic of Congo (MGVP 2009). They have been continually updated during years of operation by MGVP and may also be applied to Grauer's gorillas and chimpanzees. To reach the 'gold standard', MGVP recommends additional rules be implemented; these are marked by a footnote below.*

**Gorillas Are Endangered* Please Help Us Keep Them Healthy—Gorilla Visitation RULES for Tourists, Researchers and Staff**

**Before You Set Out**[13]

- Maximum of 8 visitors in each group, plus 2 park staff for tourist visits — 1 guide + 1 tracker [14]
- Minimum age: 15 years old
- To protect the health of the gorillas, wash your hands before setting out [15].
- Please use clean tracking clothes for EACH gorilla visit; please clean your shoes carefully BEFORE and after each visit [16].
- **If you do not feel well,** have diarrhoea or a sore throat, please report it to your guide. It is very important that people with signs of any type of infection never visit gorillas. *Depending on the country, you may be eligible for a rain check/ refund so you may visit when you are well.*
- If you have a chronic illness such as heart disease, emphysema, or arthritis, please reconsider your decision to trek. Health services are limited near the park.
- Please use the restroom before your visit, as there will be no facilities available.

**While You Are in the Park**

- Do not enter the park without a guide.
- Please keep your voice low.
- 'Leave No Trace'. If you brought it in - take it out. Do not litter. Avoid unnecessarily damaging any plants. Do not remove any plants or wildlife from the park.
- If you must relieve yourself, bury solid waste at least one foot (30 cm). If you are with a guide, ask them to dig the hole.
- Leave all backpacks, walking sticks, food and drink, at least **100 metres** from gorillas (the length of a football/soccer field). The porters and extra trackers will stay here.
- No smoking or spitting.

**When You Are With the Gorillas**

- Maintain a 7 metre (23 feet) distance from the gorillas.
- Spend a maximum of 1 hour per visit
- Do not eat or drink during the gorilla visit. Do not feed the gorillas. AGAIN Smoking is not allowed.
- Do NOT use flash photography. Ask your guide for tape to cover flash if needed.
- Speak only in a soft voice.
- All cell phones must be OFF. Radios should be turned down.

---

13 Pre-visit vaccinations have been discussed in other sites, and tourists are very likely to follow protocols if informed in advance. However this would not prevent the diseases of prime concern (influenza, common cold, TB).

14 MGVP 'gold standards' recommend the maximum number of people should be reduced to improve both the quality of the visit for tourists and the ability of the guides to enforce rules. Instead of 8 guests + 2 park staff, MGVP recommends 6+2.

15 Toilets and hand-washing facilities to be provided at morning meeting points. Hands and boots should be disinfected at entrance to park/forest—this can be carried out with hand sprayers containing disinfectant.

16 Trackers and rangers should also change clothes, shower, and clean boots before visiting a second group. During a respiratory disease outbreak, and for one week afterwards, staff should **not** move between groups.

- Do not antagonize the gorillas in any way: Do not point at the gorillas, make sudden gestures or movements or loud noises
- If a gorilla charges you, remain still, avoid eye contact BUT DO NOT turn away.
- Follow the instructions and advice of your guide.
- You MAY be asked to wear a mask BEFORE visiting the gorillas and wash your hands again/use hand sanitizer if there is a local or global disease outbreak. The park officials will institute this rule when advised by vets and other health experts [17].
- If you cough or sneeze, you should wear a mask (For tourists, guides will provide the mask and will collect them at the end of the visit [18]
- **Note: Those who do not respect the guidelines may be asked to leave the gorillas and the park; you will not receive a refund and you may be penalized.**

## B. Western Gorillas: tracking

*Note: This content is adapted from material provided by WCS (WCS Field Veterinary Program 2008) for Mondika, where tourists track habituated western lowland gorillas.*

### Gorilla tracking at Mondika

Tracking gorillas at Mondika can be physically demanding and we request that visitors are in sufficient physical condition to endure hikes of up to 3 hours in dense vegetation, often wading through water and swamps.

**Tourist Health Requirements:**
In order to ensure to the degree possible that tourists and other visitors are not carrying diseases that may be subsequently transmitted to the Mondika gorillas, the following regulations have been instituted:

Prior to arrival in Congo, each visitor will be required to furnish proof of current vaccination against the following:

- Polio (attenuated)
- Measles* (*It is contraindicated that immunocompromised individuals be vaccinated against measles)
- Yellow fever (this is also required for entering many African countries)

In addition, each visitor must provide proof of negative tuberculosis (TB) status:

- Negative TB test (Mantoux skin test or other recognised test) obtained in the last six months prior to arrival.

This information will be verified on arrival at Bomassa Base before granting permission to visit Mondika. Failure to provide the necessary information, or falsifying such information, can result in being refused access to the Mondika site and/or gorilla viewing. Anyone exhibiting signs of potentially transmissible disease, such as influenza, may be refused access to Mondika Camp and gorilla viewing. Anyone with an active herpes outbreak (cold sores) or diarrhoea will also be denied entry to the forest. Staff at Bomassa and Mondika retain the right to deny access to the gorillas to anyone believed to be currently ill with a transmissible disease.

For the health and well-being of the visitors, the following are also strongly recommended:

- Tetanus vaccination
- Hepatitis A vaccination
- Hepatitis B vaccination.

17 MGVP “gold standards” recommend that everyone should be made to wear an N95 mask – staff and tourists. If N95 masks are unobtainable and/or too expensive, a standard surgical mask should be used. This is particularly important in light of the increasing severity and frequency of influenza virus infections among people.

18 For tourist groups, the gorilla guide should be assigned the role of collecting used masks and disposing of them properly. For research groups and routine monitoring, the lead tracker is assigned this task.

**Tourist Visit Health and Safety Regulations**

1. The minimum age of visitors for gorilla viewing is 15 years.
2. The maximum number of visitors viewing the gorillas at any one time is limited to two people. Visitors will be accompanied by one tracker and one guide, so that the viewing group is limited to a total of four people. This is because of the small size of the gorilla group, the fact that the group is often very spread out and dispersed, the terrain and disease concerns.
3. Visits with the gorillas will be limited to one hour. Guides will make every reasonable attempt to insure good viewing of the gorillas, but such may not always be possible. The guides' decision on when to terminate the visit is final.
4. A maximum of two gorilla visits will be facilitated on any given day. Each of these visits will have a maximum of two visitors and viewing will be for a maximum one hour.
5. All visitors must maintain a minimum distance of 7 meters from the gorillas at all times. If during the visit a gorilla approaches to within that 7m distance, your guides will have you retreat to a safe distance.
6. All visitors must wear the provided facemasks (covering nose and mouth) at all times when observing the gorillas. These facemasks will not in any way negatively affect your experience with the gorillas, but can play an important role in minimising transmission of diseases such as the common cold or other respiratory conditions, which are frequently picked up on long-haul flights. These facemasks must be returned to the guide at the end of the visit.
7. Visitors must remain with their guide at all times. Speak and move quietly in the forest. You will see much more. In the event that an animal displays or charges, remain calm and avoid movements that may further excite the animal, avoid eye contact and follow the directions of your guide.
8. Do not attempt to touch, point at or otherwise interact with the gorillas or other wildlife.
9. No defecating in the forest. Please take care of any needs before leaving the base camp.
10. No urinating within 100m of the gorillas, nor in any water source. If at all possible, a small hole should be dug and the urine covered over with dirt.
11. No coughing, sneezing or spitting in proximity to the gorillas. If you do have to sneeze or blow your nose, please turn away and cover your mouth with a tissue.
12. No littering of any kind will be permitted; everything carried into the forest must be carried out.
13. No smoking is permitted in the forest.
14. No eating is permitted within 100m of the gorillas. All food packaging and utensils must be carried out of the forest.
15. No feeding of the gorillas or any other animals.
16. Do not attempt to attract the attention of the gorillas or animals for a photo opportunity and do not use flash photography.
17. Do not leave bags or other belongings unattended in the forest in proximity to the gorillas.

## C. Western Gorillas: bai visits

*Note: This content is adapted from material provided by WCS for tourism at Mbeli Bai in the Republic of Congo (WCS Field Veterinary Program 2008), and is an example of tourism regulations at 'bai' sites, in which visitors observe gorillas, if they are present, along with other species that visit a forest clearing. Viewing at these sites tends to be from platforms on the edge of the clearing, in this case called the 'mirador'. For regulations from additional bai sites, see WCS Gabon (2006) for Langoué Bai, Gabon, and for Bai Hokou in CAR: http://www.dzanga-sangha.org/drupal/node/516*

**Guidelines for visitors to Mbeli Bai, Nouabalé-Ndoki National Park**

These brief guidelines should help you prepare for the tropical rain forest and for visiting Mbeli Bai. The Nouabalé-Ndoki National Park is an intact forest ecosystem with healthy populations of wild animals. These instructions are for your safety and for the health of the animals. They will also ensure that your experience of the Nouabalé-Ndoki National Park is as enjoyable and memorable as possible. Please do not hesitate to contact Nouabalé-Ndoki National Park (NNNP) staff or researchers at the Mbeli Bai Study for any questions regarding health, safety and wildlife. It is important that you always follow the instructions of NNNP staff (both guides and researchers) carefully during your visit.

**Illness**

- No visitor should visit the forest if they have any symptoms of illness. If you become ill during your visit, please notify the Park staff or research team leader immediately. Cases of human viruses and bacteria that can be transmitted from

humans to apes include influenza and the common cold. Therefore, these illnesses could prove harmful to chimpanzees and gorillas.

**Behaviour in the camp**

- Your accommodation is situated 2.7 km from Mbeli Bai and it will take you around 45 minutes at a leisurely pace on a well-trodden path to reach the clearing. You are in the middle of the rainforest and it is not uncommon to encounter wild animals in the camp or on the path. Wild animals are potentially dangerous and should always be treated with the utmost respect.
- Extreme care should be taken if moving between your house and your toilet during the night, and you should not move around the camp at night without a guide.
- In the NNNP we are trying to integrate research and eco-tourism at one site. We do however ask you to respect the camp workers and researchers who live in the camp, and avoid leaving the tourist camp to visit the research camp.
- Please do not drop litter.

**Behaviour in the forest**

- Do not walk in the forest without a guide or a researcher
- Always stay in visual contact with park staff, guides or researchers. Park staff have years of experience with wild animals and will provide instructions in the event that you meet an animal on the path to the bai.
- Follow the instructions of park staff, guides and researchers when encountering an elephants, gorillas or other wild animals.
- Never run or shout while in proximity to wildlife.
- Walk silently and always be vigilant while in the forest.
- Do not approach any large animals, including chimpanzees, gorillas and elephants. Never try to touch or in any way physically contact any of the animals in the forest.
- Act submissively towards all animals in the forest and do not exhibit any behaviour that may threaten or harass the animal.
- If you meet a gorilla in the forest, you must remain where you are, keep quiet and still and don't run away.
- Avoid making any noise or other disturbance while in the presence of wildlife. (If you have to communicate with your guide or your group, use low and hushed voices).
- Do not use flashes or artificial lights when photographing or filming wildlife. Also, please keep any equipment noise to a minimum. Wear appropriate field clothes, preferably in forest colours such as green and brown.
- Do not drop litter. Human refuse (food remains, garbage, personal items, etc.) is often attractive to wildlife and should be transported from the forest to designated latrines and disposed of properly. Ziploc bags should be included in hiking gear to store and transport trash generated while in the forest.
- Smoking is prohibited in the forest.
- Please refrain from coughing, sneezing, or nose blowing in proximity to animals.
- Please use designated latrines at either Mbeli Bai camp or Mbeli mirador, and avoid using the forest as a toilet!

**Behaviour at the bai**

- All the animals visiting Mbeli Bai are wild and habituated only to the presence of researchers on the observation platform (mirador). In order to minimise disturbance and maximise your time with the animals please when on the platform:
- Speak quietly, move slowly.
- Do not smoke, do not cook food.
- Do not walk in the forest behind the mirador.
- Avoid wearing colourful clothes, such as bright red, yellow.
- Always listen to the advice of the researchers.
- Do not walk to the toilet without a tracker.
- Do not lean over the edge of the mirador.
- Be aware of snakes!

## D. Western Gorillas: forest walk/chance observation

*Note: These recommendations were adapted from a Zoological Society of London visitor information leaflet provided to tourists who visit Mikongo in Gabon (ZSL 2009). During guided walks through the forest, visitors could on occasion encounter gorillas.*

### Requirements and recommendations to tour operators

Requirements:

- **Age limit:** no less than 15 years old - this is primarily because children of less than 15 years old can still be vectors of childhood diseases and might not be able to deal in an appropriate manner with a dangerous situation – there is no official upper age limit.
- **Good physical fitness:** guests have to be fit enough to hike for a minimum of 2–3 hours in a dense and humid environment.

Recommendations:

- **Guests should have updated vaccinations for the following diseases:** polio (attenuated), measles, tetanus, hepatitis A, yellow fever (compulsory in Gabon). At this stage, as guests are not in close contact with habituated gorillas, vaccinations are only recommended. There is no way for us to check that guests are actually vaccinated against these diseases before they arrive at MCC and it is difficult to make sure that tour operators actually provide these recommendations to their customers. If tourists are to be taken for habituated gorillas viewing in the future, vaccinations will be compulsory and ways of control implemented.
- **Clothing:** Guests should wear comfortable outdoor clothes of neutral colours (avoid visible colours such as white, bright blue and red, as well as black), preferably long trousers and long-sleeved tops.

Checking for guests' health status

- **Visitor health information form:** at their arrival, guests are given a health form to fill in as part of an indemnity form package (cf. annexe I). The health form should be used as a support to raise guests' awareness about anthropozoonotic diseases and as a means to check for guests' healthiness from their arrival.
- **Direct observations:** ecoguides and management staff have to pay attention to any sign of illness (fever, weakness, dizziness, sneezing/coughing/sniffing, diarrhoea/vomiting, injury) shown by guests. Guests also have to be encouraged to self-report any health problem occurring during their stay. In case a guest shows any signs of illness, the management staff has to strongly recommend guests to stay at camp. The management staff retain the right to deny access to the forest to any guest believed to be ill with a transmissible disease (e.g., cold, diarrhoea) or with any affliction likely to compromise their safety.
- **Awareness:** posters summarising primate health rules have been designed, and posted in all guest rooms.

Applying responsible behaviours

- **Informing guests upon their arrival:** in the indemnity form package to be signed by tourists at their arrival (cf. Annexe I), a sheet summarises the main safety rules and recommendations corresponding to responsible behaviours to follow while in the camp and in the forest. These rules and recommendations are similar to the ones provided to forest workers. One important additional rule is that guests have to respect and follow ecoguides' directives during walks in any case. To empower and increase the sense of responsibilities of ecoguides, ecoguides have to be the ones explaining the rules and recommendations to the guests from their arrival: the ecoguide has to go through them with the guests and check that they are well understood. So particular attention should be given to refresh ecoguide training on these rules and check on how they apply them.
- **Group size:** whatever their size, all groups have to be accompanied by 2 ecoguides, one leading and one at the back. The maximum group size for guided walks is recommended to be no more than 7 persons, including ecoguides, for safety but also to increase wildlife viewing opportunities. Larger groups should then be encouraged to split into smaller ones. This question needs to be addressed in advance with tour operators when discussing bookings so that guests and tour leaders are aware before their arrival.
- **Introductory talk and check-up by guides:** before going for walks in the forest, leading guides have to explain again the rules and recommendations to the guests and check that all are dressed appropriately and look in good shape.
- **Boot cleaning and disinfection:** before and after each walk, guides and guests have to dip their boot soles into the disinfectant solution.
- **During walks:** ecoguides have to avoid interfering with the habituation work by preparing walks with guests in advance and checking with the habituation team that areas involved do not overlap. Regular radio checks between teams during walks have to be made to check on their respective position and adapt tourism circuits accordingly. It is strictly forbidden that ecoguides and guests purposefully join the habituation team in the forest.

## E. Chimpanzees

*Note: Extracted from the Jane Goodall Institute–Uganda Ecotourism Health Protocols (JGI-Uganda 2006), which cover a range of visitor categories. The excerpt below is for 'Day Visitors', i.e. tourists. Regulations vary slightly between JGI and other chimpanzee sites - see also regulations from Gombe (Collins 2003; Gombe Stream Research Centre and Wilson 2006) and from Mahale Mountains National Park (TANAPA and FZS 2007).*

**Age Limits:**
Minimum age is 15 years.

Maximum age is 65 years; this is also dependant on size and fitness level of the person. Management will assess all clients prior to starting the walk. If managers are concerned, you may be refused entry with the chimpanzees.

**Health Clearance:**
All visitors that participate in the chimp walks are required to be free of any flu-like disease at the time of the walk. Anyone with a herpes (cold sores) outbreak will also be denied entry to the forest. If the project supervisor is at all concerned about the visitors' present state of health, participation on the walk will be denied. JGI management staff will have the final say on who can go on the walk; this is not negotiable.

**All visitors must be given the following instructions:**

1. **If you are sick, you are not allowed to enter the forest to follow the chimpanzees.** Human illnesses can infect and kill these animals. Do not approach them if they arrive in camp. Even if you are not visibly sick, you may be carrying a disease that can kill them which is why following these rules is so crucial.
2. **It is crucial that you remain a minimum of 10 metres/33 feet from chimpanzees and baboons at all times.** If an animal starts to approach, move away to a distance of 10 metres. It is your responsibility to keep the safe and proper distance.
3. **The number of people in your group must never exceed six (6), excluding your guide, while following the chimps.** You must be accompanied by a Guide at all times in the forest. If you encounter another group of people observing chimps or baboons, wait patiently at a distance until they move away. Children under the age of 7 are not permitted in the forest.
4. **You are allowed to remain with a group of chimpanzees for one hour, after which you may encounter other parties briefly and visit the many scenic areas of the forest.**
5. **It is very important that you stay together in your group.** Never spread out or surround animals you are observing. When you come upon chimps or baboons in the forest it is best that you sit quietly. You will see more natural behaviour if the chimps are relaxed.
6. **If you must talk in the forest, speak quietly.** Do not use arm gestures while talking. This may be seen as a threat by baboons and chimps. Never stare at a baboon, as it is taken as a threat.
7. **Carry your equipment, backpacks and other items at all times.** Both chimps and baboons will steal anything left unattended. These unfortunate incidents increase the risk of disease transfer and result in damage to your belongings. Be especially careful with bandanas and tissues. And never leave belongings outside unattended in camp.
8. **Do not spit or nose blow on the ground. Suppress sneezes and coughs while in forest**. If you must, cover your face and turn away from the animals being observed.
9. **Do not smoke or eat in the forest.** Always eat indoors behind a latched door. Visitors have been seriously injured by baboons that have tried to steal food.
10. **Never feed the chimpanzees, baboons or other wildlife.**
11. **Use the latrine and wash hands with soap before entering the forest and upon return.** You are responsible for digging a 1 ft deep hole in the forest for burying faeces when a latrine is not available.
12. **Never attempt flash photography or use reflective devices.** Wild animals are unpredictable when startled. Visitors have been seriously threatened by chimpanzees after ignoring this rule. Never try to attract an animal's attention in order to take a better photograph.
13. **Littering of any kind is forbidden.** Never throw food, candy wrappers, cigarette butts, or any other man-made product onto the ground. Transporting the rubbish you bring back out of the forest and reserve/park would be greatly appreciated.

## F. Orangutans: wild

### GUIDELINES FOR TOURISTS VISITING THE RED APE ENCOUNTERS, MALAYSIA WILD HABITUATED ORANGUTANS

The most important thing for a visitor to remember is to always follow the tour leader's recommendations for the safety of both the orangutan and the people.

**RULE 1: Number of people limited to 5 tourists per group (RAE staff not included).**

- *Reasons: control the risk of human impacts*
  *optimise the encounter and viewing opportunities for tourists*

**RULE 2: Duration of an orangutan viewing time limited to one hour maximum**

- *Reasons: reduce orangutan exposure to potential germ-carrying people*
  *minimise behavioural disturbance and associated stress in the animals*

If orangutans are not visible when the visitors arrive at the site, they can wait in stand-by with their guide at a minimum of 100 metres from the tree where the animal stays.

**RULE 3: Frequency of visits limited to 1 visit per day and per habituated orangutan**

- *Reasons: minimise stress of the animals*
  *minimise the negative impacts of heavy human presence on RAE natural environment (trampling, disturbance to the ecosystem, etc.).*

**RULE 4: Ill people cannot visit the orangutan**

Tourists are asked to self-report any sickness to the RAE staff and their visit will be refunded or rescheduled. RAE staff can refuse a visit to any visitor showing obvious signs of disease.

- *Reasons: minimise risks of disease transmission*

**RULE 5: Not closer than 10 metres from an orangutan**

- *Reasons: minimise risks of disease transmission*

**RULE 6: Adopt an appropriate behaviour during the close contact with the orangutan**

- *Reasons: minimise the stress and disturbance to the animals*
- *Proper behaviours*:
  - ✓ Refrain from smoking, eating, sneezing and coughing in the presence of orangutans
  - ✓ visitors should remain in a tight group, without losing contact with the RAE staff
  - ✓ where possible, visitors should sit whilst watching the apes
  - ✓ body language is important and visitors should stay as quiet as possible during their entire visit (no screaming, no brisk movements, no running, etc…). Show respect to the animals and try to remain as silent as possible with them.
  - ✓ do not clear vegetation to get a better view of the orangutans
  - ✓ do not stare at the orangutans and do not use binoculars, photographic lenses and/or video cameras if the animals are disturbed (kiss-squeak vocalisations).
  - ✓ do not try to approach an orangutan (especially a newcomer) unless a guide is with you.

**RULE 7: Adopt an appropriate behaviour during all times in the forest**

- *Reasons: minimise disturbance to the ecosystem*
- *Proper behaviours:*
  - ✓ all faecal material and papers must be buried (a parang can be borrowed anytime from the RAE staff).
  - ✓ littering is strictly prohibited at RAE site and all types of rubbish must be carried outside of the forest.
  - ✓ do not collect any living organisms from the forest (flowers, insects, seeds, etc.).

## G. Orangutans: ex-captives and wild

### Sumatran Orangutan Health Protocols and Guidelines for Visitors to the Bukit Lawang Eco-tourism Site (SOS 2008)

As you trek through the forest at Bukit Lawang, it is important to remember that you are entering the habitat of one of the rarest great ape species on Earth.

The population of Sumatran orangutans at Bukit Lawang is from two different origins:

1. Ex-captive individuals who have been rehabilitated and released in the forest. Captive and rehabilitation experiences often result in released rehabilitant orangutans not fearing humans and even expecting to interact with them.
2. Wild individuals, some of whom have become habituated to human presence, with the remaining being naïve (i.e. not used to people's presence in their forest habitat).

Inappropriate behaviour by visitors may affect the behaviour and health of orangutans from both populations negatively, which places them at increased risk of becoming stressed and falling ill. By following these simple guidelines, visitors are able to see the Sumatran orangutans at Bukit Lawang in a way that is both safe for themselves and safe for the orangutans, whilst at the same time, experiencing a more natural, unique experience in the forest.

**Group Responsibilities**

- A maximum group size of seven visitors is to be adhered to whilst in the forest. Research from other eco-tourist sites that allow great ape trekking has shown that visitor group size can affect the behaviour of the great apes encountered and (as a result), the visitors' experience. Where groups of visitors are too high in number, the animals become stressed and nervous and move away from visitor groups.
- Every member of a visitor group should maintain a minimum distance of TEN METRES from the closest orangutan. The potential for disease transfer, both humans to orangutan and orangutan to human, is very high due to the close genetic relationship humans share with great apes. Pneumonia, influenza, tuberculosis, hepatitis A, B, C and E, cholera, herpes, parasites and even the common cold can all be passed between great apes and humans.
  - ☐ This distance serves to protect visitors from the possibility of attack by orangutans. This is a real factor in ex-captive orangutans, since most are not afraid of humans after having lived as human captives and being rehabilitated by humans; it is not a serious concern with wild orangutans.
  - ☐ If an orangutan moves towards a visitor group or any member of the group, it is primarily the responsibility of the guide to move the whole visitor group back (maintaining the minimum distance at all times). Every member of a visitor group should nonetheless move away from any orangutan that approaches and alert others of the approach.
- Once in the presence of orangutans (less than 50 metres away, the distance at which orangutans are considered to be associating with one another), visitors may stay NO LONGER THAN ONE HOUR. The visit will be formally timed from the point of entering the orangutans' presence. When this period is over, the group is to leave the area that the orangutan is in.
  - ☐ Timing is the guide's responsibility and the viewing period CANNOT be extended.
- Remember that visitors are guests in the Gunung Leuser National Park, which is the orangutans' home and that what is best for the orangutans is to freely roam and forage naturally in the forest without excessive disturbance.

**Orangutan Viewing**

Sumatran orangutans share over 96.5% of their genetic DNA with humans and as a result they are like us in many ways. It is important to remember that orangutans are highly intelligent, thinking, feeling beings and should be treated with due care and respect. Visitors to the Bukit Lawang site are to observe the following 'orangutan etiquette' guidelines:

- Visitors should not touch the orangutans under any circumstances. Touching is very dangerous, for various reasons: diseases, infections and even parasites can easily pass between orangutans and humans and physical contact makes the likelihood of this higher. Touching also gives the orangutans the chance to grab; some of them do, with all four hands and feet, typically to steal food or other goods. A mature orangutan is approximately four times stronger than a human and can inflict serious or fatal injuries if they feel threatened, irritated or upset.
  - ☐ Binoculars may be useful because they allow close up views of orangutans from safe distances. Please do not use binoculars unless orangutans are relaxed and stop using them if orangutans show signs of becoming uneasy. Binocular lenses pointed at an orangutan can look like 'big eyes' and orangutans sometimes seem to find this uncomfortable.

- ☐ Camera usage must also follow the same guidelines for binoculars. Camera lenses may often be larger than those of binoculars and thus may irritate the orangutans. Also limit the use of flash photography as this may also affect the orangutans.

- Visitors must not feed the orangutans under any circumstances.
- Visitors should not under any circumstances move to or stay in a location that puts them between two orangutans, especially a mother and her infant or a male and his female consort. Orangutan mothers are extremely protective of their young and can become aggressive if they feel that their infant is being threatened. Male orangutans can become aggressive if anyone approaches their consort and may threaten, chase or even attack.
- Visitors or guides should not call out to the orangutans or otherwise lure them to change their behaviour. Calling or luring the orangutans can cause stress and it automatically disrupts natural behaviour.
- Visitors should refrain from making any sudden movements and should not attempt to gain the attention of the orangutans by waving their arms, etc., for the same reasons given above. In addition to disrupting their behaviour, this can annoy orangutans and evoke threats or more serious aggression.
- Visitors should refrain from making too much noise within the forest and try to talk quietly. Loud noise can be interpreted as a threat by the orangutans and they can respond either by fleeing or threatening back.
  - ☐ If an orangutan begins to make kiss-squeak vocalisations, throaty grunts or growls, or 'raspberry' sounds, breaking and throwing branches, or shakes trees, these are signs of irritated disturbance and aggressive threats. It is best to move on and leave the orangutan alone.

**Visitor Responsibilities**

- Visitors must not enter the forest if they are feeling unwell or recently had an illness and/or diarrhoea. It is each visitor's moral responsibility to report any sign of disease to their guide before entering the forest. Spending time around the orangutans whilst unwell can seriously risk infecting them, which could easily result in their death—and has, in the past. Any orangutan infected by humans could potentially infect other orangutans as well.
  - ☐ If the guide feels that a visitor is not well enough to enter the forest, it is within his/her authority to refuse entry to the visitor.

**A not uncommon scene at tourism sites involving ex-captive orangutans, illustrating the potential for both aggressive encounters and disease transmission. Photo © Steve Unwin.**

- No food should be brought into the forest by visitors. If necessary (for longer treks or in special cases), all food should be carried by the guide for safe-keeping.
  - ☐ Eating or even having food visible whilst in the forest increases the risk of both disease transmission and attacks from orangutans. One of the main reasons that orangutans contact and attack humans is to steal food, and seeing food is therefore a major provocation. If no food is brought in, the orangutans will learn that there is nothing to attack for, which will make a safer experience for ALL of the orangutans and ALL future visitors and guides.
- Visitors should take any litter they have out of the forest when they leave.
  - ☐ This includes fruit skins as discarded foods may later attract orangutans and allow for disease transfer
  - ☐ It is most preferable to bring as little as possible into the forest, only the essentials should be taken in. This will limit chances of loss/damage.
  - ☐ Refrain from smoking in the forest. Smoking is NOT permitted when in the presence of orangutans.
- If the visitor needs to defecate within the forest, he/she must ensure that it is away from the orangutans and that a hole is dug (at least 30cm deep) and subsequently filled in. Where possible, visitors should try and wait until they are out of the forest.

**Forest Responsibilities**

Like any tropical forest, Bukit Lawang and its surrounding areas represent a complicated and diverse (but above all, fragile) habitat. The whole forest system is a delicately balanced network of animal and plant species and many species are heavily dependent upon one-another. We therefore ask visitors to follow this simple guideline:

- Visitors should not remove, damage, or alter any of the vegetation within the forest. Leaves, seeds and shells all play a role within the forest ecosystem and should not be taken out.

**It is the responsibility of every person entering the forest to help ensure the survival of this critically endangered species and its habitat. Visitors should discourage other members in their party, including their guides, from acting in a way that contradicts these guidelines and should express their disapproval and report to the national park office any activity which puts either the visitors or the orangutans at risk.**

**With your help and cooperation, the orangutan can continue to flourish in Bukit Lawang and visitors for years to come will also be able to enjoy and appreciate them in their natural forest home.**

## Appendix II – Information on Face Masks/N95 Respirator Masks

**Facemasks/Surgical Masks vs. N95 respirator masks:** This document has recommended as best practice that all visitors, including staff, tourists and researchers, who approach to a distance of 10 metres or less from wild great apes wear surgical N95 respirators. As there are a large variety of masks on the market, variously called 'face masks', 'surgical masks' or 'respirators', the following information describes the differences in mask types and provides additional information. All of this information is adapted from material produced by human health networks (CDC 2004; CDC 2006; Dreller *et al.* 2006; FDA 2009) and/or adapted from recommendations from great ape veterinary experts (MGVP 2008; MGVP 2009).

**Facemasks:** A facemask is a loose-fitting, disposable device that creates a physical barrier between the mouth and nose of the wearer and potential contaminants in the immediate environment. Facemasks may be labelled as surgical, laser, isolation, dental or medical procedure masks. Facemasks are made in different thicknesses and with different abilities to protect the wearer from contact with liquids. These properties may also affect how easily the wearer can breathe through the facemask and how well the facemask protects the wearer. If worn properly, a facemask is meant to help block large-particle droplets (greater than 50-100µm diameter), splashes, sprays or splatter that may contain infectious agents from reaching the wearer's mouth and nose. Facemasks may also help reduce exposure of others to respiratory secretions of the wearer. While a facemask may be effective in blocking splashes and large-particle droplets, a facemask, by design, does not filter or block very small particles in the air that may be transmitted by coughs or sneezes. Facemasks also do not provide complete protection because of the loose fit between the surface of the facemask and the wearer's face.

**N95 Respirators:** Although appearing similar to face masks to the layperson, an N95 respirator is a respiratory protective device designed to achieve a close facial fit and efficient filtration of airborne particles including very small airborne particles. The 'N95' designation means that in laboratory tests, the respirator blocks at least 95% of very small (less than 10 µm) particles, which include

small particle aerosols generated directly from a cough or sneeze. Mask ratings above N95, i.e. N99 or N100, are also acceptable as they block a higher percentage of particles. An N95 respirator requires a proper fit, tight but comfortable, to the wearer's face to be effective. A proper fit check is relatively simple: when inhaling, the respirator should collapse, and when exhaling there should be no leakage around the face. If properly fitted, the filtration capabilities of N95 respirators exceed those of face masks. However, even a properly fitted N95 respirator does not completely eliminate the risk of disease transmission. N95 respirators are not designed for children or people with facial hair, because a proper fit cannot be achieved. As N95 respirators achieve a tighter facial fit, they may require more effort to breathe and this should be explained to the wearer before use. Some people with chronic respiratory, cardiac, or other medical conditions find it harder to wear N95 masks, but great ape tourism activities, especially those that require strenuous hiking, will probably not attract this sort of tourist. Some N95 models have exhalation valves that can make breathing out easier and help reduce heat build-up, although these will be more expensive. A type of N95 respirator called the Duck-Bill N95 respirator allows more room and has been tested by the MGVP (MGVP 2008) for comfort and reduced fogging of binoculars and glasses.

**'Surgical' N95 Respirators:** There are N95 respirators sold for use in construction or other dusty situations to protect the wearer from inhaling noxious particles. Surgical quality N95 respirators are approved for use in medical situations and meet additional performance standards for surgical face masks, and therefore it is the 'Surgical N95 Respirator' that is recommended as best practice for great ape tourism.

**Mask Information Sources:** More information on the types of masks and respirators described above can be found on a number of public health information websites. An excellent resource, including pictures of the different types, can be found at the website below, which also describes in great detail the host, pathogen and environmental factors that affect a particle's infectivity: *http://pandemicflu.gov/plan/healthcare/maskguidancehc.html*

**Disposal of Used Masks and Respirators:** Masks and respirators may only be used once. Used masks or respirators must be placed in a plastic bag and carried out of great ape habitat or back to a base camp and disposed of hygienically – as they are paper based, they can be burned. Staff members should wash hands or used a hand sanitizer after handling used masks.

**Mask Procurement:** As this document is intended to be a global resource, it is difficult to provide a list of mask suppliers. Veterinary support networks and relevant public health ministries should be able to provide guidance on mask procurement options in each geographic region.

**Ranger wearing a duck-billed N95 surgical mask, Virunga National Park, DRC. Photo © Christina Ellis**

# Occasional Papers of the IUCN Species Survival Commission

1. *Species Conservation Priorities in the Tropical Forests of Southeast Asia.* Edited by R.A. Mittermeier and W.R. Konstant, 1985, 58pp.
2. *Priorités en matière de conservation des espèces à Madagascar.* Edited by R.A. Mittermeier, L.H. Rakotovao, V. Randrianasolo, E.J. Sterling and D. Devitre, 1987, 167pp.
3. *Biology and Conservation of River Dolphins.* Edited by W.F. Perrin, R.K. Brownell, Zhou Kaiya and Liu Jiankang, 1989, 173pp.
4. *Rodents. A World Survey of Species of Conservation Concern.* Edited by W.Z. Lidicker, Jr., 1989, 60pp.
5. *The Conservation Biology of Tortoises.* Edited by I.R. Swingland and M.W. Klemens, 1989, 202pp.
6. *Biodiversity in Sub-Saharan Africa and its Islands: Conservation, Management, and Sustainable Use.* Compiled by S.N. Stuart and R.J. Adams, with a contribution from M.D. Jenkins, 1991, 242pp.
7. *Polar Bears: Proceedings of the Tenth Working Meeting of the IUCN/SSC Polar Bear Specialist Group,* 1991, 107pp.
8. *Conservation Biology of Lycaenidae (Butterflies).* Edited by T.R. New, 1993, 173pp.
9. *The Conservation Biology of Molluscs: Proceedings of a Symposium held at the 9th International Malacological Congress, Edinburgh, Scotland, 1986.* Edited by A. Kay. Including a Status Report on Molluscan Diversity, by A. Kay, 1995, 81pp.
10. *Polar Bears: Proceedings of the Eleventh Working Meeting of the IUCN/SSC Polar Bear Specialist Group, January 25–28 1993, Copenhagen, Denmark*. Compiled by Ø. Wiig, E.W. Born and G.W. Garner, 1995, 192pp.
11. *African Elephant Database 1995.* M.Y. Said, R.N. Chunge, G.C. Craig, C.R. Thouless, R.F.W. Barnes and H.T. Dublin, 1995, 225pp.
12. *Assessing the Sustainability of Uses of Wild Species: Case Studies and Initial Assessment Procedure.* Edited by R. and C. Prescott-Allen, 1996, 135pp.
13. *Tecnicas para el Manejo del Guanaco [Techniques for the Management of the Guanaco].* Edited by S. Puig, South American Camelid Specialist Group, 1995, 231pp.
14. *Tourist Hunting in Tanzania.* Edited by N. Leader-Williams, J.A. Kayera and G.L. Overton, 1996, 138pp.
15. *Community-based Conservation in Tanzania.* Edited by N. Leader-Williams, J.A. Kayera and G.L. Overton, 1996, 226pp.
16. *The Live Bird Trade in Tanzania.* Edited by N. Leader-Williams and R.K. Tibanyenda, 1996, 129pp.
17. *Sturgeon Stocks and Caviar Trade Workshop: Proceedings of a Workshop, 9–10 October 1995 Bonn, Germany.* Federal Ministry for the Environment, Nature Conservation and Nuclear Safety and the Federal Agency for Nature Conservation. Edited by V.J. Birstein, A. Bauer and A. Kaiser-Pohlmann, 1997, 88pp.
18. *Manejo y Uso Sustentable de Pecaries en la Amazonia Peruana.* R. Bodmer, R. Aquino, P. Puertas, C. Reyes, T. Fang and N. Gottdenker, 1997, 102pp.
19. *Proceedings of the Twelfth Working Meeting of the IUCN/SSC Polar Bear Specialist Group, 3–7 February 1997, Oslo, Norway.* Compiled by A.E. Derocher, G.W. Garner, N.J. Lunn and Ø. Wiig, 1998, 159pp.
20. *Sharks and their Relatives – Ecology and Conservation.* Compiled by M. Camhi, S. Fowler, J. Musick, A. Bräutigam and S. Fordham, 1998, 39pp. (Also in French)
21. *African Antelope Database 1998.* Compiled by R. East and the IUCN/SSC Antelope Specialist Group, 1999, 434pp.
22. *African Elephant Database 1998.* R.F.W. Barnes, G.C. Craig, H.T. Dublin, G. Overton, W. Simons and C.R. Thouless, 1999, 249pp.
23. *Biology and Conservation of Freshwater Cetaceans in Asia.* Edited by R.R. Reeves, B.D. Smith and T. Kasuya, 2000, 152pp.
24. *Links between Biodiversity Conservation, Livelihoods and Food Security: The Sustainable Use of Wild Species for Meat.* Edited by S.A. Mainka and M. Trivedi, 2002, 137pp. (Also in French)
25. *Elasmobranch Biodiversity, Conservation and Management. Proceedings of the International Seminar and Workshop, Sabah, Malaysia, July 1997.* Edited by S.L. Fowler, T.M. Reed and F.A. Dipper, 2002, 258pp.
26. *Polar Bears: Proceedings of the Thirteenth Working Meeting of the IUCN/SSC Polar Bear Specialist Group, 23–28 June 2001, Nuuk, Greenland.* Compiled by N.J. Lunn, S. Schliebe and E.W. Born, 2002, 153pp.
27. *Guidance for CITES Scientific Authorities: Checklist to Assist in Making Non-detriment Findings for Appendix II Exports.* Compiled by A.R. Rosser and M.J. Haywood, 2002, 146pp.
28. *Turning the Tide: The Eradication of Invasive Species. Proceedings of the International Conference on Eradication of Island Invasives.* Edited by C.R. Veitch and M.N. Clout, 2002, 414pp.
29. *African Elephant Status Report 2002: An Update from the African Elephant Database.* J.J. Blanc, C.R. Thouless, J.A. Hart, H.T. Dublin, I. Douglas-Hamilton, C.G. Craig and R.F.W. Barnes, 2003, 302pp.
30. *Conservation and Development Interventions at the Wildlife/Livestock Interface: Implications for Wildlife, Livestock and Human Health.* Compiled by S.A. Osofsky and S. Cleaveland, W.B. Karesh, M.D. Kock, P.J. Nyhus, L. Starr and A. Yang, 2005, 220pp.
31. *The Status and Distribution of Freshwater Biodiversity in Eastern Africa.* Compiled by W. Darwall, K. Smith, T. Lower and J.-C. Vié, 2005, 36pp.
32. *Polar Bears: Proceedings of the 14th Working Meeting of the IUCN/SSC Polar Bear Specialist Group, 20–24 June 2005, Seattle, Washington, USA.* Compiled by J. Aars, N.J. Lunn and A.E. Derocher, 2006, 189pp.
33. *African Elephant Status Report 2007: An Update from the African Elephant Database.* Compiled by J.J. Blanc, R.F.W. Barnes, C.G. Craig, H.T. Dublin, C.R. Thouless, I. Douglas-Hamilton and J.A. Hart, 2007, 275pp.
34. *Best Practice Guidelines for Reducing the Impact of Commercial Logging on Great Apes in Western Equatorial Africa*. D. Morgan and C. Sanz, 2007, 32pp. (Also in French)
35. *Best Practice Guidelines for the Re-introduction of Great Apes.* B. Beck K. Walkup, M. Rodrigues, S. Unwin, D. Travis, and T. Stoinski, 2007, 48pp. (Also in French and Bahasa Indonesia)
36. *Best Practice Guidelines for Surveys and Monitoring of Great Ape Populations.* H. Kühl, F. Maisels, M. Ancrenaz and E.A. Williamson, 2008, 32 pp. (Also in French)
37. *Best Practice Guidelines for the Prevention and Mitigation of Conflict Between Humans and Great Apes.* K. Hockings and T. Humle, 2009, 41pp. (Also in French and Bahasa Indonesia)

Many of these publications are available online at: www.iucn.org/themes/ssc/publications/thematic_pubs.htm

INTERNATIONAL UNION
FOR CONSERVATION OF NATURE

WORLD HEADQUARTERS
Rue Mauverney 28
1196 Gland, Switzerland
mail@iucn.org
Tel +41 22 999 0000
Fax +41 22 999 0002
**www.iucn.org**


Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

74

# Infections Acquired from Animals Other Than Pets

DANIEL S. SHAPIRO

## KEY CONCEPTS

- Half of the estimated 1500 human infectious diseases are zoonotic in origin.
- As many zoonotic agents are uncommon in humans and, for a number, have been established as causes of laboratory-acquired infections, good communication with the clinical microbiology laboratory is essential.
- Although the number of infectious agents potentially transmissible from a specific animal to humans may be great, many of these infections are limited geographically and need not be considered unless a bioterrorist event or the introduction of an infection to a new area is a possibility.
- Bats are reservoirs for such emerging diseases as those caused by Nipah virus, Australian bat lyssavirus, the SARS coronavirus, and Ebola virus. While there are more rodent species than there are bat species, bats host more viral zoonoses per species than do rodents.
- The majority of potential agents of bioterrorism are zoonotic.

Zoonotic infections are defined as infections that are transmitted from nonhuman vertebrates to humans. These are acquired from farm animals, pets, beasts of burden, fish, and wild animals via a number of routes (Figure 74-1).

The approach to the patient with a potential zoonotic infection involves the generation of a differential diagnosis that includes those infectious agents that are potentially transmissible from the specific animal(s) to which the patient was exposed. Historical points to consider are summarized in Table 74-1.

Although the number of infectious agents potentially transmissible from a specific animal to humans may be great, many of these infections are limited geographically and need not be considered unless a bioterrorist event or the introduction of an infection to a new area is a possibility. Examples include the lack of plague transmission outside endemic areas, countries that are free of brucellosis, and the limitation of tularemia to the northern hemisphere.

In some cases a good animal exposure history will be elicited but a review of the medical literature will not be able to identify any relevant diseases from that specific animal.

The lack of an effective veterinary or human public health infrastructure in a given country may result in a lack of knowledge of those zoonotic infections transmitted from even commonly encountered animals. For example, camels have been noted to have serologic evidence of infection with *Coxiella burnetii*, but human cases of Q fever as a result of contact with camels or ingestion of camel milk have often been poorly documented.

When there are few data about a particular animal and its role as a reservoir of zoonotic agents, it is worth considering biologically similar animals from which zoonoses have been acquired; for example, *Escherichia coli* O157:H7 infections have been most commonly transmitted to humans via the ingestion of undercooked ground beef. Deer, like cattle, are large grazing herbivores. Humans have been infected after eating venison.

Other important clinical clues to consider include:

- The environment of the animal. For example, shark bite wounds may be infected with *Vibrio* spp., which are commonly found in salt water and as part of the normal oral flora of sharks, whereas freshwater alligator bites are most commonly infected with *Aeromonas hydrophila*, an organism that is found in fresh water and as part of the normal alligator oral flora.
- Consider the diet of the animal. Cattle that have been fed material that includes nervous tissue are at increased risk of having bovine spongiform encephalopathy (BSE).
- Consider other species with which the animal has had contact, including contact with humans while in captivity. Tuberculosis, measles and shigellosis are not normally infectious agents of nonhuman primates. Rather, they are acquired from human contact. Similarly, the housing of camels indoors with cattle increases the risk that the camels will acquire bovine tuberculosis.
- An occupational history, obtained in some detail, can provide important information on those zoonotic agents to which an individual may have been exposed.[1]

As many zoonotic agents are uncommon in humans and, for a number, have been established as causes of laboratory-acquired infections, good communication with the clinical microbiology laboratory is essential. In some cases the diagnosis is established serologically, whereas in others a particular pathogen, perhaps one that requires special culture media or handling, may be isolated. In addition to increasing the probability of correctly identifying the etiology of the patient's illness, good communication is essential for safety, especially when infections due to *Francisella tularensis*, *Brucella* spp., Macacine herpesvirus-1 (cercopithecine herpesvirus type 1; herpesvirus simiae; B virus) and other highly biohazardous agents are under consideration.[2] In those cases in which the pathogen is a potential agent of bioterrorism or is uncommon in humans, even a well-equipped clinical microbiology laboratory may be unable to perform the necessary testing on-site.

The following discussion is organized by type of animal, as this is helpful for the clinician who is attempting to generate a reasonable differential diagnosis.

## Domesticated Herbivores (Cattle, Sheep, Goats, Pigs, Camels, Horses and Related Animals)

### BACTERIAL INFECTIONS

See also Chapter 72 for a further discussion of occupational risks associated with these infections.

*Brucella melitensis* is most commonly acquired from goats and has been acquired from sheep and dromedary camels. *Brucella abortus* is associated with cattle. Although horses can occasionally become infected, transmission to humans from horses, if it occurs, is very rare. *Brucella suis* has been transmitted to humans from both domesticated and feral pigs. The specificity of the association between the species of *Brucella* and the animal host is not absolute.

Anthrax is most commonly acquired from large domesticated herbivores. Cutaneous anthrax, inhalation anthrax (woolsorter's disease)

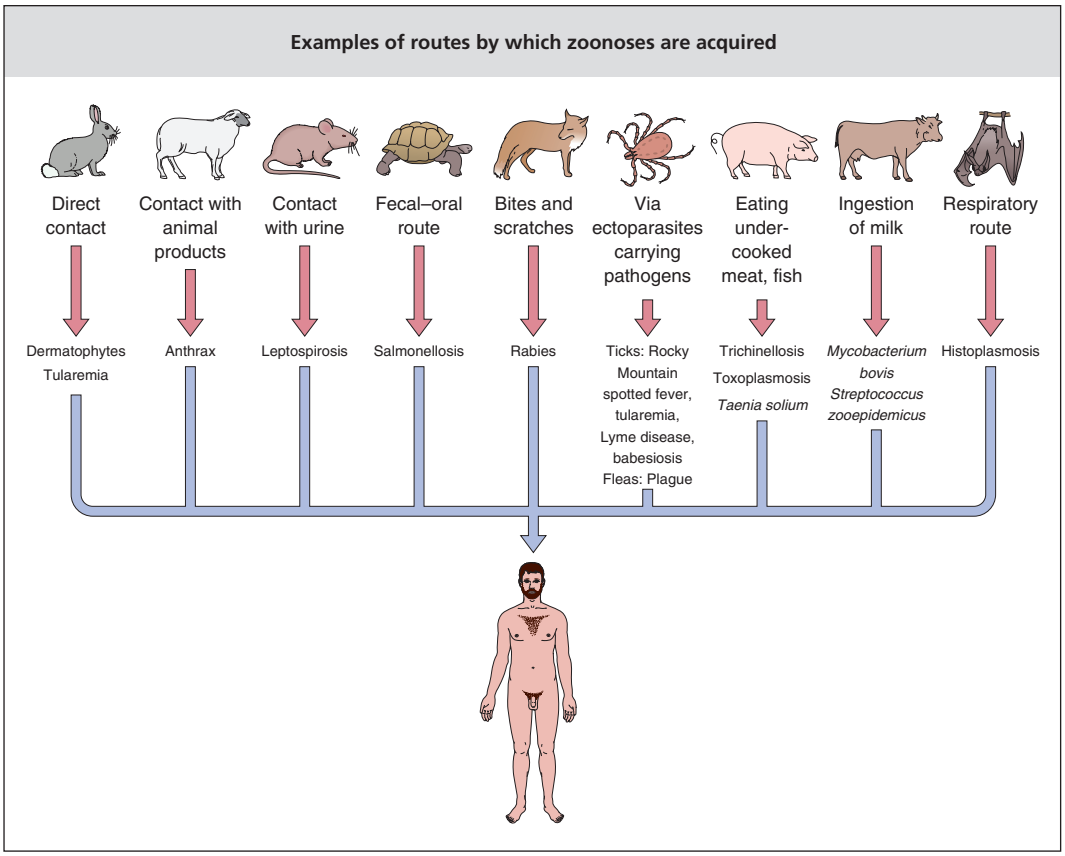



**Figure 74-1** Examples of routes by which zoonoses are acquired.

**TABLE 74-1 Selected Historical Points in Patient Exposure History**

| Historical Finding | Worth Adding to Differential Diagnosis |
|---|---|
| Contact with any vertebrate, especially reptiles | Salmonellosis |
| Exposure to urine, either directly or via contaminated water | Leptospirosis, as essentially all mammals can become infected with *Leptospira interrogans* and shed infectious organisms in the urine |
| Bites from wild mammals, with the exception of those from rodents other than groundhogs (*Marmota monax*) | Evaluate risk of rabies and the potential need for rabies prophylaxis |
| Itching and a history of cutaneous contact with a mammal | Allergic reaction, dermatophyte infection or infestation with ectoparasites, such as species-specific varieties of *Sarcoptes scabiei* |
| Consumption of undercooked wild mammals | Trichinellosis and toxoplasmosis |
| Consumption of fermented fish or marine mammals | Botulism, most commonly due to the type E toxin |
| Consumption of uncooked fish | Any of more than 50 parasitic infections, depending upon the species of fish eaten and the geographic locale |

and gastrointestinal anthrax are associated with the domestication of sheep, goats, and cattle. In parts of the world in which water buffalo are domesticated they have served as the source of outbreaks of human anthrax, as have oxen. Animal products can transmit this disease.

Epizootics of tularemia, associated with heavy infestation by the wood tick, *Dermacentor andersoni*, occur in sheep. Human cases have included infections in sheep shearers, owners, and herders. In a review published in 1955, 189 human cases of tularemia were reported in association with the sheep industry.[3]

Tuberculosis due to *Mycobacterium bovis* subsp. *bovis* was the impetus for pasteurization of cow's milk. Infection with *M. bovis* subsp. *bovis* is also associated with occupational exposure, as in slaughterhouse workers.

Infection with *Listeria monocytogenes* occurs via ingestion of contaminated food, usually meat and dairy products, and rarely by direct cutaneous exposure during parturition. Cutaneous listeriosis has been reported among veterinarians and other individuals delivering animals.[4] Infections transmitted by ingestion of milk products are listed in Table 74-2.

*Yersinia enterocolitica*, normally found in the fecal flora of pigs, has been transmitted from pigs to humans via contact and by ingestion of chitterlings (pig intestines).[5]

*Erysipelothrix rhusiopathiae* has been acquired from many different animals and animal products. It typically is an occupational illness, often acquired via a hand wound while handling animal material. Alerting the clinical microbiology laboratory to its possibility is of great help, as the organism's identification is not difficult if it is suspected.[6]

*Streptococcus suis*, especially type 2, a pathogen of pigs, is a common cause of bacteremia and bacterial meningitis among individuals working with pigs in Asia.

*Rhodococcus equi* is commonly found in the feces of horses and in the soil. Exposure to farm animals, including horses, has been reported in some cases of human infection.

The association of leptospirosis with swine is well known. It has been called swineherd's disease. Cattle, goats, camels, dogs, and rats are all sources of human infection.

**TABLE 74-2 Agents Transmitted Via Milk Products and Cheese**

| Disease | Source |
|---|---|
| *Clostridium botulinum* toxin | Yogurt, cheese |
| *Brucella* spp. | Many animals' milk and cheese |
| *Campylobacter fetus* | Cow's milk |
| *Campylobacter jejuni* | Cow's milk, cheese from goats |
| *Campylobacter laridis* | Cow's milk, contaminated by birds |
| Central European tick-borne encephalitis | Goat's milk, cheese from goats and sheep |
| *Corynebacterium diphtheriae* | Cow's milk |
| *Corynebacterium ulcerans* | Cow's milk |
| *Escherichia coli* O157:H7 and other strains | Cow's and goat's milk, cream, cheese |
| *Listeria monocytogenes* | Cow's milk, cheese |
| *Mycobacterium bovis* subsp. *bovis* | Cow's milk |
| *Salmonella* spp. | Many animals' milk, cheese, ice cream |
| *Staphylococcus aureus* | Cow's milk |
| *Streptobacillus moniliformis* | Cow's milk (single outbreak in 1926) |
| *Streptococcus zooepidemicus* | Cow's milk, cheese |
| *Toxoplasma gondii* | Goat's milk |
| *Yersinia enterocolitica* | Cow's milk |

Exposure of pregnant women to the birth products of sheep and goats that are infected with *Chlamydophila abortus* (*Chlamydia psittaci*, serotype 1) has been reported in both Europe and the USA, and can be severe, resulting in abortion.[7]

Salmonellosis has been transmitted to humans by each of these animals. Pigs have been documented as a source of human cases of multidrug-resistant *Salmonella enterica* serotype *typhimurium* definitive phage type 104 (DT104) infection.[8]

*Escherichia coli* O157:H7 is often present in the gastrointestinal tract of cattle and is most commonly acquired via ingestion of undercooked ground beef. Transmission due to fecal contamination of food products can occur, such as from unpasteurized apple cider prepared from apples that were on the ground in a cattle pasture and used for cider production. Deer, like cattle, are large grazing herbivores and have been reported to transmit this infection to humans who have consumed venison. Outbreaks have been associated with visits to petting zoos. Shiga toxin-producing *E. coli* other than *E. coli* O157:H7 cause approximately half of human Shiga toxin-producing *E. coli* infections.

*Pasteurella aerogenes* is the most commonly isolated organism from human infections following the bites of swine.[9] A number of other gram-negative organisms have also been isolated from these infections. Camel bite injuries typically become infected and are particularly likely from male camels during the rutting season. Members of the genus *Actinobacillus* have been recovered from bites of horses and cattle. *Pasteurella caballi* has been isolated from wounds following horse bites. Rabies has been reported in all of these animals as well as in llamas.

Human cases of Q fever are acquired from birth products of sheep, goats, and cattle, as well as from cats. Airborne infection can occur over significant distances. The data on human acquisition via contaminated milk are less compelling.

Glanders, due to *Burkholderia mallei*, has been transmitted to humans via equids. The disease is limited geographically so its isolation from a patient in North America or Europe must be assumed to be due to bioterrorism until proven otherwise.

## VIRAL INFECTIONS

Localized cutaneous involvement can be due to infection with parapoxviruses that include orf virus (which causes contagious ecthyma and is transmitted by sheep and goats either directly or via fomites), bovine papular stomatitis virus and pseudocowpox virus; and by the orthopoxviruses cowpox virus (which is more commonly transmitted to humans via cats than cattle) and buffalopox virus. The host range of influenza A virus includes many mammals, including marine mammals, swine and horses.

Variant Creutzfeldt–Jakob disease (variant CJD) has been reported from the UK, France, Japan and other countries. It is associated with the consumption of meat from cattle that were infected with BSE. Although cases of BSE have been identified in the USA, no cases of variant CJD have been identified from consumption of US cattle. Prion diseases of large herbivores in the USA, including chronic wasting disease of cervids, have raised the possibility of the introduction of additional prion diseases into the human food supply. A detailed discussion of the molecular aspects of prion-associated disease and the clinical manifestations of the spongiform encephalopathies is found in Chapter 23.

Many cases of the Middle East Respiratory Syndrome (MERS) have occurred in people who had contact with dromedary camels. Viruses isolated from infected camels are indistinguishable from those isolated from people. As of April 2015, 1123 cases and 463 deaths have been reported due to MERS. Cases from the Middle East have been imported into a number of countries. On the basis of DNA sequencing, there appear to be multiple independent viruses causing MERS.

Rift Valley fever, which infects domestic ruminants, can be transmitted to humans by mosquitoes and by contact with the tissues of slaughtered, infected animals such as sheep.[10] Similarly, Crimean–Congo hemorrhagic fever infects a variety of animals, including cattle and sheep, and is transmitted to humans via ticks (especially *Hyalomma* spp.), via contact with blood of infected animals, and in the hospital setting.

Hendra virus, a paramyxovirus, caused infections of horses and a few individuals in contact with these horses in Australia. The natural reservoir is a flying fox (bat). Nipah virus was the cause of an epidemic of encephalitis that affected more than 250 people in Malaysia and Singapore, killing 105 people. More recent outbreaks have occurred in India in West Bengal in 2001 when it killed three-quarters of the 66 infected people and in Bangladesh in 2004 when it killed 18 of 30 infected people. While in early outbreaks infected people had contact with pigs, which were culled to stop the epidemic, more recent outbreaks in Bangladesh have been associated with the consumption of fresh date palm sap that had been contaminated by bats. There has been concern about the possibility that some cases were due to person-to-person transmission. The natural reservoir of Nipah virus, a paramyxovirus that is related most closely to the Hendra virus, has been identified as a bat. Menangle virus, also a paramyxovirus, caused infections of pigs and in humans in contact with infected pigs in Australia. The natural reservoir has been identified as a flying fox (bat).

There is concern of the possibility of certain endogenous porcine retrovirus infections causing disease in humans following xenotransplantation of organ tissues from pigs. Some of these retroviruses can propagate in human cell lines and they could potentially induce immunodeficiency in experimental systems.[11] This poses a potential risk of activation of porcine retroviruses in the setting of an unnatural host such as an immunosuppressed, solid organ human transplant recipient. Porcine heterografts for heart valve replacement surgery are unlikely to be complicated by inadvertent activation of porcine retroviruses. Glutaraldehyde fixation and sterilization of porcine heart valves eliminates infectivity of endogenous retroviruses.[12]

There have been outbreaks in Brazil among cattle and people who had contact with cattle infected with strains of vaccinia virus. In some cases there have been significant deletions of parts of the viral genome.

### PARASITIC INFECTION

A 1993 epidemic of cryptosporidiosis occurred in Milwaukee, Wisconsin, in which the public water supply was contaminated and infected more than 400 000 people. The epidemic was traced to untreated water from Lake Michigan from which the causative organism was incompletely removed by water filtration. Possible sources included cattle along two rivers, slaughterhouses and human sewage.[13] Human cases of cryptosporidiosis also occur via direct contact with cattle and sheep (the disease primarily occurs in lambs).

Echinococcal disease, although not transmitted to humans directly from sheep, occurs in areas of the world in which sheep serve as an intermediate host and in which dogs ingest sheep viscera, subsequently excreting infective eggs in their feces.

The pig ascarid *Ascaris suum* has caused human infection.[14]

*Taenia solium*, the pork tapeworm, is acquired via the ingestion of undercooked infected pork. Alternatively, infection may occur as a consequence of ingestion of infective eggs, as when someone infected by *T. solium* prepares food and contaminates the food with infective eggs that are present in his or her feces.[15] *Trichinella spiralis* is most commonly acquired from eating undercooked pork. Trichinellosis has also been acquired following the ingestion of horsemeat.[16]*Taenia saginata*, the beef tapeworm, is acquired via the ingestion of undercooked beef. Toxoplasmosis can be acquired via the ingestion of undercooked meat, especially lamb, as well as from contaminated goat's milk.

### DERMATOPHYTE INFECTION

Infection with zoophilic dermatophytes commonly occurs following contact with these animals. This includes, for example, *Trichophyton verrucosum* spread from cattle to humans, and *T. equinum* from horses.[17]

## Bats

There is great interest in viral infections of bats. Rabies virus is known to occur in many species of bat. Transmission of rabies to humans follows bite, scratch and, far less often, inhalation of aerosolized saliva. Bats also account for many cases of rabies in livestock. Other Lyssaviruses that have been transmitted to humans from bats include European bat Lyssavirus-1, European bat Lyssavirus-2 and Australian bat Lyssavirus.[18] Most recent reports of human rabies from bat exposure find no clear evidence of a documented bat bite. Transmission apparently occurs from inadvertent bites or from unrecognized contact with the bat saliva. This forms the rationale for the administration of rabies immune globulin and rabies vaccine when a bat is found in the room upon awaking from sleep, in the room of a small child, or in the room of an intoxicated or mentally challenged person[19] (see Chapter 171). However, given the large number of people (approximately 2.7 million with bedroom exposure and without a bite) who would have to be treated with rabies immune globulin and the rabies vaccine in order to prevent a single case of human rabies, this recommendation is controversial.[20] As noted above, bats have been found to be reservoirs of the zoonotic paramyxoviruses Nipah virus, Hendra virus and Menangle virus.[21] In addition, after decades of active research, bats have been identified as the reservoirs of both Ebola virus[22] and Marburg virus.[23]

Outbreaks of histoplasmosis due to *Histoplasma capsulatum* have been associated with exposure to bat guano in caves, after disturbing piles of bat guano in old buildings[24] and clearing debris from a bridge.[25]

While bacteria that are established as pathogens of humans, including members of the genera *Salmonella, Shigella, Campylobacter, Yersinia, Leptospira,* and *Pasteurella,* have been isolated from bats, transmission from bats to humans of these organisms has not been documented.

## Nonhuman Primates

The pathogens found in nonhuman primates (NHPs) include many human pathogens that have subsequently caused human illness when the infected primates transmit these infections back to humans. These pathogens include bacterial (*Shigella* and *Salmonella* spp.), mycobacterial (*M. tuberculosis*), viral (hepatitis A virus), parasitic (*Entamoeba histolytica*), and fungal (dermatophyte) agents. In addition, there are infectious agents of human origin that infect NHPs and that have not been reported to be transmitted back to humans. These include measles virus and (human) herpes simplex virus type 1.

The host range of viral pathogens of NHPs may include humans. Some of these viruses are particularly virulent in humans. Historically, it is worth noting that molecular evidence suggests that HIV-1 was originally a pathogen of chimpanzees, *Pan troglodytes troglodytes*, and that HIV-2 was originally a pathogen of sooty mangabeys. There are numerous simian immunodeficiency virus (SIV) strains and it is possible that one or more might be transmitted to humans via contact such as through butchering, ingestion or by growing the pathogen and subsequently be efficiently spread from human to human. Transmission of SIV occurred in a laboratory worker.[26] Infections due to simian foamy viruses, which are also retroviruses, have been well documented following exposure to NHPs in zoos, primate centers, and in people who hunt and butcher primates in Africa. Human infections by simian foamy viruses originating in such diverse species as gorillas, chimpanzees, baboons and macaques (in Asia) have all been documented, though no long-term health effects on humans from these infections have been demonstrated.

The possibility of life-threatening infection with the neurotropic Macacine herpesvirus-1 (also known as B virus, as cercopithecine herpesvirus-1, and as herpesvirus simiae) must be considered in bites, scratches and contact with tissue or saliva from the rhesus monkey, *Macaca mulatta*.[27] There are distinct genotypes of the virus and the isolates from different primate species vary in their pathogenicity for humans. The National B Virus Resource Center at Georgia State University (website: http://www2.gsu.edu/~wwwvir/) is the reference laboratory for the USA.

There has been transmission from NHPs of filovirus infections, including both Ebola strains of African origin and the Reston strain of Ebola, which is less pathogenic for humans than other strains of Ebola. Marburg virus, a filovirus causing hemorrhagic fever with high mortality, was first transmitted from vervet (or green) monkeys to humans.

Monkeypox, an orthopoxvirus, was initially identified in human cases of illness that were clinically consistent with smallpox, though adenopathy occurs in these infections. It is found in NHPs and in squirrels and other rodents in Africa and has been transmitted from human to human. Tanapox (benign epidermal monkeypox) has been transmitted to humans both via mosquitoes and by direct contact with monkeys in primate centers in the USA, but has not been transmitted from human to human. Yabapox virus has, rarely, caused subcutaneous growths at the site of inoculation.

Kyasanur forest disease virus, a member of the tick-borne encephalitis subgroup, is found in Karnataka, a state in India, and has a number of NHP reservoirs. The presence of dead monkeys in the endemic area, which is expanding, may precede an epidemic.

Rabies has been reported in NHPs. With the exception of a report in which the white-tufted-ear marmoset (*Callithrix jacchus*) was the source of eight human cases of rabies in Brazil,[28] transmission of rabies from NHPs to humans is rare.

## Mustelids (Ferrets, Skunks, Otters, Mink, Weasels, Badgers, Martens)

Influenza A virus was transmitted in a laboratory setting when a researcher was infected by a ferret that had been infected with a strain of influenza A virus and which 'sneezed violently at close range' while it was being examined.[29] Ferrets are susceptible to influenza A and B viruses. Mink that are in mink farms have been found to be infected with influenza A viruses.[30]

There is a report of *M. bovis* subsp. *bovis* infection of the right palm more than 20 years following a ferret bite.[31]*M. bovis* subsp. *bovis* is known to infect wild ferrets and badgers. There is a case report of sporotrichosis complicating a badger bite. Rabies infection is known

to occur in skunks, otters, badgers, weasels, mink and ferrets (including pet ferrets). Transmission of rabies from skunks to humans has been documented.[32] A rabies vaccine has been licensed in the USA for use in ferrets; recommendations are for primary immunization at 3 months and booster immunizations annually.[33] The recommendations regarding a healthy ferret that bites a human are the same as those for dogs and cats with respect to confinement and observation for 10 days, with evaluation by a veterinarian at the first sign of illness.[33]

Rat-bite fever as a result of ferret and weasel bites was reported in the medical literature between 1910 and 1920. Only in a report of a weasel bite was there isolation of an organism from the patient's blood.[34] Trichinellosis has been reported in people who ate inadequately cooked or raw liver, spleen, blood and muscle of a badger.[35]

## Rodents

*Yersinia pestis* is transmitted in epidemics from rats to humans via the rat flea, *Xenopsylla cheopis*. Numerous rodents and other mammals serve as reservoirs of *Y. pestis*, some of which have been responsible for cases of human plague. Similarly, tularemia is widely distributed in nature and has been transmitted to humans by many different rodents.

Leptospirosis is commonly associated with skin or mucous membrane exposure to water contaminated by the urine of rodents, including rats, mice and voles. It has rarely been reported to be transmitted via rodent bite.[36] Other uncommonly reported bacterial infections following rodent bites include *Pasteurella multocida*, the *Pasteurella* 'SP' group and sporotrichosis. Rat-bite fever can be due to either *Streptobacillus moniliformis* or *Spirillum minus*. The former has been transmitted to humans not only by wild rats but also by laboratory rats, mice and other rodents.

It is unclear how often rodents cause cases or outbreaks of human salmonellosis. There have been multi-state outbreaks of human salmonellosis that originated in frozen 'feeder' mice that were fed to reptile and amphibian pets[37] and from pet rodents.[38] Given that *Salmonella* spp. are commonly recovered from rodent feces, the serotypes commonly recovered from rodents are similar to those recovered from cases of human disease, and as rodents often infest human dwellings, restaurants and food production facilities, it is likely that rodents account for some fraction of human salmonellosis cases.

Many of the tick-borne relapsing fevers have wild rodents as reservoirs. This is also the case for *Babesia microti*, Lyme disease and human granulocytic anaplasmosis. The reservoirs of Colorado tick fever include squirrels, chipmunks and other rodents. Similarly, Powassan encephalitis, tick-borne encephalitis, and Omsk hemorrhagic fever virus are transmitted via ticks and have small mammals as reservoirs. *Leishmania* spp. often have rodents as reservoirs.

Those members of the Hantavirus genus that are known to cause hantavirus pulmonary syndrome (HPS) are carried by New World rats and mice, family Muridae, subfamily Sigmodontinae, and are transmitted via the inhalation of rodent excreta or saliva or, rarely, via rodent bite. In the USA and Canada, the viruses include Sin Nombre virus, the main cause of HPS, transmitted by the deer mouse (*Peromyscus maniculatus*) and other less common rodent-borne hantaviruses. In South America, viruses include Andes virus in Argentina, Chile and Uruguay transmitted by the long-tailed pygmy rice rat (*Oligoryzomys longicaudatus*), a virus for which there is epidemiologic evidence of person-to-person transmission; Juquitiba virus in Brazil; Laguna Negra virus in Paraguay, transmitted by the vesper mouse (*Calomys laucha*); and Bermejo virus in Bolivia. Additional hantaviruses have been discovered as well. Hantaviruses that are associated with hemorrhagic fever with renal syndrome in Europe and Asia include Hantaan virus, transmitted by the murine field mouse (*Apodemus agrarius*); Dobrava virus transmitted by the murine field mouse (*Apodemus flavicollis*); Seoul virus, transmitted by the Norway rat (*Rattus norvegicus*) in Asia; and Puumala virus transmitted by the bank vole (*Clethrionomys glareolus*).[39]

Arenaviruses are transmitted from rodents via the excreta and urine. These include lymphocytic choriomeningitis virus, which is found worldwide and has been transmitted to humans by hamsters[40] as well as mice; Machupo virus, which causes Bolivian hemorrhagic fever and is transmitted by *Calomys callosus*; Junin virus, which causes Argentinian hemorrhagic fever and is transmitted by *Calomys* spp.; Guanarito virus, which is found in Venezuela; Lassa fever virus, which is found in Africa and is transmitted by the multimammate rat, *Mastomys natalensis*; and a recently described New World arenavirus that caused three fatal infections in California and shared 87% identity with the Whitewater Arroyo virus at the nucleotide level.[39]

Reservoirs of cowpox virus include several rodents. This is consistent with the epidemiology of cowpox in which cat contact is implicated. Cowpox, or a similar virus, has also been transmitted via rat bite.[41]

A multi-state outbreak of more than 70 cases of monkeypox occurred in the USA following the importation of exotic rodents from Ghana and affected people who had contact with pet prairie dogs that had been in contact with the African rodents at an animal distributor.

Rickettsialpox has been associated with infestation of mice (*Mus musculus*) with mites which serve as the vector for human disease.[42] Rodents serve as reservoirs for many other rickettsial diseases, including murine typhus in which rats have historically been the reservoir, though in areas of California and Texas cats and opposums serve that role; *Rickettsia prowazekii*, which has been associated with flying squirrels;[43] scrub typhus, in which rats are hosts of the trombiculid mite vectors; and members of the spotted fever group.

Although the issue of whether giardiasis is commonly zoonotic in origin is debated, beavers may have been the source of an outbreak of water-borne giardiasis.[44]

Ingestion of rodents has been associated with rare cases of trichinellosis, such as following the ingestion of squirrel and bamboo rat.[45] There has been speculation on whether consumption of squirrel brains causes a spongiform encephalopathy, but data are limited.[46] Eating fermented beaver has resulted in botulism.[47]

*Trichophyton mentagrophytes* var. *mentagrophytes* is a common zoophilic dermatophyte, infecting humans and domestic animals. Rodents are regarded as the reservoir of this mold.

## Lagomorphs (Rabbits, Hares)

Tularemia, also known as rabbit fever, has been acquired from rabbits and hares as a result of cutaneous contact and skinning of the animals, presumably by entering via microabrasions in the skin or via the conjunctiva, and following ingestion.[4,48] Transmission via infectious aerosol has been reported as a result of mowing over a rabbit.[49] Tularemia transmission to humans has not been reported from domesticated rabbits. Although uncommon, eight cases of human bubonic plague from 1950 to 1974 were reported as a result of contact (e.g. skinning) with rabbits and hares[50] in plague-endemic areas of the USA. Q fever has been transmitted to humans following contact with wild rabbits.[51]

A patient with *Bordetella bronchiseptica* respiratory infection was shown to be due to a strain that was indistinguishable by pulsed-field gel electrophoresis from the strain isolated from a respiratory tract isolate from one of 20 farm rabbits that slept with a cat with which the patient had contact.[52]

## Raccoons

The raccoon ascarid, *Baylisascaris procyonis*, has caused cases, including fatal ones, of meningoencephalitis, often with an associated cerebral spinal fluid (CSF) eosinophilia and usually in young children who accidentally ingest infectious ova.[53] Ocular involvement has also been reported. Leptospirosis has been reported from contact with raccoons.[54] Rabies is common in raccoons, although transmission of the strain found in raccoons to humans in the USA has only been rarely reported.

## Mongooses

Leptospirosis is common among mongooses in Hawaii[55] and a number of Caribbean islands.[56] Rabies is quite common among many species

of mongoose and accounts for a significant number of cases of human exposure to rabies in the Caribbean. It is the principal rabies reservoir in South Africa and it may be an important source of wildlife rabies in India.[57]

## Insectivores

Hedgehog contact, notably with pet hedgehogs, has transmitted salmonellosis[58] and dermatophyte infections due to *Trichophyton erinacei*.[55] In an outbreak of leptospirosis in Italy in which 32 of 33 confirmed cases were contracted by drinking water at the same water fountain, a dead hedgehog was found in a water reservoir connected to the system, although isolation of *Leptospira* spp. from the hedgehog was not attempted.[59]

The Asian house shrew, *Suncus murinus*, may be infested with the oriental rat flea, *Xenopsylla cheopis*, and infected with *Yersinia pestis*. It may well be important in the maintenance of plague between epidemics. Insectivores also appear to be reservoirs of tick-borne encephalitis and tularemia.

## Marine Mammals (Seals, Sea Lions, Walrus, Whales, Dolphins, Porpoises, Manatees)

At the case report level, there are several infections that have been transmitted from marine mammals to humans. Leptospirosis, which is commonly encountered in seals and the California sea lion, was transmitted from an infected sea lion pup to a human. Two people developed leptospirosis after performing a necropsy on a sea lion that died of leptospirosis.[60] Human infection with *Erysipelothrix rhusiopathiae* has been reported among veterinarians and veterinary students caring for or performing autopsies on cetaceans.[61] In these reports, the isolation of the organism was not made from the human cases. Two of three people who cared for affected gray seals developed 'single milker's nodule-like lesions' on the fifth finger of the right hand. The lesions from the seal handlers demonstrated virus particles that were identical to the virus particles from the seals' pox lesions and were characteristic of the paravaccinia subgroup of poxviruses.[62] In 2005, a marine mammal technician who was bitten by a seal developed an orf-like lesion that was ultimately demonstrated to be due to seal pox on the basis of polymerase chain reaction (PCR) and sequencing of the amplified DNA.

Pulmonary tuberculosis due to a member of the *Mycobacterium tuberculosis* complex that is similar to *M. bovis* has been transmitted from seals in a marine park in Western Australia to a seal trainer who developed pulmonary tuberculosis 3 years after his last exposure to the animals with an isolate of the *Mycobacterium* that could not be distinguished from the seal isolates on the basis of DNA restriction endonuclease analysis.[63] Seal trainers are in very close contact with seals which, by barking and coughing, are potentially able to transmit infection via the aerosol route.

Four people involved in necropsies of harbor seals from which influenza A virus A/Seal/Mass/1/80 (H7N7) was isolated developed purulent conjunctivitis but did not have detectable antibodies in single serum samples 3–6 months after the exposure to the influenza A virus isolated from the seals.[64] A seal that was known to be infected with the influenza A virus sneezed into the face and right eye of a person who subsequently developed conjunctivitis from which the virus was isolated.[65] Influenza A virus has also been isolated from cetaceans.

Numerous cases of 'seal finger' have been reported in people who have been bitten or scratched by seals and from skinning or handling seals. Seal finger often responds to tetracycline therapy. The etiologic agent has not been established. Other organisms that have been transmitted via the bite of marine mammals include a single case report of *Mycoplasma phocacerebrale*, which was isolated from the drainage material from a patient's fingers and swabs from the seal's front teeth.[66]

Consumption of whale, seal and walrus meat is not uncommon among the Inuit in Canada, Alaska, Greenland and Siberia. There have been large epidemics of salmonellosis resulting from consumption of whale meat from floating and beached whale carcasses that have been used as the source of food. Trichinellosis (trichinosis) has been acquired following the consumption of raw or undercooked walrus meat. The clinical presentation in arctic trichinellosis due to *Trichinella nativa* differs from that of classic trichinellosis caused by *Trichinella spiralis* in that the most prominent clinical symptoms in arctic trichinellosis are gastrointestinal, with prolonged diarrhea.[67] Food-borne botulism, typically due to *Clostridium botulinum* type E, has been acquired from the consumption of fermented foods including beluga whale meat, seal meat, seal flippers and walrus meat.

## Armadillos

Both experimental and naturally occurring leprosy in nine-banded armadillos has been noted and there has been a body of literature (reviewed by Blake *et al.*[68]) that suggests that contact with armadillos may have been the source of leprosy in some patients in the USA and Mexico. Sporotrichosis has been found to be highly associated with armadillo contact in Uruguay.[69]

## Birds

Psittacosis is transmitted to humans not only via pet birds, but also via turkeys, wild and domestic pigeons, ducks, and other birds.[70]

Salmonellosis has been acquired from contact with birds and from consumption of birds (e.g. chicken, turkey) and eggs.[71] *Campylobacter jejuni* and *C. laridis* infections have been associated with both the consumption of birds and, interestingly, consumption of milk that has been pecked by magpies (*Pica pica*) and jackdaws (*Corvus monedula*).[72] *Erysipelothrix rhusiopathiae* has been acquired from bird contact. Newcastle disease virus of fowl, an occupational disease, causes an acute conjunctivitis that may be associated with preauricular adenitis.[73]

Histoplasmosis, often in large outbreaks, has been the result of inhalation of bird excreta.[74] Infection with *Cryptococcus neoformans*, which is known to be found in bird droppings, has at the case report level been linked to exposure to pet birds[75] and fancy pigeons.[76]

Avian strains of influenza A virus represent a global concern, as the host range of the viruses may include humans. There exists the potential for pandemic influenza as a result of the introduction of an avian virus with a hemagglutinin to which humans lack immunity.[77] For a detailed discussion of the risks associated with avian influenza please refer to Chapter 172.

The epidemic of West Nile virus infection in the USA and Canada is largely attributable to the introduction of this flavivirus into a new ecologic niche in wild birds in North America.[78] Blackbirds, crows, other wild birds and domestic chickens are susceptible to this viral illness and this forms the reservoir for this mosquito-transmitted infection that is responsible for a potentially lethal form of viral encephalitis.[79]

Tularemia has been, at the several case report level, acquired from wild birds. A case of Crimean–Congo hemorrhagic fever in an ostrich farm worker who was involved in the slaughter of ostriches, *Struthio camelus*, and handled the fresh blood and tissues of the birds, has been reported. There were numerous adult *Hyalomma* ticks on the ostriches and he likely was infected either directly due to skinning the ostriches or as a result of the presence of the ticks on the ostriches.[80]

## Fish

In addition to the normal flora of the fish, a wound can become infected with environmental bacteria. The species of bacteria that live in water are dependent on both salinity and temperature. Estuarine and freshwater bacteria include members of the genera *Vibrio*, *Aeromonas* and *Plesiomonas*. As a result, the etiologic agents isolated from an infected wound from a fish bite, spine, or fin injury that occurs in salt water may well be different from one that occurs in fresh water. The normal flora of teeth in salt-water sharks includes, for example, *Vibrio* spp., including *V. harveyi* (formerly *V. carchariae*), an organism that was the cause of infection following the bite of a great white shark.[81]

By contrast, *Edwardsiella tarda* is commonly isolated from catfish injuries occurring in fresh water. Other organisms that have caused wound injuries as a result of injuries from fish include *Aeromonas* spp., *Erysipelothrix rhusiopathiae*, *Mycobacterium marinum*, *Mycobacterium terrae*, *Streptococcus iniae*, *Vibrio vulnificus* and *Vibrio vulnificus* serovar E (biotype 2; indole-negative) from eels.[82] *Vibrio alginolyticus*, *Photobacterium damselae* subsp. *damselae* (formerly *Vibrio damsela*), *Shewanella putrefaciens*, *Pseudomonas aeruginosa* and *Halomonas venusta* have been isolated from fish bites and injuries. It is not always clear whether the source of the organism is the fish or the water.

Ingestion of fish or fish products can pose a significant risk of acquiring both bacterial and parasitic infections unless the fish has been well cooked.

*Vibrio* spp., including *V. fluvialis*, *V. hollisae*, *V. parahaemolyticus* and *V. cholerae* O1,[83] have all been associated with fish consumption, as has *P. shigelloides*. Eel consumption has been associated with *Photobacterium damselae* subsp. *damselae* (formerly *Vibrio damsela*).[84] *Listeria monocytogenes* infections have been associated with the consumption of fish, including vacuum-packed salmon and cold-smoked rainbow trout.[85]

Fish-associated botulism is usually due to type E toxin and in the USA is most common among Alaskans. Fermented fish eggs, fish eggs, home-marinated fish and dry salted fish have all been implicated. Consumption of apparently fresh (unpreserved and unfermented) fish in Hawaii resulted in three adults with botulism due to type B toxin.[86] Numerous parasitic infections have been reported following the consumption of raw, undercooked, pickled and lightly or cold-smoked fish. Selected cestodes, trematodes and nematodes acquired from the consumption of fish are listed in Table 74-3.

**TABLE 74-3 Selected Parasites Transmitted Via Consumption of Fish**

| Parasite | Type of Parasite | Types of Fish |
|---|---|---|
| *Diphyllobothrium latum* | Cestode | Salmon, pike, perch, burbot |
| *Diphyllobothrium pacificum* | Cestode | Marine fish |
| *Diphyllobothrium ursi* | Cestode | Salmon |
| *Nanophyetus salmincola* | Trematode | Usually salmonids |
| *Heterophyes heterophyes* | Trematode | Mullet, tilapia, mosquito fish |
| *Haplorchis yokogawai* | Trematode | Mullet |
| *Haplorchis taichui* | Trematode | Mullet |
| *Opisthorchis sinensis* | Trematode | Freshwater fish |
| *Opisthorchis viverrini* | Trematode | Freshwater fish |
| *Opisthorchis felineus* | Trematode | Freshwater fish |
| *Metorchis conjunctus* | Trematode | Freshwater fish |
| *Anisakis simplex* | Nematode | Salmon, tuna, herring, mackerel, others |
| *Pseudoterranova decipiens* | Nematode | Cod, pollock, haddock, salmon, Pacific rockfish |
| *Eustrongyloides* spp. | Nematode | Killifish, estuarine fish, minnows |
| *Dioctophyma renale* | Nematode | Freshwater, estuarine fish |
| *Capillaria philippinensis* | Nematode | Freshwater, estuarine fish |
| *Gnathostoma spinigerum* | Nematode | Freshwater fish |

## Amphibians

Contact with amphibians has rarely transmitted salmonellosis, but has transmitted sparganosis due to *Diphyllobothrium* (*Spirometra*) *mansoni* via the use of contaminated frog flesh as a poultice (reviewed by Huang and Kirk[87]) and, rarely, intraocular *Alaria* spp., as reported in a woman with a long history of frog collection and food preparation.[88]

Ingestion of frogs has transmitted sparganosis. Infection with the trematode *Fibricola seoulensis* occurred after 10 Korean soldiers ate raw or undercooked flesh of snakes or frogs during survival training.[89] Two cases of intraocular infection with an *Alaria* spp. occurred in Asian-Americans in California who consumed cooked frogs' legs in Chinese dishes.[90] Frogs' legs have a very high rate of contamination with *Salmonella*.

## Bears

There is a published report of transmission of leptospirosis to two zoo employees in which the most likely source was an ill polar bear cub.[91] There are few published reports on infections following bear bites. A man shot and killed a grizzly bear in Alaska and scratched his left index finger on one of the bear's teeth while removing the bear's tongue, resulting in a *Mycobacterium chelonae* subsp. *abscessus* infection.[92]

In multiple reports, consumption of undercooked bear meat has caused trichinellosis. Bear steaks are often served rare, in part because they are somewhat 'tough' if they are fully cooked. Bears are known to have a high rate of toxoplasmosis and the possibility of a dual infection (trichinellosis and toxoplasmosis) in a person who ingested undercooked bear meat has been reported.[93] Note that acute hypervitaminosis A occurs following the ingestion of polar bear liver.

## Large Herbivores (Elephants, Rhinoceroses)

The few infections transmitted to humans include *M. tuberculosis* from elephants,[94] *M. bovis* from rhinoceroses[95] and an orthopoxvirus (possibly cowpox) from elephants to humans. It is likely that many cases of tuberculosis in elephants, which are almost all due to *M. tuberculosis*, are due to human-to-elephant transmission. In the USA, approximately 3% percent of elephants are infected with *M. tuberculosis*.[96]

**References available online at expertconsult.com.**

## KEY REFERENCES


Bartlett P.C., Vonbehren L.A., Tewari R.P., et al.: Bats in the belfry: an outbreak of histoplasmosis. *Am J Public Health* 1982; 72:1369-1372.

Centers for Disease Control and Prevention: Botulism outbreak associated with eating fermented food – Alaska, 2001. *MMWR Morb Mortal Wkly Rep* 2001; 50:680-772.

De Serres G., Skowronski D.M., Mimault P., et al.: Bats in the bedroom, bats in the belfry: reanalysis of the rationale for rabies postexposure prophylaxis. *Clin Infect Dis* 2009; 48:1493-1499.

Duma R.J., Sonenshine D.E., Bozeman F.M., et al.: Epidemic typhus in the United States associated with flying squirrels. *JAMA* 1981; 245:2318-2323.

Evans M.E., Gregory D.W., Schaffner W., et al.: Tularemia: a 30-year experience with 88 cases. *Medicine (Baltimore)* 1985; 64:251-269.

Hirsch M.S., Moellering R.C. Jr, Pope H.G., et al.: Lymphocytic-choriomeningitis-virus infection traced to a pet hamster. *N Engl J Med* 1974; 291:610-612.

Khabbaz R.F., Heneine W., George J.R., et al.: Brief report: infection of a laboratory worker with simian immunodeficiency virus. *N Engl J Med* 1994; 330:172-227.

MacKenzie W.R., Hoxie N.J., Proctor M.E., et al.: A massive outbreak in Milwaukee of cryptosporidium infection transmitted through the public water supply. *N Engl J Med* 1994; 331:161-167.

Mikota S.K., Larsen R.S., Montali R.J.: Tuberculosis in elephants in North America. *Zoo Biol* 2000; 19:393-403.

Miller J.M., Astles R., Baszler T., et al.: Guidelines for safe work practices in human and animal medical diagnostic laboratories: Recommendations of a CDC-convened, Biosafety Blue Ribbon Panel. *MMWR Morb Mortal Wkly Rep* 2012; 61(Suppl.):1-105.

St Louis M.E., Morse D.L., Potter M.E., et al.: The emergence of grade A eggs as a major source of *Salmonella enteritidis* infections. New implications for the control of salmonellosis. *JAMA* 1988; 259:2103-2107.

Weber D.J., Rutala W.A.: Zoonotic infections. *Occup Med* 1999; 14:247-284.

## REFERENCES


1. Weber D.J., Rutala W.A.: *Zoonotic infections*. *Occup Med* 1999; 14:247-284.
2. Miller J.M., Astles R., Baszler T., et al.: Guidelines for safe work practices in human and animal medical diagnostic laboratories: Recommendations of a CDC-convened, Biosafety Blue Ribbon Panel. *MMWR Morb Mortal Wkly Rep* 2012; 61(Suppl.):1-105.
3. Jellison W.L., Kohls G.M.: *Tularemia in sheep and sheep industry workers in western United States*. Public Health Monograph, Vol. 28. Washington DC: US Department of Health, Education, and Welfare; 1955.
4. Cain D.B., McCann V.L.: An unusual case of cutaneous listeriosis. *J Clin Microbiol* 1986; 23:976-1107.
5. Lee L.A., Gerber A.R., Lonsway D.R., et al.: *Yersinia enterocolitica* O:3 infections in infants and children, associated with the household preparation of chitterlings. *N Engl J Med* 1990; 322:984-1107.
6. Dunbar S.A., Clarridge J.E. 3rd: Potential errors in recognition of *Erysipelothrix rhusiopathiae*. *J Clin Microbiol* 2000; 38:1302-1304.
7. Jorgensen D.M.: Gestational psittacosis in a Montana sheep rancher. *Emerg Infect Dis* 1997; 3:191-224.
8. Molbak K., Baggesen D.L., Aarestrup F.M., et al.: An outbreak of multidrug-resistant, quinolone-resistant *Salmonella enterica* serotype typhimurium DT104. *N Engl J Med* 1999; 341:1420-1425.
9. Lester A., Gerner-Smidt P., Gahrn-Hansen B., et al.: Phenotypical characters and ribotyping of *Pasteurella aerogenes* from different sources. *Zentralbl Bakteriol* 1993; 279:75-82.
10. Woods C.W., Karpati A.M., Grein T., et al.: An outbreak of Rift Valley fever in northeastern Kenya, 1997–98. *Emerg Infect Dis* 2002; 8:138-144.
11. Tacke S.J., Kurth R., Denner J.: Porcine endogenous retroviruses inhibit human immune cell function: risk for xenotransplantation? *Virology* 2000; 268:87-93.
12. Moza A.K., Mertsching H., Herden T., et al.: Heart valves from pigs and porcine endogenous retrovirus: experimental and clinical data to assess the probability of porcine endogenous retrovirus infections in human subjects. *J Thorac Cardiovasc Surg* 2001; 121:697-701.
13. MacKenzie W.R., Hoxie N.J., Proctor M.E., et al.: A massive outbreak in Milwaukee of cryptosporidium infection transmitted through the public water supply. *N Engl J Med* 1994; 331:161-167.
14. Phills J.A., Harrold A.J., Whiteman G.V., et al.: Pulmonary infiltrates, asthma and eosinophilia due to *Ascaris suum* infestation in man. *N Engl J Med* 1972; 286:965-970.
15. Schantz P.M., Moore A.C., Munoz J.L., et al.: Neurocysticercosis in an Orthodox Jewish community in New York City. *N Engl J Med* 1992; 327:692-695.
16. Ancelle T., Dupouy-Camet J., Desenclos J.C., et al.: A multifocal outbreak of trichinellosis linked to horse meat imported from North America to France in 1993. *Am J Trop Med Hyg* 1998; 59:615-619.
17. Shwayder T., Andreae M., Babel D.: *Trichophyton equinum* from riding bareback: first reported U.S. case. *J Am Acad Dermatol* 1994; 30:785-787.
18. McCall B.J., Epstein J.H., Neill A.S., et al.: Potential exposure to Australian bat lyssavirus, Queensland, 1996–1999. *Emerg Infect Dis* 2000; 6:259-264.
19. CDC: Human rabies – California, 2002. *MMWR Morb Mortal Wkly Rep* 2002; 51:686-778.
20. De Serres G., Skowronski D.M., Mimault P., et al.: Bats in the bedroom, bats in the belfry: reanalysis of the rationale for rabies postexposure prophylaxis. *Clin Infect Dis* 2009; 48:1493-1499.
21. Philbey A.W., Kirkland P.D., Ross A.D., et al.: An apparently new virus (family Paramyxoviridae) infectious for pigs, humans, and fruit bats. *Emerg Infect Dis* 1998; 4:269-271.
22. Leroy E.M., Kumulungui B., Pourrut X., et al.: Fruit bats as reservoirs of Ebola virus. *Nature* 2005; 438:575-576.
23. Towner J.S., Pourrut X., Albarino C.G., et al.: Marburg virus infection detected in a common African bat. *PLoS ONE* 2007; 2:e764.
24. Bartlett P.C., Vonbehren L.A., Tewari R.P., et al.: Bats in the belfry: an outbreak of histoplasmosis. *Am J Public Health* 1982; 72:1369-1372.
25. Sorley D.L., Levin M.L., Warren J.W., et al.: Bat-associated histoplasmosis in Maryland bridge workers. *Am J Med* 1979; 67:623-626.
26. Khabbaz R.F., Heneine W., George J.R., et al.: Brief report: infection of a laboratory worker with simian immunodeficiency virus. *N Engl J Med* 1994; 330:172-227.
27. Holmes G.P., Chapman L.E., Stewart J.A., et al.: Guidelines for the prevention and treatment of B-virus infections in exposed persons. The B virus Working Group. *Clin Infect Dis* 1995; 20:421-439.
28. Favoretto S.R., de Mattos C.C., Morais N.B., et al.: Rabies in marmosets (*Callithrix jacchus*), Ceara, Brazil. *Emerg Infect Dis* 2001; 7:1062-1065.
29. Smith W., Stuart-Harris C.H.: Influenza infection of man from the ferret. *Lancet* 1936; 2:121-123.
30. Englund L.: Studies on influenza viruses H10N4 and H10N7 of avian origin in mink. *Vet Microbiol* 2000; 74:101-107.
31. Jones J.W., Pether J.V., Rainey H.A., et al.: Recurrent *Mycobacterium bovis* infection following a ferret bite. *J Infect* 1993; 26:225-226.
32. Hattwick M.A., Hochberg F.H., Landrigan P.J., et al.: Skunk-associated human rabies. *JAMA* 1972; 222:44-47.
33. Jenkins S.R., Auslander M., Conti L., et al.: Compendium of animal rabies, prevention and control, 2001. *J Am Vet Med Assoc* 2001; 218:26-31.
34. Dick G.F., Tunnicliff R.: A streptothrix isolated from the blood of a patient bitten by a weasel (*Streptothrix putorii*). *J Infect Dis* 1918; 23:183-227.
35. Sohn W.M., Kim H.M., Chung D.I., et al.: The first human case of *Trichinella spiralis* infection in Korea. *Korean J Parasitol* 2000; 38:111-115.
36. Gollop J.H., Katz A.R., Rudoy R.C., et al.: Rat-bite leptospirosis. *West J Med* 1993; 159:76-77.
37. CDC: Notes from the field: Infections with *Salmonella* I 4,[5],12:i:- linked to exposure to feeder rodents – United States, August 2011-February 2012. *MMWR Morb Mortal Wkly Rep* 2012; 61:277.
38. Swanson S.J., Snider C., Braden C.R., et al.: Multidrug-resistant *Salmonella enterica* serotype Typhimurium associated with pet rodents. *N Engl J Med* 2007; 356:21-28.
39. Fatal illnesses associated with a New World arenavirus. *MMWR Morb Mortal Wkly Rep* 2000; 49:709-711.
40. Hirsch M.S., Moellering R.C. Jr, Pope H.G., et al.: Lymphocytic-choriomeningitis-virus infection traced to a pet hamster. *N Engl J Med* 1974; 291:610-612.
41. Postma B.H., Diepersloot R.J., Niessen G.J., et al.: Cowpox-virus-like infection associated with rat bite. *Lancet* 1991; 337:733-734.
42. Brettman L.R., Lewin S., Holzman R.S., et al.: Rickettsialpox: report of an outbreak and a contemporary review. *Medicine (Baltimore)* 1981; 60:363-372.
43. Duma R.J., Sonenshine D.E., Bozeman F.M., et al.: Epidemic typhus in the United States associated with flying squirrels. *JAMA* 1981; 245:2318-2323.
44. Dykes A.C., Juranek D.D., Lorenz R.A., et al.: Municipal waterborne giardiasis: an epidemiologic investigation. Beavers implicated as a possible reservoir. *Ann Intern Med* 1980; 92:165-170.
45. Wang Z.Q., Cui J.: The epidemiology of human trichinellosis in China during 1964–1999. *Parasite* 2001; 8:S63-S66.
46. Kamin M., Patten B.M.: Creutzfeldt–Jakob disease. Possible transmission to humans by consumption of wild animal brains. *Am J Med* 1984; 76:142-145.
47. Botulism outbreak associated with eating fermented food – Alaska, 2001. *MMWR Morb Mortal Wkly Rep* 2001; 50:680-772.
48. Evans M.E., Gregory D.W., Schaffner W., et al.: Tularemia: a 30-year experience with 88 cases. *Medicine (Baltimore)* 1985; 64:251-269.
49. McCarthy V.P., Murphy M.D.: Lawnmower tularemia. *Pediatr Infect Dis J* 1990; 9:298-300.
50. von Reyn C.F., Barnes A.M., Weber N.S., et al.: Bubonic plague from exposure to a rabbit: a documented case, and a review of rabbit-associated plague cases in the United States. *Am J Epidemiol* 1976; 104:81-87.
51. Marrie T.J., Schlech W.F., Williams J.C., et al.: Q fever pneumonia associated with exposure to wild rabbits. *Lancet* 1986; 1:427-429.
52. Gueirard P., Weber C., Le Coustumier A., et al.: Human *Bordetella bronchiseptica* infection related to contact with infected animals: persistence of bacteria in host. *J Clin Microbiol* 1995; 33:2002-2006.
53. Sorvillo F., Ash L.R., Berlin O.G., et al.: *Baylisascaris procyonis*: an emerging helminthic zoonosis. *Emerg Infect Dis* 2002; 8:355-359.
54. Falk V.S.: Leptospirosis in Wisconsin: report of a case associated with direct contact with raccoon urine. *Wisconsin Med J* 1985; 84:14-15.
55. Middleton C.R., Ansdell V.E., Sasaki D.M.: Of mice and mongooses … a history of leptospirosis research in Hawaii. *Hawaii Med J* 2001; 60:179-181, 184-6.
56. Jones C.J., Taylor K.D., Myers D.M., et al.: Pathogenic *Leptospira* isolates from the Caribbean island of Barbados. *Int J Zoonoses* 1982; 9:138-146.
57. Everard C.O., Everard J.D.: Mongoose rabies. *Rev Infect Dis* 1988; 10(Suppl. 4):S610-S614.
58. Anand C.M., Fonseca K., Longmore K., et al.: Epidemiologic investigation of *Salmonella tilene* by pulsed-field gel electrophoresis and polymerase chain reaction. *Can J Infect Dis* 1997; 8:318-322.
59. Philpot C.M., Bowen R.G.: Hazards from hedgehogs: two case reports with a survey of the epidemiology of hedgehog ringworm. *Clin Exp Dermatol* 1992; 17:156-158.
60. Cacciapuoti B., Ciceroni L., Maffei C., et al.: A waterborne outbreak of leptospirosis. *Am J Epidemiol* 1987; 126:535-545.
61. Dilbone R.P.: Erysipelas suspected in two porpoises. *J Am Vet Med Assoc* 1965; 147:1085.
62. Hicks B.D., Worthy G.A.: Sealpox in captive grey seals (*Halichoerus grypus*) and their handlers. *J Wildl Dis* 1987; 23:1-6.
63. Thompson P.J., Cousins D.V., Gow B.L., et al.: Seals, seal trainers, and mycobacterial infection. *Am Rev Respir Dis* 1993; 147:164-167.
64. Geraci J.R., St Aubin D.J., Barker I.K., et al.: Mass mortality of harbor seals: pneumonia associated with influenza A virus. *Science* 1982; 215:1129-1131.
65. Webster R.G., Geraci J., Petursson G., et al.: Conjunctivitis in human beings caused by influenza A virus of seals. *N Engl J Med* 1981; 304:911.
66. Baker A.S., Ruoff K.L., Madoff S.: Isolation of *Mycoplasma* species from a patient with seal finger. *Clin Infect Dis* 1998; 27:1168-1170.
67. MacLean J.D., Viallet J., Law C., et al.: Trichinosis in the Canadian Arctic: report of five outbreaks and a new clinical syndrome. *J Infect Dis* 1989; 160:513-520.
68. Blake L.A., West B.C., Lary C.H., et al.: Environmental nonhuman sources of leprosy. *Rev Infect Dis* 1987; 9:562-577.
69. Conti Díaz I.: Esporotricosis. *Rev Méd Uruguay* 1987; 3:135-147.
70. Henry K., Crossley K.: Wild-pigeon-related psittacosis in a family. *Chest* 1986; 90:708-710.
71. St Louis M.E., Morse D.L., Potter M.E., et al.: The emergence of grade A eggs as a major source of *Salmonella enteritidis* infections. New implications for the control of salmonellosis. *JAMA* 1988; 259:2103-2107.
72. Riordan T., Humphrey T.J., Fowles A.: A point source outbreak of campylobacter infection related to bird-pecked milk. *Epidemiol Infect* 1993; 110:261-335.
73. Trott D.G., Pilsworth R.: Outbreaks of conjunctivitis due to the Newcastle disease virus among workers in chicken-broiler factories. *Br Med J* 1965; 5477:1514-1517.
74. Latham R.H., Kaiser A.B., Dupont W.D., et al.: Chronic pulmonary histoplasmosis following the excavation of a bird roost. *Am J Med* 1980; 68:504-508.
75. Nosanchuk J.D., Shoham S., Fries B.C., et al.: Evidence of zoonotic transmission of *Cryptococcus neoformans* from a pet cockatoo to an immunocompromised patient. *Ann Intern Med* 2000; 132:205-208.
76. Bauters T.G., Moerman M., Pini G., et al.: Colonization of a voice prosthesis by *Cryptococcus neoformans*. *Med Mycol* 2001; 39:379-381.
77. Horimoto T., Kawaoka Y.: Pandemic threat posed by avian influenza A viruses. *Clin Microbiol Rev* 2001; 14:129-149.
78. Lin Y.P., Shaw M., Gregory V., et al.: Avian-to-human transmission of H9N2 subtype influenza A viruses: relationship between H9N2 and H5N1 human isolates. *Proc Natl Acad Sci USA* 2000; 97:9654-9658.

79. CDC: West Nile Virus activity – United States July 31–August 7, 2002, Louisiana January 1–August 7. *MMWR Morb Mortal Wkly Rep* 2002; 51:681-773.
80. Shepherd A.J., Swanepoel R., Leman P.A., et al.: Field and laboratory investigation of Crimean–Congo haemorrhagic fever virus (Nairovirus, family Bunyaviridae) infection in birds. *Trans Roy Soc Trop Med Hyg* 1987; 81:1004-1007.
81. Pavia A.T., Bryan J.A., Maher K.L., et al.: *Vibrio carchariae* infection after a shark bite. *Ann Intern Med* 1989; 111:85-86.
82. Veenstra J., Rietra P.J., Stoutenbeek C.P., et al.: Infection by an indole-negative variant of *Vibrio vulnificus* transmitted by eels. *J Infect Dis* 1992; 166:209-210.
83. McIntyre R.C., Tira T., Flood T., et al.: Modes of transmission of cholera in a newly infected population on an atoll: implications for control measures. *Lancet* 1979; 1:311-314.
84. Shin J.H., Shin M.G., Suh S.P., et al.: Primary *Vibrio damsela* septicemia. *Clin Infect Dis* 1996; 22:856-857.
85. Ericsson H., Eklow A., Danielsson-Tham M.L., et al.: An outbreak of listeriosis suspected to have been caused by rainbow trout. *J Clin Microbiol* 1997; 35:2904-2907.
86. Fish botulism – Hawaii, 1990. *MMWR Morb Mortal Wkly Rep* 1991; 40:412-414.
87. Huang C.T., Kirk R.: Human sparganosis in Hong Kong. *J Trop Med Hyg* 1962; 65:133-138.
88. Shea M., Maberley A.L., Walters J., et al.: Intraretinal larval trematode. *Trans Am Acad Ophthalmol Otolaryngol* 1973; 77:OP784-OP791.
89. Hong S.-T., Chai J.-Y., Lee S.-H.: Ten human cases of *Fibricola seoulensis* infection and mixed one with Stellantchasmus and Metagonimus. *Korean J Parasitol* 1986; 24:94-96.
90. McDonald H.R., Kazacos K.R., Schatz H., et al.: Two cases of intraocular infection with *Alaria mesocercaria* (Trematoda). *Am J Ophthalmol* 1994; 117:447-455.
91. Anderson D.C., Geistfeld J.G., Maetz H.M., et al.: Leptospirosis in zoo workers associated with bears. *Am J Trop Med Hyg* 1978; 27:210-231.
92. Evans T.G., Burgert S.J.: The culprit: grizzly bear or plastic surgeon? *Clin Infect Dis* 1993; 17:1067-1068.
93. Jordan G.W., Theis J., Fuller C.M., et al.: Bear meat trichinosis with a concomitant serologic response to *Toxoplasma gondii*. *Am J Med Sci* 1975; 269:251-257.
94. Michalak K., Austin C., Diesel S., et al.: *Mycobacterium tuberculosis* infection as a zoonotic disease: transmission between humans and elephants. *Emerg Infect Dis* 1998; 4:283-337.
95. Dalovisio J.R., Stetter M., Mikota-Wells S.: Rhinoceros' rhinorrhea: cause of an outbreak of infection due to airborne *Mycobacterium bovis* in zookeepers. *Clin Infect Dis* 1992; 15:598-600.
96. Mikota S.K., Larsen R.S., Montali R.J.: Tuberculosis in elephants in North America. *Zoo Biol* 2000; 19:393-403.

Tuberculosis 91 (2011) 208–211

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

REVIEW

# Tuberculosis at the human–animal interface: An emerging disease of elephants

Susan K. Mikota [a,d], Joel N. Maslow [b,c,d,*]

[a] Elephant Care International, Hohenwald, TN, United States
[b] Section of Infectious Diseases, VA Medical Center, 3800 Woodland Ave., Philadelphia, PA 19104, United States
[c] Division of Infectious Diseases, University of Pennsylvania, Philadelphia, PA, United States

ARTICLE INFO

*Article history:*
Received 7 January 2011
Received in revised form
10 February 2011
Accepted 14 February 2011

*Keywords:*
Tuberculosis
Multi-drug resistant tuberculosis (MDR-TB)
*Mycobacterium tuberculosis*
*Mycobacterium bovis*
Zoonosis

SUMMARY

Over the past 15 years, cases of infection with organisms of the *Mycobacterium tuberculosis* complex have been diagnosed among captive elephants in the United States and worldwide. Outbreak investigations have documented that among staff employed at facilities housing infected animals, skin test conversion to purified protein derivative have been documented. Clonal spread among animals in close contact and even inter-species spread between elephant and human has been documented. Detection of actively infected animals relies on samples obtained by trunk wash. Diagnosis has been augmented by the development of a multi-antigen serologic assay with excellent specificity and sensitivity. Treatment regimens are still in development with efficacy largely unknown due to a paucity of both premortem follow-up and necropsy data of treated animals. The epidemiology, diagnosis and treatment of tuberculosis in elephants require additional careful study of clinical data.

Published by Elsevier Ltd.

## 1. Introduction

Over the past 2 decades, tuberculosis (TB) has seen a resurgence initially associated with the epidemic of human immunodeficiency virus (HIV) infection and more recently with the emergence of multi-drug resistant (MDR) and extremely drug resistant (XDR) strains. Surprisingly overlooked in the fight against TB is the potential for transmission at the human–animal interface.[1] This interface includes not only domestic livestock such as cattle and buffalo but also non-human primates, elephants, and other species that interact with people in zoos, circuses, temples, and tourist facilities around the world and that represent potential reservoirs of both drug-susceptible and resistant strains of TB. In fact, the isolation of MDR-TB from an elephant in the United States (U.S.)[27] highlights what was heretofore a theoretical concern in the nation's population of approximately 450 elephants.

While elephants are maintained in many zoos and circuses worldwide, Asia in particular hosts a large population of captive elephants including 3400–3600 in India alone.[2] Reports from India, Sri Lanka, and other Asian countries indicate that TB is not an unusual finding on post-mortem examination in captive elephants.[3,4] Moreover, unofficial reports from Asia and the U.S. indicate that some elephants with apparent active disease have been treated with short courses using single anti-mycobacterial drug regimens at doses that would be considered ineffective to achieve therapeutic serum levels creating the potential for drug resistance.

The pathogenesis of human TB has been studied for many centuries with the introduction of drug treatment in the 1940's. In contrast, TB in elephants has been studied for only 14 years with limited, poorly funded research and reluctance to publish and/or share data.

While inter-species transmission of TB between elephants and humans has been described,[1] and public health evaluations have documented a risk for human exposure from infected elephants,[1,5,6] the risk to animal handlers or to the general public of acquiring TB from non-humans is incompletely understood.

## 2. History and current status of tuberculosis in elephants

Descriptions of a disease in elephants resembling TB were reported by Ayurvedic physicians in Asia over 2000 years ago.[7,8] Based on characteristic skeletal lesions a TB pandemic has been implicated as a causative factor in the extinction of the mastodon (*Mammut americanum*).[9] Although case reports appeared in the 1800s[10] and the early 1900s,[11] TB "emerged" in elephants in 1996 with the death of two circus elephants.[12] Notably, two cases from this herd were reported in 1983[13] and 1994.[12]

* Corresponding author. Section of Infectious Diseases, VA Medical Center, 3800 Woodland Ave., Philadelphia, PA 19104, United States. Tel.: +1 215 823 4307; fax: +1 215 823 5171.
*E-mail address:* joel.maslow@va.gov (J.N. Maslow).
[d] The authors contributed equally to this study.

1472-9792/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.tube.2011.02.007

TB was subsequently identified in five additional elephant herds[12] and prompted a collaborative effort by the United States Department of Agriculture (USDA), the American Association of Zoo Veterinarians (AAZV), zoos, circuses, and experts representing the veterinary and human healthcare communities to develop the Guidelines for the Control of Tuberculosis in Elephants that were first published in 1997. The Guidelines, which recommend diagnostic methods and treatment protocols, were revised in 2000, 2003, 2008, and 2010[14] as new information became available.

Between 1994 and November 2010, TB was confirmed by culture in 50 U.S. elephants. *Mycobacterium tuberculosis* was isolated from 46 Asian elephants (*Elephas maximus*) and 3 African elephants (*Loxodonta africana*) and *Mycobacterium bovis* from 1 African elephant. Thirty-one cases were diagnosed antemortem and 19 post-mortem, most lacking clinical signs consistent with TB.[14] Among the current population of 246 Asian elephants in the U.S. the approximate prevalence is 18% versus 2% among the 204 African elephants. As culture has poor sensitivity, the true prevalence may be higher. *Mycobacterium avium* and a variety of non-tuberculous mycobacteria are frequent isolates[15] but have not been associated with pathology with the exception of two cases of *Mycobacterium szulgai* in African elephants.[16]

Epidemiologic and outbreak investigations of TB in elephants in the U.S. is challenged by movement of elephants between facilities and changes in ownership. Additionally, reluctance of private owners to provide information regarding heritage and movements and privacy concerns relating to human caretakers complicates contact tracing.

Evaluation of elephants for TB worldwide has also begun. In Sweden TB was confirmed by culture in 5 elephants post-mortem.[17,18] Other European countries have initiated testing campaigns, although prevalence rates have not yet been reported. Surveillance in Asia began in 2006 when Elephant Care International (www.elephantcare.org), initiated a surveillance program in Nepal. Of 211 elephants screened (90% of the known captive population), greater than 20% were seroreactive (Mikota, unpublished). A survey conducted in India found that 15% of 387 temple, government, and privately owned elephants were seroreactive by the commercially available Elephant TB Stat-Pak® assay (ChemBio, Medford, NY) detailed below.[19] Elephants housed at religious temples, the group with the greatest human contact, had the highest rate of seroreactivity (25%) versus 12–15% in other groups.[19] And this year, 4 cases of culture confirmed disease in Thai elephants were reported.[20] The sensitivity and specificity of serodiagnosis for TB in elephants are discussed below. Culture provides a lower limit of detection but likely underestimates disease burden in this species of animal.

No formal studies to delineate the exposure risks for elephants have been performed. An unproven supposition is that index infections occur due to prolonged close contact with an infected human. Transmission between elephants with close contact has been documented by molecular typing.[12,15]

## 3. Clinical disease and diagnosis in elephants

TB in elephants may present as a chronic wasting disease with weight loss, exercise intolerance, and occasionally coughing or abnormal discharges. Frequently, clinical signs are lacking until the disease is quite advanced.[12] *M. tuberculosis* has been isolated premortem from respiratory secretions, feces, and vaginal discharges. On post-mortem, some elephants have significant abscess formation and casseation of the lungs, thoracic and abdominal lymph nodes, and liver. Other cases have been diagnosed incidentally at necropsy by identification and culture of small, focal granulomas.[11,12] Chest radiographs are impossible in adult elephants and the intradermal tuberculin test has proven to be unreliable as a screening test.[12,17] Culture has served as the "gold-standard" for diagnosis. Nucleic acid amplification to detect mycobacterial DNA in primary specimens obtained by trunk wash has comparable diagnostic capability as for humans.[15] The problem of PCR inhibitors due to contamination with organic material and soil may be minimized by use of modifications using common laboratory and commercially available specimen decontamination systems.[21]

The trunk wash has been devised to collect samples from elephants for culture. Sterile saline is instilled into the trunk, the trunk is elevated, and the sample is collected into a sterile plastic bag as the elephant forcibly exhales.[22,23] The behaviors necessary for the trunk wash require training and can be dangerous in certain elephants. If the elephant fails to forcibly exhale, only the distal trunk is sampled rather than the respiratory tract. Bacterial and fungal sample contamination is common because elephants use their trunk for a variety of functions. Moreover, elephants shed organisms intermittently as exemplified by the Swedish experience where only 7 of 189 trunk wash samples collected from five infected elephants yielded *M. tuberculosis*.[18] Similar results were experienced by investigators in Thailand where only 2 of 60 trunk wash cultures were positive in four infected elephants.[20]

Other techniques including an experimental ELISA assay[24] and a formerly commercially available Blood TB Test[12] that combines serologic detection and lymphocyte transformation in response to purified protein derivative (PPD)-A derived from *M. tuberculosis* and PPD-B derived from *M. bovis*[25] have been studied.[26] A commercial assay based on serologic detection of pooled *M. tuberculosis* complex antigens as a screening assay (TB Rapid Test or ElephantTB STAT PAK® assay, ChemBio Inc., Medford, NY) with a confirmatory antigen-specific multi-antigen print immunoassay (MAPIA®, ChemBio) has been shown to be accurate and reproducible for elephants.[27,28] The Stat Pak® assay is licensed by the United States Department of Agriculture (USDA) as a screening test of TB in elephants. The sensitivity and specificity of the STAT PAK® to diagnose *M. tuberculosis* complex infection is 100% and 95%, respectively.[29] Sequential application of the confirmatory assay, increases the accuracy to approximately 100%.[27,29] This assay has identified infected elephants 8 years prior to diagnosis by culture[18,27] and was useful as a screening tool for outbreaks in Sweden and in Thailand.[17,18,20] Thus, the assay may have utility to detect latent infection. Moreover, the finding that treatment of culture-positive elephants yielded a decline in antigenic reactivity suggested that the assay may have utility to monitor therapy.[27]

## 4. Treatment

Treatment recommendations were modeled on regimens from the American Thoracic Society with the assumption that drug acceptance may be erratic, pharmacokinetics could differ for elephants, and that disease might be more difficult to eradicate in elephants. At the time that the first treatment protocols were published in 1997, these issues were still unresolved. Whereas there was consensus regarding the treatment of animals that were actively shedding tubercle bacilli, the same was not the case for exposed elephants. Further, a key untested assumption was that treatment was curative.

Elephants with active disease receive 3 drugs for 2 months followed by 2 drugs for 10 months. Isoniazid (INH) and rifampin (RIF) were considered the 2 key drugs with either pyrazinamide (PZA) or ethambutol (EMB) as the third drug. A 12-month treatment course was chosen due to uncertainties regarding the extent of disease and treatment requirements in elephants. Efficacy was determined by ascertainment of serum drug levels. Due to concerns for toxicity, targets were set as the lower of the human therapeutic

ranges.[30] Because oral dosing was poorly tolerated, rectal administration was explored.

Pharmacokinetic trials were conducted to determine optimal dosing regimens and routes. Since dosing was conducted as part of actual treatment regimens, pharmacokinetic (PK) data was analyzed from composite dosing trials. The results of PK studies in elephants was published for four of the primary anti-tuberculous drugs INH, RIF, EMB, and PZA.[31–34] Additionally, single dose trials were performed in bongo antelope for amikacin (AMK), INH and EMB[35] that provides comparative data for another large mammal, albeit a ruminant with different gastrointestinal and drug absorption physiology. Prior PK studies from bongo antelope suggested that dosing anti-tuberculous drugs obeys allometric scaling, a zoologic concept that the dose divided by the log-mass of the animal is a constant for select drugs.[36] However, data from elephants suggests linear scaling of dosing is more appropriate. INH, PZA, and EMB were well absorbed rectally with the maximal serum concentration (Cmax), area under the curve (AUC), and elimination parameters similar to that for oral dosing[31,32,34] whereas RIF was absorbed poorly via the rectum[33] presumably due to its higher lipophilic nature. Moreover, PK data in elephants indicated absorption and elimination characteristics similar to humans. Recent unpublished studies have demonstrated that INH absorption via the rectum may actually be as rapid as 7.5–15 min (Maslow and Mikota unpublished data). While prior publications reported Cmax at 1 or 2 h, these times represented the first blood draw.

Other considerations that may affect PK studies and dose relationships relate to vehicles used to administer drug to elephants and to the necessity of obtaining specimens in the field. INH when provided as a suspension is particularly volatile in food especially in acidic vehicles such as colas or other foods with a low pH.[37] Additionally, INH quickly degenerates after blood draw necessitating samples be maintained on ice and then rapidly processed and frozen.

## 5. Drug resistance

There have been two elephants reported with drug resistant TB.[27] One elephant was diagnosed with pan-susceptible infection from positive cultures obtained via trunk wash and from vaginal discharge. Despite 10 months of two drug treatment with INH and PZA administered rectally followed by an additional 10 months of three-drug treatment with INH, PZA, and RIF the animal developed recurrent culture positive vaginal discharge with MDR-TB a year after treatment was completed ([27] and G Dumonceaux, personal communication). The second animal is stated to have developed recurrent pulmonary infection with a RIF-resistant strain following a treatment course with INH and PZA, i.e. there was no documented exposure to RIF.

The efficacy of treatment is unknown. Although treated animals are required to undergo quarterly trunk-wash evaluations, there is no central repository for results. Also there is limited post-mortem data with no requirement for reporting. As noted above, the observation that treated animals manifest loss of seroreactivity to a combined antigen panel[27] may be useful.

Recurrent infection has been documented in at least 4 cases. Two cases were cited above; a third case has also been published.[27] A fourth case of recurrent infection occurred in an animal that had achieved target serum levels with 2 drugs.[12] The latter case was considered to arise from a peri-bronchial lymph node that eroded into the respiratory tree; re-treatment was apparently successful without second recurrence. In contrast, for some herds that achieved subtherapeutic levels, attack rates of recurrent infection in other herd members have approached 50% (S Mikota, unpublished data). Fortunately, the latter have developed recurrent infection with susceptible strains.

## 6. Zoonotic implications

Elephants can spray many feet and often place their trunks inside the mouths of other elephants presenting risks for both zoonotic and animal-to-animal transmission. Michalak et al. reported on the investigation of the animal handlers at a facility with three known active cases.[1] Of 22 animal handlers tested, 11 had reactions to intradermal PPD from *M. tuberculosis*; 3 were PPD converters, including one individual without direct involvement in elephant care. The other 8 reactive individuals had either unknown prior PPD status or were previously PPD-positive. One elephant handler had a chest radiograph suggestive of active tuberculosis and sputum was culture positive for *M. tuberculosis* that had an IS6100 restriction fragment length polymorphism (RFLP) pattern matching the elephants and confirming inter-species transmission of infection. And while the route of infection was presumed to be elephant to human, the index case was not known.

A subsequent paper reported on an outbreak investigation at the Los Angeles Zoo following the identification of *M. tuberculosis* in 2 Asian elephants, 3 Rocky Mountain goats, and a black rhinoceros.[6] IS6110 RFLP typing demonstrated clonal spread of infection. Of 307 individuals screened by skin testing, 55 (18%) were reactive at baseline and 15 (5%) demonstrated PPD conversion for whom risk for conversion included elephant training, attendees at the first elephant necropsy, and groundskeepers. A third outbreak investigation was conducted of employees at an elephant refuge following the identification of active infection in an elephant.[38] Nine employees demonstrated PPD conversion including 8 of 13 quarantine area workers of whom 3 were administrators who did not have direct elephant contact.[38]

A fourth outbreak investigation involved the potential for transmission of *M. bovis* in captivity, albeit not in elephants. Necropsy of a rhinoceros, with unsuspected *M. bovis* infection resulted in multiple PPD conversions[39] and resulted in the infection of non-human primates housed near the rhino barn and was the likely source of infection in a bongo antelope diagnosed years later.[35] Zoonotic transmission of *M. bovis* is well described among abattoir workers and was a cause of gastrointestinal TB from ingestion of infected milk or meat. Deer and wild animals such as badgers[40] continue as a reservoir of under-appreciated infection.

## 7. Conclusion

Tuberculosis in elephants and other wildlife poses the potential for animal and human disease. Collaborative efforts began in 1996 among regulatory bodies, animal and human medical providers, and the zoological and circus communities to identify sources of infection, develop and evaluate potential diagnostic tests, and share treatment information. These efforts represent a beginning to understand this disease in animals beyond commercially used hoofstock. Without a concerted effort among the diverse stakeholders, TB will continue to affect exotic animals posing the risk for morbidity and death for endangered species and potential risks for dissemination of resistant strains between animals and to people.

To attain the goal of TB eradication sharing of treatment outcomes and protocols is needed. Secondly to enable this to happen is to guarantee that privacy concerns are addressed to protect facilities from backlash that would derail efforts to collect epidemiologic data and thus analyze population results. Only when treatment decisions can be based on fact rather than anecdotal experience can veterinary care move forward and the public health be promoted.

## Disclaimer

The views cited in this article are those of the authors.

**Ethical approval:** Not required.

**Funding:** None.

**Competing interests:** None declared.

## References

1. Michalak K, Austin C, Diesel S, Bacon JM, Zimmerman P, Maslow JN. *Mycobacterium tuberculosis* infection as a zoonotic disease: transmission between humans and elephants. *Emerg Infect Dis* 1998;**4**:283–7.
2. Elephant database. http://www.elephant.se/country.php?name=india; [accessed 8.02.11].
3. Cheeran J, Nair N. *Techniques & procedure for post-mortem of elephants*. URL:. New Delhi, India: Project Elephant and Central Zoo Authority, Ministry of Environment and Forests http://cheerans.com/files/postmortem.pdf; 2003.
4. Dangolla A, Silva I. The status and veterinary problems in captive elephants in Sri Lanka. http://www.aserc.org/index.php?option=com_content&task=view&id=37; [accessed 8 02 11].
5. Murphree R, Warkentin J, Dunn J, Schaffner W, Jones T. Outbreak of *Mycobacterium tuberculosis* infection among employees of an elephant refuge. In: 2010 National tuberculosis conference; June 22–24 2010.
6. Oh JY, Kim KS, Jeong YW, Cho JW, Park JC, Lee JC. Human exposure following *Mycobacterium tuberculosis* infection of multiple animal species in a metropolitan zoo. *Emerg Infect Dis* 2002;**8**(11):1290–3.
7. Iyer AK. Veterinary science in India, ancient and modern with special reference to tuberculosis. *Agric Livest India* 1937;**7**:718–24.
8. McGaughey CA. Diseases of elephants: part III. *Ceylon Vet J* 1961;**9**:94–8.
9. Rothschild B, Laub R. Hyperdisease in the late Pleistocene: validation of an early 20th century hypothesis. *Naturwissenschaften* 2006;**93**:557–64.
10. Garrod AH. Report on the Indian elephant which died in the society's gardens on July 7th, 1875. *Proc Zool Soc Lond* 1875;**1875**:542.
11. Mikota S. Tuberculosis in elephants. In: Fowler M, Miller R, editors. *Zoo and wild animal medicine, current therapy*. 6th ed. St. Louis, MO: Saunders/Elsevier; 2008. p. 355–68.
12. Mikota SK, Peddie L, Peddie J, Isaza R, Dunker F, West G, et al. Epidemiology and diagnosis of *Mycobacterium tuberculosis* in captive Asian elephants (*Elephas maximus*). *J Zoo Wildl Med* 2001;**32**:1–16.
13. Saunders G. Pulmonary *Mycobacterium tuberculosis* infection in a circus elephant. *J Am Vet Med Assoc* 1983;**183**:1311–2.
14. Miller M. Update on elephant tuberculosis guidelines; report of the scientific subcommittee for tuberculosis in elephants. In: Meeting of the United States Animal Health Association (USAHA). Minneapolis, MN; 2010.
15. Payeur JB, Jarnagin JL, Marquardt JG, Whipple DL. Mycobacterial isolations in captive elephants in the United States. *Ann N Y Acad Sci* 2002;**969**:256–8.
16. Lacasse C, Terio K, Kinsel MJ, Farina LL, Travis DA, Greenwald R, et al. Two cases of atypical mycobacteriosis caused by *Mycobacterium szulgai* associated with mortality in captive African elephants (*Loxodonta africana*). *J Zoo Wildl Med* 2007;**38**(1):101–7.
17. Lewerin SS, Olsson S-L, Eld K, Roken B, Ghebremichael S, Koivula T, et al. Outbreak of *Mycobacterium tuberculosis* infection among captive Asian elephants in a Swedish zoo. *Vet Rec* 2005;**156**:171–5.
18. Moller T, Roken B, Petersson L, Vitaud C, Lyashchenko K. Preliminary results of a new serological test for detection of TB-infection (*Mycobacterium tuberculosis*) in elephants (*Elaphas maximus* and *Loxodonta africanum*) Swedish case studies. *Verh ber Erkg Zootiere* 2005;**42**:173–81.
19. Abraham D, Cheeran JV, Sukumar R, Mikota SK, Rao S, Ganguly A, et al., 20 June 2008. (Project Elephant, Ministry of Environment and Forests, Government of India, Parayavaran Bahvan, CGO Complex, Lodhi Road). Health assessment of captive Asian elephants in India with special reference to tuberculosis.
20. Angkawanish T, Wajjwalku W, Sirimalaisuwan A, Mahasawangkul S, Kaewsakhorn T, Boonsri K, et al. *Mycobacterium tuberculosis* infection in domesticated Asian elephants, Thailand. *Emerg Infect Dis* 2010;**16**:1949–51.
21. Kay M, Linke L, Triantis J, Salman M, Larsen R. Evaluation of DNA extraction techniques for detecting *Mycobacterium tuberculosis*-complex organisms in Asian elephant trunk washes. *J Clin Microbiol*; 2011 [epub ahead of print].
22. Abraham D, Davis J. Revised trunk wash collection procedure for captive elephants in a range country setting. *Gajah* 2008;**28**:53–4.
23. Isaza R, Ketz C. A trunk wash technique for the diagnosis of tuberculosis in elephants. *Verh ber Erkg Zootiere* 1999;**29**:121–4.
24. Larsen RS, Salman MD, Mikota SK, Isaza R, Montali RJ, Triantis J. Evaluation of a multiple-antigen enzyme-linked immunosorbent assay (ELISA) for detection of *Mycobacterium tuberculosis* in captive elephants. *J Zoo Wildl Med* 2000;**31**:291–302.
25. Griffin J, Buchan G. Aetiology, pathogenesis and diagnosis of *Mycobacterium bovis* in deer. *Vet Microbiol* 1994;**40**:193–205.
26. Mikota SK, Maslow JN. Theoretical and technical aspects of diagnostic techniques for mammalian tuberculosis. In: Baer CK, editor. *Annual conference of the American association of zoo veterinarians*; 1997. p. 162–5. Houston, TX.
27. Lyashchenko KP, Greenwald R, Esfandiari J, Olsen JH, Ball R, Dumonceaux G, et al. Tuberculosis in elephants: antibody responses to defined antigens of *Mycobacterium tuberculosis*, potential for early diagnosis, and monitoring of treatment. *Clin Vaccine Immunol* 2006;**13**(7):722–32.
28. Lyashchenko KP, Singh M, Colangeli R, Gennaro ML. A multi-antigen print immunoassay for the development of serological diagnosis of infectious diseases. *J Immunol Methods* 2000;**242**:91–100.
29. Greenwald R, Lyashchenko O, Esfandiari J, Miller M, Mikota S, Olsen JH, et al. Highly accurate antibody assays for early and rapid detection of tuberculosis in African and Asian elephants. *Clin Vaccine Immunol* 2009;**16**(5):605–12.
30. Peloquin CA. Therapeutic drug monitoring in the treatment of tuberculosis. *Drugs* 2002;**62**(15):2169–83.
31. Maslow JN, Mikota SK, Zhu M, Isaza R, Peddie LR, Dunker F, et al. Pharmacokinetics of isoniazid (INH) in the treatment of *Mycobacterium tuberculosis* among Asian and African elephants (*Elephas maximus* and *Loxodonta africana*). *J Vet Pharmacol Ther* 2005;**28**:21–8.
32. Maslow JN, Mikota SK, Zhu M, Riddle H, Peloquin CA. Pharmacokinetics of ethambutol (EMB) in the treatment of *Mycobacterium tuberculosis* in Elephants. *J Vet Pharmacol Ther* 2005;**28**:321–3.
33. Peloquin CA, Maslow JN, Mikota SK, Forrest A, Dunker F, Isaza R, et al. Dose selection and pharmacokinetics of rifampin in elephants for the treatment of tuberculosis. *J Vet Pharmacol Ther* 2006;**29**:581–5.
34. Zhu M, Maslow JN, Mikota SK, Isaza R, Dunker F, Riddle H, et al. Population pharmacokinetics of pyrazinamide in elephants. *J Vet Pharmacol Ther* 2005;**28**:403–9.
35. Auclair B, Mikota SK, Peloquin CA, Aguilar R, Maslow JN. Population pharmacokinetics of antituberculous drugs and treatment of *Mycobacterium bovis* infection in bongo antelope (*Tragelaphus eurycerus isaaci*). *J Zoo Wildl Med* 2002;**33**(3):193–203.
36. Hunter R, Isaza R. Concepts and issues with interspecies scaling in zoological pharmacology. *J Zoo Wildl Med* 2008;**39**(4):517–26.
37. Peloquin CA, Durbin D, Childs J, Sterling T, Weiner M. Stability of antituberculosis drugs mixed in food. *Clin Infect Dis* 2007;**45**:521.
38. Murphree R, Warkentin J, Dunn J, Schaffner W, Jones T. Tuberculosis outbreak at Elephant refuge, Tennessee, USA, 2009. *Emerg Infect Dis*; 2011 March [Epub ahead of print].
39. Dalovisio JR, Stetter M, Mikota-Wells S. Rhinoceros' Rhinorrhea: cause of an outbreak of infection due to airborne *Mycobacterium bovis* in zookeepers. *Clin Infect Dis* 1992;**15**:598–600.
40. Gallagher J, Clifton-Hadley R. Tuberculosis in badgers; a review of the disease and its significance for other animals. *Res Vet Sci.* 2000;**69**:203–17.

**Susan K. Mikota** D.V.M. is the Director of Veterinary Programs and Research for Elephant Care International (ECI) and heads ECI's Elephant TB Initiative. She is a member of the Elephant TB Subcommittee of the United States Animal Health Association and the Asian Elephant Specialist Group of the IUCN. She works in the USA and Asia. Her research interests include TB and other infectious diseases of elephants.

**Joel Maslow** M.D. Ph.D. M.B.A. is the Associate Chief of Research at the Philadelphia VA Medical Center and the Associate Dean for Research and Professor of Medicine at the University of Pennsylvania. Dr. Maslow is an infectious diseases physician with specialty in mycobacterial diseases, HIV, and research in both the pathogenesis of mycobacterial disease and molecular epidemiology. Dr. Maslow has served on the TB advisory board of Elephant Care International and has been a consultant to zoos, circuses, the USDA, OSHA, and other organizations on mycobacterial disease in animals since 1996.

# Elephant Tuberculosis References (By date; most recent first)
Elephant Care International Database
www.elephantcare.org
Accessed May 2022

Szydlowski, M. (2022). "Elephants in Nepal: Correlating disease, tourism, and welfare." Journal of Applied Animal Welfare Science.

Asian elephants and humans have long shared their lives, but recent changes in human perspectives on animal use have created ripples through the small country of Nepal. Captive elephants are caught in the crossfire between local communities, elephant owners, mahouts, and NGOs in debates over their treatment, health, welfare and use in tourism. In addition, zoonotic disease, natural disasters and political strife affect the lives of captive elephants and mahouts. For example, during the COVID-19 pandemic, elephants, caregivers and owners found themselves facing income loss, decreased welfare from housing and husbandry issues, and food shortages. Many owners sold elephants, fired mahouts, and “quit” the tourism industry. Others sought help from outside organizations, community members, and governmental agencies to retain ownership of what they viewed as valuable commodities. NGOs and grassroots organizations assisted in the hopes of keeping elephants in Nepal, thus preventing them from long, treacherous walks across the border and into situations where they might face further welfare decreases. This article combines elephant stable visits and interviews with mahouts, owners, NGO, and government staff between January 2019 and December 2021. It highlights the ongoing health and welfare challenges faced by elephants and mahouts in Nepal. 

Shah, Y., S. Paudel, K. Pandey, G. P. Gupta, E. S. Solo, J. Joshi, D. K. Pant and B. D. Pandey (2022). "Insights into transmission dynamics of Mycobacterium tuberculosis complex in Nepal." Tropical Medicine and Health 50(1).

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis complex (MTBC) in humans and animals. Numbers of multi drug resistance TB (MDR-TB), extrapulmonary TB (EPTB) and zoonotic TB cases are increasingly being reported every year in Nepal posing a major public health problem. Therefore, the Government of Nepal should act immediately to strengthen the screening facilities across the country to be able to identify and treat the TB infected patients as well as detect zoonotic TB in animal species. Endorsement of One Health Act by the Government of Nepal is an opportunity to initiate the joint programs for TB surveillance among human and animal species using one health approach to reduce the TB burden in Nepal. 

Ishikawa, S., Y. Ozeki, S. Suga, Y. Mukai, H. Kobayashi, E. Inouchi, S. A. Kaboso, G. Gebretsadik, D. Dewi, A. Nishiyama, Y. Tateishi, H. Takihara, S. Okuda, S. Yoshida, N. Misawa and S. Matsumoto (2022). "Monitoring IgG against Mycobacterium tuberculosis " Sci Rep 12(1): 4310.

Tuberculosis (TB) is fatal in elephants, hence protecting elephants from TB is key not only in the conservation of this endangered animal, but also to prevent TB transmission from elephants to humans. Most human TB cases arise from long-term asymptomatic infections. Significant diagnostic challenges remain in the detection of both infection and disease development from latency in elephants due to their huge bodies. In this study, we assessed cryopreserved sera collected for over

16 years, from the first Japanese treatment case of elephant TB. Semi-quantification of IgG levels to 11 proteins showed high detection levels of 3 proteins, namely ESAT6/CFP10, MPB83 and Ag85B. The level of IgG specific to these 3 antigens was measured longitudinally, revealing high and stable ESAT6/CFP10 IgG levels regardless of onset or treatment. Ag85B-specifc IgG levels were largely responsive to onset or treatment, while those of MPB83 showed intermediate responses. These results suggest that ESAT6/CFP10 is immunodominant in both asymptomatic and symptomatic phases, making it useful in the detection of infection. On the other hand, Ag85B has the potential to be a marker for the prediction of disease onset and in the evaluation of treatment effectiveness in elephants.

Goosen, W. J., L. Kleynhans, T. J. Kerr, P. D. van Helden, P. Buss, R. M. Warren and M. A. Miller (2022). "Improved detection of Mycobacterium tuberculosis and M. bovis in African wildlife samples using cationic peptide decontamination and mycobacterial culture supplementation." J Vet Diagn Invest 34(1): 61-67.

In South Africa, mycobacterial culture is regarded as the gold standard for the detection of Mycobacterium tuberculosis complex (MTBC) infection in wildlife even though it is regarded as "imperfect." We compared a novel decontamination and mycobacterial culture technique (TiKa) to the conventional mycobacterium growth indicator tube (MGIT) system using known amounts of bacilli and clinical samples from MTBC-infected African buffaloes (Syncerus caffer), white rhinoceros (Ceratotherium simum), and African elephants (Loxodonta africana). Use of the TiKa-KiC decontamination agent on samples spiked with 10,000 to 10 colony forming units (cfu) of M. bovis (SB0121) and M. tuberculosis (H37Rv) had no effect on isolate recovery in culture. In contrast, decontamination with MGIT MycoPrep resulted in no growth of M. bovis samples at concentrations < 1,000 cfu and M. tuberculosis samples < 100 cfu. Subsequently, we used the TiKa system with stored clinical samples (various lymphatic tissues) collected from wildlife and paucibacillary bronchoalveolar lavage fluid, trunk washes, and endotracheal tube washes from 3 species with known MTBC infections. Overall, MTBC recovery by culture was improved significantly ($p < 0.01$) by using TiKa compared to conventional MGIT, with 54 of 57 positive specimens versus 25 of 57 positive specimens, respectively. The TiKa mycobacterial growth system appears to significantly enhance the recovery of MTBC members from tissue and paucibacillary respiratory samples collected from African buffaloes, African elephants, and white rhinoceros. Moreover, the TiKa system may improve success of MTBC culture from various sample types previously deemed unculturable from other species.

Tollis, M., E. Ferris, M. S. Campbell, V. K. Harris, S. M. Rupp, T. M. Harrison, W. K. Kiso, D. L. Schmitt, M. M. Garner, C. A. Aktipis, C. C. Maley, A. M. Boddy, M. Yandell, C. Gregg, J. D. Schiffman and L. M. Abegglen (2021). "Elephant Genomes Reveal Accelerated Evolution in Mechanisms Underlying Disease Defenses." Mol Biol Evol 38(9): 3606-3620.

Disease susceptibility and resistance are important factors for the conservation of endangered species, including elephants. We analyzed pathology data from 26 zoos and report that Asian elephants have increased neoplasia and malignancy prevalence compared with African bush elephants. This is consistent with observed higher susceptibility to tuberculosis and elephant endotheliotropic herpesvirus (EEHV) in Asian elephants. To investigate genetic mechanisms underlying disease resistance, including differential responses between species, among other elephant traits, we sequenced multiple elephant genomes. We report a draft assembly for an Asian elephant, and defined 862 and 1,017 conserved potential regulatory elements in Asian and African bush elephants, respectively. In the genomes of both elephant species, conserved elements were significantly enriched with genes differentially expressed between the species. In Asian elephants, these putative regulatory regions were involved in immunity pathways including tumor-necrosis factor, which plays an important

role in EEHV response. Genomic sequences of African bush, forest, and Asian elephant genomes revealed extensive sequence conservation at TP53 retrogene loci across three species, which may be related to TP53 functionality in elephant cancer resistance. Positive selection scans revealed outlier genes related to additional elephant traits. Our study suggests that gene regulation plays an important role in the differential inflammatory response of Asian and African elephants, leading to increased infectious disease and cancer susceptibility in Asian elephants. These genomic discoveries can inform future functional and translational studies aimed at identifying effective treatment approaches for ill elephants, which may improve conservation.

Suga, S., Y. Mukai, S. Ishikawa, S. Yoshida, S. Paudel and T. Wada (2021). "Intensive treatment of a captive bornean elephant (elephas maximus borneensis) infected with mycobacterium caprae in Japan." Journal of Zoo and Wildlife Medicine 51(4): 1062-1066.

In 2015, an estimated 17-year-old female Bornean elephant (Elephas maximus borneensis) at Fukuyama Zoo in Japan exhibited anorexia and significant weight loss. Pan-susceptible Mycobacterium tuberculosis complex (MTBC) was isolated from vaginal discharge, oral mucus, urine, and fecal samples by culture. The isolate was identified as Mycobacterium caprae by genetic analysis. Isoniazid, pyrazinamide, and levofloxacin were administered rectally. Body weight increased to normal, but subsequently decreased again. Elevation of liver enzymes occurred, likely related to the increase in isoniazid dosage. After recovery from side effects, the elephant's weight increased further. However, isoniazid-resistant M. caprae was isolated from oral mucus after anti-tuberculosis drug treatment for 9 mo. The regimen was changed to rifampicin, pyrazinamide, ethambutol, and levofloxacin, administered orally or rectally. The 18-mo treatment was completed in October 2018. This elephant has shown no clinical sign since. No MTBC-positive sample had been obtained as of March 2020. 

Shah, Y. and S. Paudel (2021). "Protect elephants from tuberculosis." Science 374(6569): 832-833.

Sahoo, N., S. K. Sahu, A. K. Das, D. Mohapatra, S. K. Panda, S. K. Gupta, B. K. Behera, A. Pahari and M. Dash (2021). "ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS HEMORRHAGIC DISEASE OUTBREAK IN AN INDIAN ZOO." J Zoo Wildl Med 52(4): 1286-1297.

Elephant endotheliotropic herpesvirus hemorrhagic disease (EEHV HD) is an acute viral infection of growing Asian elephants (Elephas maximus). Four apparently healthy subadult Asian elephants aged between 6 and 10 yr at Nandankanan Zoological Park (NKZP), India, died of EEHV HD during August-September 2019. All four elephants were rescued from different reserved forests of Odisha state at less than 1 yr of age and hand reared in the NKZP. Elephants exhibited the clinical signs of lethargy, head swelling, fever, loss of appetite, abdominal distension, scant urination and defecation, signs of colic, lameness, trunk discharge, cyanosis/ulceration of tongue, erratic behavior, and recumbence before death. Period of illness varied between 28 and 42 h. Thrombocytopenia was the common significant hematological observation. No significant biochemical alterations were recorded except for higher creatinine concentrations. Analysis of blood samples in RT-PCR assay using two different sets of primers and probes that targeted terminase gene and major DNA-binding protein gene followed by cPCR and sequencing was positive for EEHV-1A in all four animals. Postmortem examination of all four carcasses showed hemorrhages in internal organs, including the hard palate, heart, lungs, stomach, mesenteric lymph nodes, mesentery, colon serosa, spleen, liver, kidney, and meninges. Histopathology showed congestion and/or hemorrhages in heart, lung, brain, kidney, and liver. There was presence of intranuclear inclusion bodies in the sinusoidal epithelial cells. The outbreak of EEHV HD that resulted in the acute death of four juvenile captive Asian elephants within <30 d, the first of its kind documented in India, is increasing the fear of similar outbreaks in the future.

Rajhans, U., G. Wankhede, B. Ambore, S. Chaudhari, N. Nighot, V. Dhaygude and C. Sonekar (2021). "Sero-diagnosis of Tuberculosis in Elephants in Maharashtra, India." Journal of Threatened Taxa 13(7): 18713-18718.

Tuberculosis is a highly contagious zoonotic disease caused by Mycobacterium spp. A study was conducted to detect the presence of Mycobacterium in captive elephants. A total of 15 captive elephants were screened from various regions in Maharashtra. The blood and serum samples collected were subjected to rapid test kit, BacT/ALERT 3D system, Ziehl-Neelsen (ZN) staining and PCR. All the samples were found seronegative using rapid test kit and whole blood PCR. Whereas, all samples were signalled culture positive in BacT/ ALERT 3D system which were further subjected to PCR, only one amplicon was produced of 176bp of RD4 gene (Mycobacterium bovis) and no acid-fast organism was detected upon ZN. Due to the atypical nature of this organism, diagnosis of this disease in elephants using various tests is complicated unlike the diagnostic tests that are validated in domestic animals. Therefore, many tests have sub-optimal sensitivity and specificity in elephants. As TB is a zoonotic disease, transmission can occur between human-livestock-elephants interface. Therefore, the zoos and state forest authority should inculcate a protocol of periodic TB screening for Mahouts and elephants in captivity along with protocol of elephant-visitor interaction, thus helping in conservation of this endangered species in India. 

Paudel, S., E. P. Brenner, S. A. Hadi, Y. Suzuki, C. Nakajima, T. Tsubota, K. P. Gairhe, B. Maharjan and S. Sreevatsan (2021). "Genome Sequences of Two Mycobacterium tuberculosis Isolates from Asian Elephants in Nepal." Microbiol Resour Announc 10(36): e0061421.

This report describes the genome sequences of two Mycobacterium tuberculosis isolates, S1 and S3, recovered from Asian elephants in Nepal. These genome sequences will enhance our understanding of the genomic epidemiology of Mycobacterium tuberculosis in Asian elephants.

Miller, M. A., T. J. Kerr, C. R. de Waal, W. J. Goosen, E. M. Streicher, G. Hausler, L. Rossouw, T. Manamela, L. van Schalkwyk, L. Kleynhans, R. Warren, P. van Helden and P. E. Buss (2021). "Mycobacterium bovis Infection in Free-Ranging African Elephants." Emerg Infect Dis 27(3): 990-992.

Mycobacterium bovis infection in wildlife species occurs worldwide. However, few cases of M. bovis infection in captive elephants have been reported. We describe 2 incidental cases of bovine tuberculosis in free-ranging African elephants (Loxodonta africana) from a tuberculosis-endemic national park in South Africa and the epidemiologic implications of these infections.

Lekko, Y. M., A. Che-Amat, P. T. Ooi, S. Omar, D. T. Mohd-Hamdan, L. S. Linazah, Z. Zakaria, S. Z. Ramanoon, M. Mazlan, F. F. A. Jesse, M. F. A. Abdul-Razak, S. Jasni and N. Abdul-Hamid (2021). "Detection of Mycobacterium tuberculosis complex antibodies in free-ranged wild boar and wild macaques in selected districts in Selangor and reevaluation of tuberculosis serodetection in captive Asian elephants in Pahang, Peninsular Malaysia." J Vet Med Sci 83(11): 1702-1707.

Tuberculosis (TB) is a chronic inflammatory and zoonotic disease caused by Mycobacterium tuberculosis complex (MTBC) members, affecting several domestic animals, wildlife species and humans. The preliminary investigation was aimed to detect antibody against MTBC among indigenous wildlife which are free-ranged wild boar, free-ranged wild macaques and captive Asian elephants in selected areas of Selangor and elephant conservation centre in Pahang, respectively. The results indicate that MTBC serodetection rate in wild boar was 16.7% (7.3-33.5 at 95% confidence interval (CI)) using an in-house ELISA bPPD IgG and 10% (3.5-25.6 at 95% CI) by DPP(®)VetTB assay, while the wild

macaques and Asian elephant were seronegative. The univariate analysis indicates no statistically significant difference in risk factors for sex and age of wild boar but there was a significant positive correlation (P<0.05) between bovine TB in dairy cattle and wild boar seropositivity in the Sepang district.

Kock, R., A. L. Michel, D. Yeboah-Manu, E. I. Azhar, J. B. Torrelles, S. I. Cadmus, L. Brunton, J. M. Chakaya, B. Marais, L. Mboera, Z. Rahim, N. Haider and A. Zumla (2021). "Zoonotic Tuberculosis – The Changing Landscape." International Journal of Infectious Diseases 113: S68-S72.

Despite slow reductions in the annual burden of active human tuberculosis (TB) cases, zoonotic TB (zTB) remains a poorly monitored and an important unaddressed global problem. There is a higher incidence in some regions and countries, especially where close association exists between growing numbers of cattle (the major source of Mycobacterium bovis) and people, many suffering from poverty, and where dairy products are consumed unpasteurised. More attention needs to be focused on possible increased zTB incidence resulting from growth in dairy production globally and increased demand in low income countries in particular. Evidence of new zoonotic mycobacterial strains in South Asia and Africa (e.g. M. orygis), warrants urgent assessment of prevalence, potential drivers and risk in order to develop appropriate interventions. Control of M. bovis infection in cattle through detect and cull policies remain the mainstay of reducing zTB risk, whilst in certain circumstances animal vaccination is proving beneficial. New point of care diagnostics will help to detect animal infections and human cases. Given the high burden of human tuberculosis (caused by M. tuberculosis) in endemic areas, animals are affected by reverse zoonosis, including multi-drug resistant strains. This, may create drug resistant reservoirs of infection in animals. Like COVID-19, zTB is evolving in an ever-changing global landscape. © 2021 The Author(s)

Jia, P., S. Dai, T. Wu and S. Yang (2021). "New Approaches to Anticipate the Risk of Reverse Zoonosis." Trends in Ecology and Evolution 36(7): 580-590.

The coronavirus disease 2019 (COVID-19) pandemic can cause reverse zoonoses (i.e., human–animal transmission of COVID-19). It is vital to utilize up-to-date methods to improve the control, management, and prevention of reverse zoonoses. Awareness of reverse zoonoses should be raised at both individual and regional/national levels for better protection of both humans and animals. © 2021 Elsevier Ltd

Chaney, S. B., D. McAloose, R. Greenwald, K. P. Lyashchenko and P. P. Calle (2021). "ASSESSMENT OF MULTIANTIGEN PRINT IMMUNOASSAY AND RAPID LATERAL-FLOW TEST FOR THE DETECTION OF MYCOBACTERIUM BOVIS INFECTION IN MALAYAN TAPIR (TAPIRUS INDICUS)." J Zoo Wildl Med 52(4): 1257-1262.

A multiantigen print immunoassay (MAPIA) and rapid test (RT) developed and validated for detection of mycobacterial antibodies in elephants (Elephas maximus and Loxodonta africana) was assessed in Malayan tapir (Tapirus indicus). Retrospective analysis of banked serum from one Mycobacterium bovis infected and seven presumably uninfected tapir was performed by MAPIA and RT. A sample collected 2 mon prior to the death of a culture-confirmed M. bovis-infected tapir served as a positive control. Seroreactivity of this sample was demonstrated via both MAPIA and RT testing. Seven uninfected animals, including four without postmortem evidence of mycobacterial disease and three that remain healthy, were negative controls; none demonstrated seroreactivity to key antigens with either test. These results suggest that MAPIA and RT have potential utility for rapid detection of M. bovis infection in Malayan tapir.

Brenner, E. P., S. A. Hadi, B. Harris, S. Robbe-Austerman and S. Sreevatsan (2021). "Genome Sequences of Mycobacterium Strains Recovered from Captive Elephants with Tuberculosis." Microbiol Resour Announc 10(36): e0067121.

Members of the Mycobacterium tuberculosis complex cause tuberculosis, infamous for enormous impacts on human health. As zoonoses, they also threaten endangered species like African/Asian elephants. We report the whole-genome sequences of Mycobacterium tuberculosis bv. tuberculosis and Mycobacterium tuberculosis bv. bovis from two zoo elephants in the United States.

Verma, R., B. M. C. Swift, W. Handley-Hartill, J. K. Lee, G. Woltmann, C. E. D. Rees and P. Haldar (2020). "A novel, high-sensitivity, bacteriophage-based assay identifies low-level mycobacterium tuberculosis bacteremia in immunocompetent patients with active and incipient tuberculosis." Clinical Infectious Diseases 70(5): 933-936.

The haematogenous dissemination of Mycobacterium tuberculosis (Mtb) is critical to the pathogenesis of progressive tuberculous infections in animal models. Using a novel, phage-based blood assay, we report the first concordant evidence in well-characterized, immunocompetent human cohorts, demonstrating associations of Mtb bacteremia with progressive phenotypes of latent infection and active pulmonary tuberculosis. © The Author(s) 2019.

Unuma, K., R. Watanabe, N. Hirayama and K. Uemura (2020). "Autopsy Identification of Viable Mycobacterium Tuberculosis in the Lungs of a Markedly Decomposed Body." Journal of Forensic Sciences 65(6): 2194-2197.

Various infectious diseases, including COVID-19, MERS, and tuberculosis, are global public health issues. Tuberculosis, which is caused by Mycobacterium tuberculosis (MTB), is highly contagious and can be transmitted through inhalation of the bacteria. However, it has been assumed that the infectiousness of bacteria and viruses in dead bodies weakens as the time from death increases. In particular, there is little awareness of infection control measures concerning decomposed bodies or even the need for such measures. The deceased, in whom we discovered MTB 3 months following her death, was a woman in her 80s who died at home. We performed judicial autopsy, because police suspected homicide when her husband hanged himself. Obtained organs were used for microscopic examination by hematoxylin–eosin staining and Ziehl–Neelsen staining. In addition, real-time PCR and mycobacterial culture testing using Ogawa's medium were performed for the detection of MTB. We found that the MTB in the decomposed body remained viable and potentially infectious. To identify the bacterial strain further, we performed DNA-DNA hybridization and identified the strain as MTB complex. Potentially infectious live MTB survived in the dead body far longer than had been previously reported. Pathologists should consider microbial culture tests for all autopsied cases in which the decedent's medical history or macro-examination suggests possible infection, even when a long duration of time has passed since death. Pathologists and specialists who perform autopsies should recognize that all dead bodies are potentially infectious, including those in which long periods have elapsed since death. © 2020 American Academy of Forensic Sciences

Swift, B. M. C., N. Meade, E. S. Barron, M. Bennett, T. Perehenic, V. Hughes, K. Stevenson and C. E. D. Rees (2020). "The development and use of Actiphage® to detect viable mycobacteria from bovine tuberculosis and Johne's disease-infected animals." Microbial Biotechnology 13(3): 738-746.

Here, we describe the development of a method that exploits bacteriophage D29 as a lysis agent for efficient DNA extraction from low numbers of mycobacterial cells. This method (Actiphage®) used in combination with PCR achieved rapid and sensitive (LOD ≤ 10 cell ml−1) detection and identification of viable, pathogenic mycobacteria in blood samples within 6 h. We demonstrate that mycobacteriophage D29 can be used to detect a range of mycobacteria from clinical blood samples

including both Mycobacterium tuberculosis complex and Mycobacterium avium subsp. paratuberculosis without the need for culture and confirms our earlier observations that a low-level bacteraemia is associated with these infections in cattle. In a study of M. bovis-infected cattle (n = 41), the sensitivity of the Actiphage® method was 95 % (95 % CI; 0.84–0.99) and specificity was 100 % (95% CI; 0.92–1). We further used Actiphage® to demonstrate viable Mycobacterium avium subsp. paratuberculosis is present in the blood of Johne's infected cattle. This method provides a revolutionary new tool for the study of infections caused by these difficult to grow pathogens. © 2019 The Authors. Microbial Biotechnology published by John Wiley & Sons Ltd and Society for Applied Microbiology.

Sookaromdee, P. and V. Wiwanitkit (2020). "Zoonotic possibility of tuberculosis from domestic elephants: a case assessment from Thailand." Egyptian Journal of Chest Diseases and Tuberculosis 69(3): 447-448.

Background Tuberculosis is an important medical problem which is at present a public health problem around the world. Zoonotic tuberculosis is a new emerging problem and has become an important issue today. The elephant tuberculosis is the specific kind of animal tuberculosis. Zoonotic tuberculosis from elephants is an interesting situation that becomes the new concern in the community where domestic elephants are common. Methods In this article, the authors specifically perform a mathematical model study to assess zoonotic possibility of tuberculosis from domestic elephants based on the available data in Thailand. Results According to this study, the prediction on the transmission rate is equal to 54.5% focusing on zoonotic transmission from domestic elephants to humans. Conclusion In this article, the authors assessed the possibility of zoonotic tuberculosis from the domestic elephant. It can be seen that there is a high chance.

Songthammanuphap, S., S. Puthong, C. Pongma, A. Buakeaw, T. Prammananan, S. Warit, W. Tipkantha, E. Kaewkhunjob, W. Yindeeyoungyeon and T. Palaga (2020). "Detection of Mycobacterium tuberculosis complex infection in Asian elephants (Elephas maximus) using an interferon gamma release assay in a captive elephant herd." Sci Rep 10(1): 14551.

Tuberculosis is highly contagious disease that can be transmitted between humans and animals. Asian elephants (Elephas maximus) in captivity live in close contact with humans in many Asian countries. In this study, we developed an interferon gamma release assay (IGRA) for elephant TB detection using antigens from the MTB complex (MTBC) and nontuberculous mycobacteria (NTM) as stimulating antigens (PPD, ESAT6, CFP10) to elicit a cell-mediated immune response (CMIR). The developed assay was applied to an elephant herd of more than 60 animals in Thailand, and the results were compared with those obtained through serological detection. IGRA has sufficient sensitivity for detecting elephant interferon gamma (eIFNγ) from specific antigen-stimulated PBMCs. Among 60 animals tested, 20 samples (33.3%) showed negative results for both MTBC and NTM infection. Eighteen samples (30%) showed positive responses against PPD from M. bovis and/or ESAT6 and CFP10, indicating MTBC infection. In contrast, only 15.6% showed seropositivity in a commercial serological test kit for elephant TB. The discrepancies between serological and CMIR highlight that the two methods may detect different stages of elephant TB. Therefore, employing both tests may enable them to complement each other in correctly identifying elephants that have been exposed to MTBC.

Ruetten, M., H. W. Steinmetz, M. Thiersch, M. Kik, L. Vaughan, S. Altamura, M. U. Muckenthaler and M. Gassmann (2020). "Iron Regulation in Elderly Asian Elephants (Elephas maximus) Chronically Infected With Mycobacterium tuberculosis." Front Vet Sci 7: 596379.

Restriction of nutrients to pathogens (nutritional immunity) is a critical innate immune response mechanism that operates when pathogens such as Mycobacterium tuberculosis have the

potential to evade humoral immunity. Tuberculosis is of growing concern for zoological collections worldwide and is well-illustrated by infections of Asian and African elephants, where tuberculosis is difficult to diagnose. Here, we investigated hematological parameters and iron deposition in liver, lung, and spleen of three Asian elephants (Elephas maximus) infected with Mycobacterium tuberculosis. For reference purposes, we analyzed tissue samples from control M. tuberculosis-negative elephants with and without evidence of inflammation and/or chronic disease. Molecular analyses of bacterial lesions of post mortally collected tissues confirmed M. tuberculosis infection in three elephants. DNA sequencing of the bacterial cultures demonstrated a single source of infection, most likely of human origin. In these elephants, we observed moderate microcytic anemia as well as liver (mild), lung (moderate) and spleen (severe) iron accumulation, the latter mainly occurring in macrophages. Macrophage iron sequestration in response to infection and inflammation is caused by inhibition of iron export via hepcidin-dependent and independent mechanisms. The hepatic mRNA levels of the iron-regulating hormone hepcidin were increased in only one control elephant suffering from chronic inflammation without mycobacterial infection. By contrast, all three tuberculosis-infected elephants showed low hepcidin mRNA levels in the liver and low serum hepcidin concentrations. In addition, hepatic ferroportin mRNA expression was high. This suggests that the hepcidin/ferroportin regulatory system aims to counteract iron restriction in splenic macrophages in M. tuberculosis infected elephants to provide iron for erythropoiesis and to limit iron availability for a pathogen that predominantly proliferates in macrophages. Tuberculosis infections appear to have lingered for more than 30 years in the three infected elephants, and decreased iron availability for mycobacterial proliferation may have forced the bacteria into a persistent, non-proliferative state. As a result, therapeutic iron substitution may not have been beneficial in these elephants, as this therapy may have enhanced progression of the infection.

Peters, H., A. Sadaula, N. Masters and A. Sainsbury (2020). "Risks from disease caused by Mycobacterium orygis as a consequence of Greater one-horned Rhinoceros (Rhinoceros unicornis) translocation in Nepal." Transboundary and Emerging Diseases 67(2): 711-723.

The greater one-horned rhinoceros (Rhinoceros unicornis) is listed as vulnerable by the IUCN Red List. Mycobacterium orygis-associated disease was identified in a single greater one-horned rhino in Chitwan National Park in February 2015 prior to a planned translocation of five greater one-horned rhinoceros from Chitwan National Park to Bardia National Park for conservation purposes. This paper describes a qualitative disease risk analysis conducted retrospectively post-translocation for Mycobacterium orygis and this translocation, with the aim to improve the understanding of disease threats to the conservation of greater one-horned rhino. The disease risk analysis method used was devised by Sainsbury & Vaughan-Higgins (Conservation Biology, 26, 2017, 442) with modifications by Bobadilla Suarez et al (EcoHealth, 14, 2017, 1) and Rideout et al (EcoHealth, 14, 2017, 42) and included the use of a scenario tree and an analysis of uncertainty as recommended by Murray et al. (Handbook on import risk analysis for animals and animal products. Volume 1. Introduction and qualitative risk analysis, 2004), and the first time this combination of methods has been used to assess the risk from disease in a conservation translocation. The scenario tree and analysis of uncertainty increased the clarity and transparency of the analysis. Rideout et al.'s (EcoHealth, 14, 2017, 42) criteria were used to assess the source hazard and may be useful in comparative assessment of source hazards for future conservation translocations. The likelihood of release into the destination site of Mycobacterium orygis as a source hazard was estimated as of low risk, the risk of exposure of populations at the destination was of high risk and the likelihood of biological and environmental consequences was low. Overall, the risk from disease associated with Mycobacterium orygis as a result of this translocation was found to be low. Recommendations on disease risk management strategies could be improved with a better

understanding of the epidemiology including the presence/absence of Mycobacterium orygis in greater one-horned rhino to develop effective disease risk management strategies.

Paudel, S. and S. Sreevatsan (2020). "Tuberculosis in elephants: Origins and evidence of interspecies transmission." Tuberculosis 123.

Tuberculosis (TB) is a devastating disease in elephants caused by either Mycobacterium tuberculosis or M. bovis. It is an ancient disease, and TB in elephants was first reported over two millennia ago in Sri Lanka. Outbreaks of TB worldwide, in captive and free-ranging elephant populations, have been recorded. Interspecies transmission of TB among elephants and humans has been confirmed in several geographic localities using spoligotyping, MIRU-VNTR analysis, and/or comparative genomics. Active surveillance of TB in wild and captive elephants and their handlers is necessary to prevent TB transmission at the elephant-human interface and to aid in the conservation of Asian and African elephants. In this review, we present an overview of diagnosis, reports of TB outbreaks in the past 25 years, TB in wild elephants, its transmission, and possible prevention and control strategies that can be applied at the elephant-human interface. © 2020

Motlatso, H. T. and R. M. Mogano (2020). "Utility of xpert® MTB/RIF ultra assay in the rapid diagnosis of bovine T tuberculosis in wildlife and livestock animals from South Africa." Prev Vet Med 177.

Goosen, W. J., T. J. Kerr, L. Kleynhans, R. M. Warren, P. D. van Helden, D. H. Persing, S. D. C. Parsons, P. Buss and M. A. Miller (2020). "The Xpert MTB/RIF Ultra assay detects Mycobacterium tuberculosis complex DNA in white rhinoceros (Ceratotherium simum) and African elephants (Loxodonta africana)." Sci Rep 10(1): 14482.

The study describes the novel use of the Xpert MTB/RIF Ultra assay for detection of Mycobacterium tuberculosis complex (MTBC) DNA in samples from white rhinoceros (Ceratotherium simum) and African elephants (Loxodonta africana). Culture negative respiratory sample matrices were spiked to determine if the Ultra could detect MTBC DNA in rhinoceros and elephant samples. Rhinoceros bronchial alveolar lavage fluid (BALF) was found to have an inhibitory effect on the Ultra. In this study, the limit of detection (LOD) of M. tuberculosis H37Rv in all spiked animal samples were 2 CFU/ml compared to 15.6 CFU/ml for humans, while the LOD for M. bovis SB0121 was 30 CFU/ml compared to 143.4 CFU/ml for M. bovis BCG in humans. Screening was performed on stored tissue and respiratory samples from known MTBC-infected animals and MTBC DNA was detected in 92% of samples collected from six rhinoceros and two elephants. Conversely, 83% of culture-negative tissue and respiratory samples from uninfected animals tested negative on the Ultra. In conclusion, the Ultra assay appears to be a sensitive and rapid diagnostic test for the detection of MTBC DNA from tissue and respiratory samples collected from African elephants and rhinoceros. Furthermore, the Ultra assay could provide a new tool for the detection of MTBC in various sample types from other wildlife species.

Goosen, W. J., T. J. Kerr, L. Kleynhans, P. Buss, D. Cooper, R. M. Warren, P. D. van Helden, B. Schröder, S. D. C. Parsons and M. A. Miller (2020). "The VetMAX™ M. tuberculosis complex PCR kit detects MTBC DNA in antemortem and postmortem samples from white rhinoceros (Ceratotherium simum), African elephants (Loxodonta africana) and African buffaloes (Syncerus caffer)." BMC Vet Res 16(1): 220.

BACKGROUND: Bovine tuberculosis and tuberculosis are chronic infectious diseases caused by the Mycobacterium tuberculosis complex members, Mycobacterium bovis and Mycobacterium tuberculosis, respectively. Infection with M. bovis and M. tuberculosis have significant implications for wildlife species management, public health, veterinary disease control, and conservation endeavours. RESULTS: Here we describe the first use of the VetMAX™ Mycobacterium tuberculosis complex (MTBC) DNA quantitative real-time polymerase chain reaction (qPCR) detection kit for African wildlife samples.

DNA was extracted from tissues harvested from 48 African buffaloes and MTBC DNA was detected (test-positive) in all 26 M. bovis culture-confirmed animals with an additional 12 PCR-positive results in culture-negative buffaloes (originating from an exposed population). Of six MTBC-infected African rhinoceros tested, MTBC DNA was detected in antemortem and postmortem samples from five animals. The PCR was also able to detect MTBC DNA in samples from two African elephants confirmed to have M. bovis and M. tuberculosis infections (one each). Culture-confirmed uninfected rhinoceros and elephants' samples tested negative in the PCR assay. CONCLUSIONS: These results suggest this new detection kit is a sensitive screening test for the detection of MTBC-infected African buffaloes, African elephants and white rhinoceros.

Budvytiene, I. and N. Banaei (2020). "Simple processing of formalin-fixed paraffin-embedded tissue for accurate testing with the xpert MTB/RIF assay." Journal of Clinical Microbiology 58(3).

Rosen, L. E., F. Olea-Popelka, S. L. Deem, R. Isaza, D. Schmitt and M. Miller (2019). "SURVEY OF ANTITUBERCULOSIS DRUG ADMINISTRATION AND ADVERSE EFFECTS IN ELEPHANTS IN NORTH AMERICA." J Zoo Wildl Med 50(1): 23-32.

Tuberculosis, caused by Mycobacterium tuberculosis, is a disease causing morbidity and mortality in captive elephants (Elephas maximus and Loxodonta africana) as well as free-ranging individuals. Elephants in North America diagnosed with tuberculosis are often treated with antituberculosis drugs, unlike livestock species, which has necessitated the development of treatment guidelines adapted from recommendations for humans. There are few published reports describing empirical treatment, which may be complicated by poor patient compliance, interruptions in drug administration, and adverse effects. A survey of elephants in North America was conducted to compile information on treatment protocols, including drugs, dosages, routes of administration, serum drug concentrations, and adverse effects of antituberculosis treatment. Responses were received regarding 182 elephants, 12 of which were treated prophylactically or therapeutically with antituberculosis drugs. Treatment protocols varied among elephants, and included various combinations of isoniazid, rifampin, pyrazinamide, ethambutol, enrofloxacin, levofloxacin, and ethionamide. Serum drug concentrations also varied considerably among and within individuals. Facility staff reported 5 elephants (out of 7 treated elephants with responses) that exhibited clinical signs that may have been associated with antituberculosis drugs or treatment procedures. Anorexia, decreased water intake, constipation, depression, ataxia, limb paresis, and tremors were among the signs observed. Most adverse effects were reported to be moderate or severe, resulting in interruption of the treatment. The results from this survey provide veterinarians and elephant managers with valuable historical data to make informed clinical management decisions regarding antituberculosis therapy in elephants.

Paudel, S., T. Tsubota and S. K. Mikota (2019). "Human TB threat to wild elephants." Nature 571(7764): 174.

Paudel, S., C. Nakajima, S. K. Mikota, K. P. Gairhe, B. Maharjan, S. Subedi, A. Poudel, M. Sashika, M. Shimozuru, Y. Suzuki and T. Tsubota (2019). "Mixed Mycobacterium tuberculosis Lineage Infection in 2 Elephants, Nepal." Emerg Infect Dis 25(5): 1031-1032.

Tuberculosis in elephants is primarily caused by Mycobacterium tuberculosis. We identified mixed M. tuberculosis lineage infection in 2 captive elephants in Nepal by using spoligotyping and large sequence polymorphism. One elephant was infected with Indo-Oceanic and East African-Indian (CAS-Delhi) lineages; the other was infected with Indo-Oceanic and East Asian (Beijing) lineages.

Paudel, S., S. K. Mikota and T. Tsubota (2019). "Tuberculosis threat in Asian elephants." Science 363(6425): 356.

Miller, M. A., P. Buss, E. O. Roos, G. Hausler, A. Dippenaar, E. Mitchell, L. van Schalkwyk, S. Robbe-Austerman, W. R. Waters, A. Sikar-Gang, K. P. Lyashchenko, S. D. C. Parsons, R. Warren and P. van Helden (2019). "Fatal Tuberculosis in a Free-Ranging African Elephant and One Health Implications of Human Pathogens in Wildlife." Front Vet Sci 6: 18.

Tuberculosis (TB) in humans is a global public health concern and the discovery of animal cases of Mycobacterium tuberculosis (Mtb) infection and disease, especially in multi-host settings, also has significant implications for public health, veterinary disease control, and conservation endeavors. This paper describes a fatal case of Mtb disease in a free-ranging African elephant (Loxodonta africana) in a high human TB burden region. Necropsy revealed extensive granulomatous pneumonia, from which Mtb was isolated and identified as a member of LAM3/F11 lineage; a common lineage found in humans in South Africa. These findings are contextualized within a framework of emerging Mtb disease in wildlife globally and highlights the importance of the One Health paradigm in addressing this anthroponotic threat to wildlife and the zoonotic implications.

Martinez, L., R. Verma, J. Croda, C. R. Horsburgh, Jr., K. S. Walter, N. Degner, K. Middelkoop, A. Koch, S. Hermans, D. F. Warner, R. Wood, F. Cobelens and J. R. Andrews (2019). "Detection, survival and infectious potential of Mycobacterium tuberculosis in the environment: a review of the evidence and epidemiological implications." The European respiratory journal 53(6).

Much remains unknown about Mycobacterium tuberculosis transmission. Seminal experimental studies from the 1950s demonstrated that airborne expulsion of droplet nuclei from an infectious tuberculosis (TB) patient is the primary route of transmission. However, these findings did not rule out other routes of M. tuberculosis transmission. We reviewed historical scientific evidence from the late 19th/early 20th century and contemporary studies investigating the presence, persistence and infectiousness of environmental M. tuberculosis We found both experimental and epidemiological evidence supporting the presence and viability of M. tuberculosis in multiple natural and built environments for months to years, presumably following contamination by a human source. Furthermore, several studies confirm M. tuberculosis viability and virulence in the environment using guinea pig and mouse models. Most of this evidence was historical; however, several recent studies have reported consistent findings of M. tuberculosis detection and viability in the environment using modern methods. Whether M. tuberculosis in environments represents an infectious threat to humans requires further investigation; this may represent an untapped source of data with which to further understand M. tuberculosis transmission. We discuss potential opportunities for harnessing these data to generate new insights into TB transmission in congregate settings. Copyright ©ERS 2019.

Lipworth, S., R. Jajou, A. De Neeling, P. Bradley, W. Van Der Hoek, G. Maphalala, M. Bonnet, E. Sanchez-Padilla, R. Diel, S. Niemann, Z. Iqbal, G. Smith, T. Peto, D. Crook, T. Walker and D. Van Soolingen (2019). "SNP-IT tool for identifying subspecies and associated lineages of Mycobacterium tuberculosis complex." Emerging Infectious Diseases 25(3): 482-488.

The clinical phenotype of zoonotic tuberculosis and its contribution to the global burden of disease are poorly understood and probably underestimated. This shortcoming is partly because of the inability of currently available laboratory and in silico tools to accurately identify all subspecies of the Mycobacterium tuberculosis complex (MTBC). We present SNPs to Identify TB (SNP-IT), a single-nucleotide polymorphism-based tool to identify all members of MTBC, including animal clades. By applying SNP-IT to a collection of clinical genomes from a UK reference laboratory, we detected an unexpectedly high number of M. orygis isolates. M. orygis is seen at a similar rate to M. bovis, yet M.

orygis cases have not been previously described in the United Kingdom. From an international perspective, it is possible that M. orygis is an underestimated zoonosis. Accurate identification will enable study of the clinical phenotype, host range, and transmission mechanisms of all subspecies of MTBCin greater detail. © 2019, Centers for Disease Control and Prevention (CDC). All rights reserved.

Kerr, T. J., C. R. de Waal, P. E. Buss, J. Hofmeyr, K. P. Lyashchenko and M. A. Miller (2019). "Seroprevalence of mycobacterium tuberculosis complex in free-ranging african elephants (Loxodonta africana) in Kruger national park, South Africa." Journal of Wildlife Diseases 55(4): 923-927.

Tuberculosis (TB) is a pathogenic disease that affects a range of wildlife species, including African elephants (Loxodonta africana). The recent discovery of fatal disease caused by infection with Mycobacterium tuberculosis in a bull elephant in the Kruger National Park (KNP), which is a bovine TB endemic area, emphasizes the importance this disease could have on both wild and captive elephant populations globally. Elephants with culture-confirmed TB have previously been shown to produce strong antibody-responses before the mycobacteria can be isolated. Therefore, we used two serologic assays that detect TB antibodies to retrospectively screen a cohort of 222 free-ranging African elephants sampled between 2004 and 2018 in KNP. The estimated TB seroprevalence for this free-roam-ing elephant population was between 6% (95% confidence interval [CI], 2–12%) and 9% (95% CI, 6–15%) based on the two tests. Overall, males had a higher TB seroprevalence than females, and adults (≤25 yr) had a higher TB seroprevalence than younger elephants (≤24 yr) on both rapid tests. The relatively high TB seroprevalence that we found highlighted the value of conducting retrospective studies in free-ranging wildlife populations in order to better understand the potential risk of disease. © Wildlife Disease Association 2019.

Yano, T., S. Premashthira, T. Dejyong, S. Tangtrongsup and M. D. Salman (2018). "The Effectiveness of a Foot and Mouth Disease Outbreak Control Programme in Thailand 2008-2015: Case Studies and Lessons Learned." Vet Sci 5(4).

Three Foot and Mouth Disease (FMD) outbreaks in northern Thailand that occurred during the implementation of the national FMD strategic plan in 2008(-)2015 are described to illustrate the lessons learned and to improve the prevention and control of future outbreaks. In 2008, during a FMD outbreak on a dairy farm, milk delivery was banned for 30 days. This was a part of movement management, a key strategy for FMD control in dairy farms in the area. In 2009, more than half the animals on a pig farm were affected by FMD. Animal quarantine and restricted animal movement played a key role in preventing the spread of FMD. In 2010, FMD infection was reported in a captive elephant. The suspected source of virus was a FMD-infected cow on the same premises. The infected elephant was moved to an elephant hospital that was located in a different province before the diagnosis was confirmed. FMD education was given to elephant veterinarians to promote FMD prevention and control strategies in this unique species. These three cases illustrate how differences in outbreak circumstances and species require the implementation of a variety of different FMD control and prevention measures. Control measures and responses should be customized in different outbreak situations.

Veerasami, M., K. Venkataraman, C. Karuppannan, A. A. Shanmugam, M. C. Prudhvi, T. Holder, P. Rathnagiri, K. Arunmozhivarman, G. D. Raj, M. Vordermeier and B. Mohana Subramanian (2018). "Point of Care Tuberculosis Sero-Diagnosis Kit for Wild Animals: Combination of Proteins for Improving the Diagnostic Sensitivity and Specificity." Indian J Microbiol 58(1): 81-92.

Tuberculosis is a significant problem globally for domestic animals as well as captive and free ranging wild life. Rapid point of care (POC) serology kits are well suited for the diagnosis of TB in wild animals. However, wild animals are invariably exposed to environmental non-pathogenic

mycobacterium species with the development of cross reacting antibodies. In the present study, POC TB diagnosis kit was developed using a combination of pathogenic Mycobacteria specific recombinant antigens and purified protein derivatives of pathogenic and non-pathogenic Mycobacteria. To benchmark the TB antibody detection kit, particularly in respect to specificity which could not be determined in wildlife due to the lack of samples from confirmed uninfected animals, we first tested well-characterized sera from 100 M. bovis infected and 100 uninfected cattle. Then we investigated the kit's performance using sera samples from wildlife, namely Sloth Bears (n = 74), Elephants (n = 9), Cervidae (n = 14), Felidae (n = 21), Cape buffalo (n = 2), Wild bear (n = 1) and Wild dog (n = 1).In cattle, a sensitivity of 81% and a specificity of 90% were obtained. The diagnostic sensitivity of the kit was 94% when the kit was tested using known TB positive sloth bear sera samples. 47.4% of the in-contact sloth bears turned seropositive using the rapid POC TB diagnostic kit. Seropositivity in other wild animals was 25% when the sera samples were tested using the kit. A point of care TB sero-diagnostic kit with the combination of proteins was developed and the kit was validated using the sera samples of wild animals.

Santos, N., T. Nunes, C. Fonseca, M. Vieira-Pinto, V. Almeida, C. Gortázar and M. Correia-Neves (2018). "Spatial analysis of wildlife tuberculosis based on a serologic survey using dried blood spots, Portugal." Emerging Infectious Diseases 24(12): 2169-2175.

We investigated the spatial epidemiology of bovine tuberculosis (TB) in wildlife in a multihost system. We surveyed bovine TB in Portugal by serologic analysis of elutes of dried blood spots obtained from hunted wild boar. We modeled spatial disease risk by using areal generalized linear mixed models with conditional autoregressive priors. Antibodies against Mycobaterium bovis were detected in 2.4% (95% CI 1.5%–3.8%) of 678 wild boar in 2 geographic clusters, and the predicted risk fits well with independent reports of M. bovis culture. Results show that elutes are an almost perfect substitute for serum (Cohen unweighted κ = 0.818), indicating that serologic tests coupled with dried blood spots are an effective strategy for large-scale bovine TB surveys, using wild boar as sentinel species. Results also show that bovine TB is an emerging wildlife disease and stress the need to prevent further geographic spread and prevalence increase. 

Riojas, M. A., K. J. McGough, C. J. Rider-Riojas, N. Rastogi and M. H. Hazbón (2018). "Phylogenomic analysis of the species of the mycobacterium tuberculosis complex demonstrates that mycobacterium africanum, mycobacterium bovis, mycobacterium caprae, mycobacterium microti and mycobacterium pinnipedii are later heterotypic synonyms of mycobacterium tuberculosis." International Journal of Systematic and Evolutionary Microbiology 68(1): 324-332.

The species within the Mycobacterium tuberculosis Complex (MTBC) have undergone numerous taxonomic and nomenclatural changes, leaving the true structure of the MTBC in doubt. We used next-generation sequencing (NGS), digital DNA–DNA hybridization (dDDH), and average nucleotide identity (ANI) to investigate the relationship between these species. The type strains of Mycobacterium africanum, Mycobacterium bovis, Mycobacterium caprae, Mycobacterium microti and Mycobacterium pinnipedii were sequenced via NGS. Pairwise dDDH and ANI comparisons between these, previously sequenced MTBC type strain genomes (including ‘Mycobacterium canettii’, ‘Mycobacterium mungi’ and ‘Mycobacterium orygis’) and M. tuberculosis H37RvT were performed. Further, all available genome sequences in GenBank for species in or putatively in the MTBC were compared to H37RvT. Pairwise results indicated that all of the type strains of the species are extremely closely related to each other (dDDH: 91.2–99.2 %, ANI: 99.21–99.92 %), greatly exceeding the respective species delineation thresholds, thus indicating that they belong to the same species. Results from the GenBank genomes indicate that all the strains examined are within the circumscription of

H37RvT (dDDH: 83.5–100 %). We, therefore, formally propose a union of the species of the MTBC as M. tuberculosis. M. africanum, M. bovis, M. caprae, M. microti and M. pinnipedii are reclassified as later heterotypic synonyms of M. tuberculosis. ‘M. canettii’, ‘M. mungi’, and ‘M. orygis’ are classified as strains of the species M. tuberculosis. We further recommend use of the infrasubspecific term ‘variant’ (‘var.’) and infrasubspecific designations that generally retain the historical nomenclature associated with the groups or otherwise convey such characteristics, e.g. M. tuberculosis var. Bovis. © 2018 by American Type Culture Collection (ATCC).

Paudel, S., S. K. Mikota, J. Thapa, K. P. Lyaschenko, K. P. Gairhe, I. P. Dhakal, N. Subedi, B. Maharjan, S. Subedi, G. E. Kaufman and T. Tsubota (2018). "Serodiagnosis of elephant tuberculosis: a useful tool for early identification of infected elephants at the captive-wild interface." European Journal of Wildlife Research 64: 70.

Tuberculosis (TB) is an emerging disease in elephants primarily caused by Mycobacterium tuberculosis (M. tb) and in some occassion by M. bovis. We performed culture and three serological tests—the Elephant TB STAT-PAK,® DPP VetTB® Assay, and MAPIA (multi-antigen print immunoassay)—prospectively on samples from eight elephants in Nepal that died of suspected or confirmed tuberculosis (TB) between 2007 and 2013. Among them, all elephants were reactive to DPP VetTB® Assay, five to Elephant TB STAT-PAK,® and two were reactive to MAPIA. Similarly, six elephants were positive on culture on samples collected antemortem or postmortem.We observed antibody responses months to years before culture confirmation of TB which shows that serological tests can be highly useful for the early diagnosis of TB in elephants. Validated point-of-care serological tests are easily performed in the field and hold promise for improved TB surveillance in other non-domestic species.

Miller, M. A., M. Finnegan, T. Storms, M. Garner and K. P. Lyashchenko (2018). "OUTBREAK OF MYCOBACTERIUM TUBERCULOSIS IN A HERD OF CAPTIVE ASIAN ELEPHANTS ( ELEPHAS MAXIMUS): ANTEMORTEM DIAGNOSIS, TREATMENT, AND LESSONS LEARNED." J Zoo Wildl Med 49(3): 748-754.

Tuberculosis (TB) was diagnosed in four Asian elephants ( Elephas maximus) in a zoo in the United States. The first case was detected by isolation of Mycobacterium tuberculosis during routine trunk wash (TW) culture testing of a herd of eight elephants. Retrospective antibody analyses revealed seroconversion 1 yr before diagnosis. Serological testing of the whole elephant herd identified two additional suspect bulls with detectable antibody, but which remained culture-negative and had no clinical signs of disease. In the following months, M. tuberculosis, identical to the isolate from the index case, was isolated from TW samples of these two elephants. A fourth elephant seroconverted nearly 4 yr after the first TB case was detected, and M. tuberculosis was isolated from a TW sample collected 1 mo later. All four infected elephants received anti-TB therapy. Two treated elephants were eventually euthanized for reasons unrelated to M. tuberculosis and found to be culture-negative on necropsy, although one of them had PCR-positive lung lesions. One infected animal had to be euthanized due to development of a drug-resistant strain of M. tuberculosis; this animal did not undergo postmortem examination due to risk of staff exposure. The fourth animal is currently on treatment. Serial serological and culture results of the other four herd mates have remained negative.

Che-Amat, A. and B. L. Ong (2018). "Wildlife Tuberculosis in Southeast Asia: A Less Known Potential Hot-Spots and Issues in Disease Surveillance and Management." Journal of Dairy and Veterinary Science 6(2: 555683.).

Barandongo, Z. R., J. K. E. Mfune and W. C. Turner (2018). "DUST-BATHING BEHAVIORS OF AFRICAN HERBIVORES AND THE POTENTIAL RISK OF INHALATIONAL ANTHRAX." J Wildl Dis 54(1): 34-44.

: Anthrax in herbivorous wildlife and livestock is generally assumed to be transmitted via ingestion or inhalation of Bacillus anthracis spores. Although recent studies have highlighted the importance of the ingestion route for anthrax transmission, little is known about the inhalational route in natural systems. Dust bathing could aerosolize soilborne pathogens such as B. anthracis, exposing dust-bathing individuals to inhalational infections. We investigated the potential role of dust bathing in the transmission of inhalational anthrax to herbivorous wildlife in Etosha National Park, Namibia, an area with endemic seasonal anthrax outbreaks. We 1) cultured soils from dust-bathing sites for the presence and concentration of B. anthracis spores, 2) monitored anthrax carcass sites, the locations with the highest B. anthracis concentrations, for evidence of dust bathing, including a site where a zebra died of anthrax on a large dust bath, and 3) characterized the ecology and seasonality of dust bathing in plains zebra ( Equus quagga), blue wildebeest ( Connochaetes taurinus), and African savanna elephant ( Loxodonta africana) using a combination of motion-sensing camera traps and direct observations. Only two out of 83 dust-bath soils were positive for B. anthracis, both with low spore concentrations (</=20 colony-forming units per gram). We also detected no evidence of dust baths occurring at anthrax carcass sites, perhaps due to carcass-induced changes in soil composition that may deter dust bathing. Finally, despite observing some seasonal variation in dust bathing, preliminary evidence suggests that the seasonality of dust bathing and anthrax mortalities are not correlated. Thus, although dust bathing creates a dramatic cloud of aerosolized soil around an individual, our microbiologic, ecologic, and behavioral results in concert demonstrate that dust bathing is highly unlikely to transmit inhalational anthrax infections.

Veerasami, M., K. Venkataraman, C. Karuppannan, A. A. Shanmugam, M. C. Prudhvi, T. Holder, P. Rathnagiri, K. Arunmozhivarman, G. D. Raj, M. Vordermeier and B. Mohana Subramanian (2017). "Point of Care Tuberculosis Sero-Diagnosis Kit for Wild Animals: Combination of Proteins for Improving the Diagnostic Sensitivity and Specificity." Indian Journal of Microbiology: 1-12.

Tuberculosis is a significant problem globally for domestic animals as well as captive and free ranging wild life. Rapid point of care (POC) serology kits are well suited for the diagnosis of TB in wild animals. However, wild animals are invariably exposed to environmental non-pathogenic mycobacterium species with the development of cross reacting antibodies. In the present study, POC TB diagnosis kit was developed using a combination of pathogenic Mycobacteria specific recombinant antigens and purified protein derivatives of pathogenic and non-pathogenic Mycobacteria. To benchmark the TB antibody detection kit, particularly in respect to specificity which could not be determined in wildlife due to the lack of samples from confirmed uninfected animals, we first tested well-characterized sera from 100 M. bovis infected and 100 uninfected cattle. Then we investigated the kit's performance using sera samples from wildlife, namely Sloth Bears (n = 74), Elephants (n = 9), Cervidae (n = 14), Felidae (n = 21), Cape buffalo (n = 2), Wild bear (n = 1) and Wild dog (n = 1).In cattle, a sensitivity of 81% and a specificity of 90% were obtained. The diagnostic sensitivity of the kit was 94% when the kit was tested using known TB positive sloth bear sera samples. 47.4% of the in-contact sloth bears turned seropositive using the rapid POC TB diagnostic kit. Seropositivity in other wild animals was 25% when the sera samples were tested using the kit. A point of care TB sero-diagnostic kit with the combination of proteins was developed and the kit was validated using the sera samples of wild animals. © 2017 Association of Microbiologists of India

Liu, C., Z. Zhao, J. Fan, C. J. Lyon, H. J. Wu, D. Nedelkov, A. M. Zelazny, K. N. Olivier, L. H. Cazares, S. M. Holland, E. A. Graviss and Y. Hu (2017). "Quantification of circulating Mycobacterium tuberculosis antigen peptides allows rapid diagnosis of active disease and treatment monitoring." Proceedings of the National Academy of Sciences of the United States of America 114(15): 3969-3974.

Tuberculosis (TB) is a major global health threat, resulting in an urgent unmet need for a rapid, non–sputum-based quantitative test to detect active Mycobacterium tuberculosis (Mtb) infections in clinically diverse populations and quickly assess Mtb treatment responses for emerging drug-resistant strains. We have identified Mtb-specific peptide fragments and developed a method to rapidly quantify their serum concentrations, using antibody-labeled and energy-focusing porous discoidal silicon nanoparticles (nanodisks) and high-throughput mass spectrometry (MS) to enhance sensitivity and specificity. NanoDisk-MS diagnosed active Mtb cases with high sensitivity and specificity in a case-control study with cohorts reflecting the complexity of clinical practice. Similar robust sensitivities were obtained for cases of culture-positive pulmonary TB (PTB; 91.3%) and extrapulmonary TB (EPTB; 92.3%), and the sensitivities obtained for culture-negative PTB (82.4%) and EPTB (75.0%) in HIV-positive patients significantly outperformed those reported for other available assays. NanoDisk-MS also exhibited high specificity (87.1–100%) in both healthy and high-risk groups. Absolute quantification of serum Mtb antigen concentration was informative in assessing responses to antimycobacterial treatment. Thus, a NanoDisk-MS assay approach could significantly improve the diagnosis and management of active TB cases, and perhaps other infectious diseases as well. 

Ghielmetti, G., M. Coscolla, M. Ruetten, U. Friedel, C. Loiseau, J. Feldmann, H. W. Steinmetz, D. Stucki and S. Gagneux (2017). "Tuberculosis in Swiss captive Asian elephants: microevolution of Mycobacterium tuberculosis characterized by multilocus variable-number tandem-repeat analysis and whole-genome sequencing." Sci Rep 7(1): 14647.

Zoonotic tuberculosis is a risk for human health, especially when animals are in close contact with humans. Mycobacterium tuberculosis was cultured from several organs, including lung tissue and gastric mucosa, of three captive elephants euthanized in a Swiss zoo. The elephants presented weight loss, weakness and exercise intolerance. Molecular characterization of the M. tuberculosis isolates by spoligotyping revealed an identical profile, suggesting a single source of infection. Multilocus variable-number of tandem-repeat analysis (MLVA) elucidated two divergent populations of bacteria and mixed infection in one elephant, suggesting either different transmission chains or prolonged infection over time. A total of eight M. tuberculosis isolates were subjected to whole-genome sequence (WGS) analysis, confirming a single source of infection and indicating the route of transmission between the three animals. Our findings also show that the methods currently used for epidemiological investigations of M. tuberculosis infections should be carefully applied on isolates from elephants. Moreover the importance of multiple sampling and analysis of within-host mycobacterial clonal populations for investigations of transmission is demonstrated.

Zlot, A., J. Vines, L. Nystrom, L. Lane, H. Behm, J. Denny, M. Finnegan, T. Hostetler, G. Matthews, T. Storms and E. DeBess (2016). "Diagnosis of Tuberculosis in Three Zoo Elephants and a Human Contact - Oregon, 2013." MMWR Morb Mortal Wkly Rep 64(52): 1398-1402.

In 2013, public health officials in Multnomah County, Oregon, started an investigation of a tuberculosis (TB) outbreak among elephants and humans at a local zoo. The investigation ultimately identified three bull elephants with active TB and 118 human contacts of the elephants. Ninety-six (81%) contacts were evaluated, and seven close contacts were found to have latent TB infection. The three bulls were isolated and treated (elephants with TB typically are not euthanized) to prevent infection of other animals and humans, and persons with latent infection were offered treatment. Improved TB screening methods for elephants are needed to prevent exposure of human contacts.

Young, L., S. Scott, M. Salfinger and E. Ramsay (2016). Serum concentrations of antimycobacterial drugs in Asian Elephants (Elephas maximus). AAZV / EAZWV / IZW Joint Conference.

Mycobacterium tuberculosis is an important disease of captive Asian elephants (Elephas maximus.) In this study six adult Asian elephants which had Mycobacterium tuberculosis cultured from trunk wash samples or had reactive DPP/MAPIA serologic responses were treated, concurrently, with one to three antimycobacterial drugs. Enrofloxacin hydrochloride, 2.5 mg/kg p.o., s.i.d., was administered to all animals in various foodstuffs for 9-15 mo. Serum enrofloxacin concentrations ranged from 230-2380 µg/ml (targeted concentrations = 125-1000 µg/ml).1 Pyrazinamide (PZA), 30 mg/kg p.o., s.i.d., was administered to five elephants in various foodstuffs for 9-12 mo. Serum PZA concentrations ranged from 26-57 µg/ml (targeted concentrations = 20- 60 µg/ml).2 Ethambutol (EMB), 30 mg/kg p.o., s.i.d., was administered to one elephant for 12 mo. A serum EMB concentration of 4.07 µg/ml was achieved (targeted concentration = 2-6 µg/ml).2 Rifampin (RIF), 10 mg/kg p.o., s.i.d., was administered to one elephant for 9 mo. A serum RIF concentration of 16 µg/ml was achieved (targeted concentration = 8-24 µg/ml). All elephants were monitored for adverse clinical effects throughout treatments. Notable side effects were limited to excess, foamy lacrimation, believed to have occurred secondary to PZA administration. Clinical chemistries and complete blood counts were monitored in all animals and values remained within reference intervals throughout treatments. This study shows antimycobacterial drug dosages may require individuation, but concurrent, long-term, multidrug regimens for the treatment of Mycobacterium tuberculosis in Asian elephants can achieve appropriate therapeutic levels with minimal detrimental side effects.

Yakubu, Y., B. L. Ong, Z. Zakaria, L. Hassan, A. R. Mutalib, Y. F. Ngeow, K. Verasahib and M. F. Razak (2016). "Evidence and potential risk factors of tuberculosis among captive Asian elephants and wildlife staff in Peninsular Malaysia." Prev Vet Med 125: 147-153.

Elephant tuberculosis (TB) caused by Mycobacterium tuberculosis is an important re-emerging zoonosis with considerable conservation and public health risk. We conducted prospective cohort and cross-sectional studies in elephants and wildlife staff respectively in order to identify potential risk factors associated with TB in captive Asian elephants and their handlers in Peninsular Malaysia. Sixty elephants in six different facilities were screened for TB longitudinally using the ElephantTB STAT-PAK and DPP VetTB assays from February 2012 to May 2014, and 149 wildlife staff were examined for tuberculosis infection using the QuantiFERON-TB Gold In-tube (QFT) assay from January to April, 2012. Information on potential risk factors associated with infection in both elephants and staff were collected using questionnaires and facility records. The overall seroprevalence of TB amongst the elephants was 23.3% (95% CI: 13.8-36.3) and the risk of seroconversion was significantly higher among elephants with assigned mahouts [p=0.022, OR=4.9 (95% CI: 1.3-18.2)]. The percentage of QFT responders among wildlife staff was 24.8% (95% CI: 18.3-32.7) and the risk of infection was observed to be significantly associated with being a zoo employee [p=0.018, OR=2.7 (95% CI: 1.2-6.3)] or elephant handler [p=0.035, OR=4.1 (95% CI: 1.1-15.5)]. These findings revealed a potential risk of TB infection in captive elephants and handlers in Malaysia, and emphasize the need for TB screening of newly acquired elephants, isolating sero-positive elephants and performing further diagnostic tests to determine their infection status, and screening elephant handlers for TB, pre- and post-employment.

Steinmetz, H. and M. Rutten (2016). TB or Not TB: Diagnosis of tuberculosis in a group of Asian elephants (Elephas maximus). AAZV /EAZWV/IZW Joint Conference, Atlanta GA.

Animal and human health is inextricably interwoven; a good example is tuberculosis (TB). Although recognized as a disease of elephants for over 20 centuries, investigations into TB's prevalence in the captive Asian elephant (Elephas maximus) population only go back 20 yr.3,4 The increasing problem of human TB combined with the susceptibility of elephants and the close contact between human and elephant, makes surveillance based on reliable early diagnosis essential.3 Although the

availability of diagnostics for clinical applications has improved in recent years, there is still a wide discrepancy between their sensitivities and specificities.1,2

In a group of 10 Asian elephants, tuberculosis was suspected from clinical observations and various clinical tests. Nevertheless, despite over 200 trunk washes being taken for analysis over a period of 14 mo, culture and RT-PCR tests for M. tuberculosis were negative. Three animals were euthanatized due to severe geriatric health problems. Pathologic examination revealed typical M. tuberculosis lesions in lung and lymph nodes. Culture and RT-PCR performed from the lesions, of postmortem collected tracheal secretions and of stomach wall tissues confirmed M. tuberculosis infection.

Based on these results, utilization of a combination of clinical signs (e.g., chronic weight loss), standard tests (e.g., comparative intradermal tuberculin test, trunk wash culture or PCR) and newer serologic tests (e.g., sero-diagnostic tests - Dual Path Platform [DPP] VetTB and multiantigen print immunoassay [MAPIA]), and repeated testing to increase antemortem validity are recommended. Gastric and bronchial lavage should also be investigated to improve accuracy of antemortem diagnostics.

Paudel, S., M. A. Villanueva, S. K. Mikota, C. Nakajima, K. P. Gairhe, S. Subedi, N. Rayamajhi, M. Sashika, M. Shimozuru, T. Matsuba, Y. Suzuki and T. Tsubota (2016). "Development and evaluation of an interferon-γ release assay in Asian elephants (Elephas maximus)." Journal of Veterinary Medical Science 78(7): 1117-1121.

We developed an interferon-γ release assay (IGRA) specific for Asian elephants (Elephas maximus). Whole blood collected from forty captive Asian elephants was stimulated with three different mitogens i.e., phytohemagglutinin (PHA), pokweed mitogen (PWM) and phorbol myristate aceteate/ionomycin (PMA/I). A sandwich ELISA that was able to recognize the recombinant elephant interferon-γ (rEIFN-γ) as well as native interferon-γ from the Asian elephants was performed using anti-elephant IFN-γ rabbit polyclonal antibodies as capture antibodies and biotinylated anti-elephant IFN-γ rabbit polyclonal antibodies as detection antibodies. PMA/I was the best mitogen to use as a positive control for an Asian elephant IGRA. The development of an Asian elephant-specific IGRA that detects native IFN-γ in elephant whole blood provides promising results for its application as a potential diagnostic tool for diseases, such as tuberculosis (TB) in Asian elephants. © 2016 The Japanese Society of Veterinary Science.

Paudel, S., J. L. Brown, S. Thapaliya, I. P. Dhakal, S. K. Mikota, K. P. Gairhe, M. Shimozuru and T. Tsubota (2016). "Comparison of cortisol and thyroid hormones between tuberculosis-suspect and healthy elephants of Nepal." Journal of Veterinary Medical Science 78(11): 1713-1716.

We compared cortisol and thyroid hormone (T3 and T4) concentrations between tuberculosis (TB)-suspected (n=10) and healthy (n=10) elephants of Nepal. Whole blood was collected from captive elephants throughout Nepal, and TB testing was performed using the ElephantTB STAT-PAK® and DPP VetTB® serological assays that detect antibodies against Mycobacterium tuberculosis and M. bovis in elephant serum. Cortisol, T3 and T4 were quantified by competitive enzyme immunoassays, and the results showed no significant differences in hormone concentrations between TB-suspect and healthy elephants. These preliminary data suggest neither adrenal nor thyroid function is altered by TB disease status. However, more elephants, including those positively diagnosed for TB by trunk wash cultures, need to be evaluated over time to confirm results. © 2016 The Japanese Society of Veterinary Science.

Hildebrandt, B., J. Saragusty, I. Moser, S. Holtze, T. Voracek, A. Bernhard, F. Goritz and R. Hermes (2016). Bronchalveloar lavage technique: a new approach for diagnosis of tuberculosis infection in elephants. Joint AAZV / EAZWV / IZW.

Tuberculosis in pachyderms was put into the spotlight two decades ago when circus elephants in North America were diagnosed with Mycobacterium tuberculosis complex. Because of the close association between elephants and humans, zoonotic risk, and high susceptibility to Mycobacterium tuberculosis, periodic testing was enacted in many zoological institutions around the world.1,2 Presently the gold standard is bacterial culture of trunk wash. Trunk wash, however, puts the operator at risk, it is insensitive, and is prone to contamination. We describe here a new technique that increases the safety and sensitivity while reducing the risk of cross-contamination. It was applied in one male and five female African and one male and three female Asian elephants. The technique relies on performing standing sedation with butorphanol 0.1 mg/kg combined with detomedine hydrochloride 0.02 mg/kg i.m. and additional nerve blocks in four locations to the trunk base 10 ml per location lidocaine hydrochloride 2%. A customized 3.5-m long videochip endoscope is inserted through the trunk and up to the larynx or the trachea. A sterile newly developed 6-hole-TBH-catheter named after inventor Thomas Bernd Hildebrandt with a length of 6 m is then placed through the 4 mm working channel of the endoscope further into the respiratory system. The lavage is performed using up to 100 ml sterile saline solution. Collection of the sample is done in closed system. The technique is safe for the operator, and has higher probability of harvesting the bacteria when such are shed while keeping environmental and trunk-related contamination to a minimum.

Abraham, D. and V. Pillai (2016). Cross-species transmission of mycobacterium tuberculosis in mahouts and captive elephants: Implications to health policy. 17th International Congress on Infectious Diseases / International Journal of Infectious Diseases

Background: There are nearly a thousand captive Asian ele- phants and not less than 3,000 mahouts in southern India. In the hands-on and open systems of captive elephant management, dis- eased mahouts and captive elephants could present the risk of cross-species tuberculosis transmission. With the help of evidence based results, we intend to formulate specific policy guidelines, which can suggest locally relevant preventive and control measures to help mitigate the risk of cross-species infection.

Methods & Materials: Over a period of three years, one time screening of nearly 800 elephants and their mahouts was achieved. Tuberculosis screening of mahouts was done by clinical examina- tion, chest X-ray evaluation, sputum culture and tuberculin skin testing, as required. Screening of elephants was done using the USDA licensed serological test, DPP Vet Assay® (Chembio Diagnos- tics Inc., Medford, New York) and trunk wash culture, as required. Detailed contact investigation of traceable human and animal con- tacts of the identified diseased mahouts and elephants were done. We examined three different contexts of tuberculosis transmission among captive elephants and mahouts. First scenario is the risk of infection from an infected mahout to an elephant. Second is the risk of infection from an infected elephant to a mahout and third is the risk of infection from an infected elephant to another elephant.

Results: There is evidence to suggest cross-species tuberculosis transmission. However, under the tropical climatic conditions in southern India, the risk of infection to a captive elephant from a diseased mahout seems to far outweigh the risks of infection to a mahout or another elephant, from a diseased elephant. There are political as well as ethical consequences to the outcomes in each of the three scenarios and they are both varied and complex.

Conclusion: Mahouts and captive elephants in southern India are highly migrant and locating the subjects for contact tracing and follow-up testing is difficult. Hence, systematic and regular tuber- culosis screening of mahouts and captive elephants is a challenge. Formulating as well as implementing policy guidelines for preven- tion and control of cross-species tuberculosis transmission, in the existing cultural and religious contexts of captive elephant man- agements in southern India, appears to be an even bigger challenge.

Vogelnest, L., F. Hulst, P. Thompson, K. P. Lyashchenko and K. A. Herrin (2015). "Diagnosis and management of tuberculosis (Mycobacterium tuberculosis) in an Asian elephant (Elephas maximus) with a newborn calf." J Zoo Wildl Med 46(1): 77-85.

In 2006, five Asian elephants (Elephas maximus) were imported to Taronga Zoo, Australia, from Thailand. Pre-import and initial postarrival tuberculosis screening was performed by trunk wash (TW) culture and was negative for Mycobacterium tuberculosis. In April 2009, the ElephantTB STAT-PAK (SP) assay was used to test the elephants. A 15.5-yr-old pregnant cow was reactive. TW frequency for this cow was increased from annually to quarterly. TW cultures remained negative on all other elephants. In February 2010, the Dual Path Platform (DPP) VetTB assay was used for the first time, and the SP-reactive cow also reacted on the DPP. A SP was run concurrently and was reactive. All other elephants were nonreactive on both assays. Treatment was not initiated due to concern about the effect of antituberculous drugs on the fetus. Quarterly TW cultures continued. The cow gave birth on 2 November 2010. A routine TW on 24 November 2010 was culture positive for M. tuberculosis. Although previous shedding could not be ruled out, reactivation of latent infection or exacerbation of subclinical disease due to parturition was suspected. Treatment with isoniazid, pyrazinamide, rifampicin, and ethambutol commenced. A 12-mo treatment course was completed within a 15-mo period. The isolate was susceptible to these drugs and genotyped as a Beijing strain. Stored serum samples from 2004 and 2006 were tested retrospectively and were reactive on SP and DPP. TW, SP, and DPP screening frequency increased to monthly for the positive cow on commencement of treatment in January 2011. Monthly serum biochemistry indicated drug-induced hepatitis. Therapeutic drug monitoring was conducted to ensure therapeutic levels were achieved. The infant calf was reactive on DPP, but TW culture negative, and was not treated. Serial DPP results for the cow and calf during and after treatment indicated that the antibody levels were declining, suggesting a favorable response to therapy in the dam, and that the origin of the antibodies in the calf were maternal, rather than a response to infection.

Perera, B. V. P., M. A. Salgadu, G. S. P. d. S. Gunwardena, N. H. Smith and H. R. N. Jinadasa (2015). "First confirmed case of fatal tuberculosis in a wild Sri Lankan elephant." Gajah 41: 28-31.

Niemeier, R. T., K. Mead, M. A. dePerio, S. Martin and G. A. Burr (2015). Evaluation of Potential Employee Exposures to Mycobacterium tuberculosis at an Elephant Refuge, U.S. Department of Health and Human Services , Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health: 27.

Mikota, S. K., K. Gairhe, K. Giri, K. Hamilton, M. Miller, S. Paudel, K. Lyashchenko, R. S. Larsen, J. B. Payeur, W. R. Waters, R. Greenwald, G. Dumonceaux and B. Vincent (2015). "Tuberculosis surveillance of elephants (Elephas maximus) in Nepal at the captive-wild interface." Eur J Wildl Res 61: 221-229.

A comprehensive elephant tuberculosis (TB) survey using culture and four serological screening tests was conducted in Nepal in response to concern raised by wildlife officials that TB could threaten wild populations of elephants, rhinos, and other susceptible species. Captive elephants come into close contact with wild animals during conservation and tourism activities inside Nepal's national parks. Private and government-owned male and female captive Asian elephants (Elephas maximus) were included in the study. The mean reported age was 38 years (range 5-60 years). A total of 289 samples from 120 elephants were collected for mycobacterial culture. Culture samples were processed at the National Tuberculosis Centre (NTC) in Nepal and the National Veterinary Services Laboratories (NVSL) in Ames, IA. Acid-fast organisms were observed in 11 and 21 samples processed at NTC and NVSL, respectively, and nontuberculous mycobacteria (NTMs) were isolated from six elephants. There were

no isolations of Mycobacterium tuberculosis or Mycobacterium bovis. Blood samples were also collected from 115 of the elephants for serological testing using the Chembio ElephantTB STAT-PAK®, the Chembio MultiAntigen Print Immunoassay test, a multi-antigen ELISA, and an immunoblot assay. Culture and serological results were variable and required careful interpretation to develop criteria to assess TB risk. Elephants were assigned to one of four disease risk groups (high, moderate, low, and undetermined), and management recommendations for each group were made to government authorities. Serological results were prioritized in developing recommendations because of culture limitations and inconclusive culture results. This strategy was based on evidence for the early predictive value of serological tests and the urgent need expressed by wildlife authorities in Nepal to protect their captive elephants, mitigate TB at the captive-wild interface, and safeguard tourism.

Lassausaie, J., A. Bret, X. Bouapao, V. Chanthavong, J. Castonguay-Vanier, F. Quet, S. K. Mikota, C. Theoret, Y. Buisson and B. Bouchard (2015). "Tuberculosis in Laos, who is at risk: the mahouts or their elephants?" Epidemiol Infect 143(5): 922-931.

SUMMARY Tuberculosis (TB) in elephants has the potential to infect humans and is an increasing public health concern. Lao PDR is one of the last countries where elephants are still used for timber extraction and where they live in close contact with their mahouts. There are 500 animals at work in the country, some interacting with wild herds. Although human TB prevalence is known to be high in Laos, studies on elephant TB had yet to be undertaken. From January to July 2012, screening was performed using the ElephantTB Stat-Pak assay on 80 elephants working around the Nam Pouy National Park in Sayaboury Province. This represents more than 18% of the total registered national working elephant population. Here we report that 36% of the elephants were seroreactive to the test. Of these, 31% had contacts with wild individuals, which suggests potential transmission of mycobacteria to the local wild herds. Clinical examination, chest X-rays, sputum microscopy and culture were performed on their 142 mahouts or owners. Despite high TB seroreactivity in elephants, no participant was smear- or culture-positive for Mycobacterium tuberculosis or M. bovis, although atypical mycobacteria were isolated from 4% of participants.

Lassausaiae, J., A. Bret, X. Bouapao, V. Chanthavong, J. Castonguay-Vanier, F. Quet, S. K. Mikota, C. Theoret, Y. Buisson and B. Bouchard (2014). "Tuberculosis in Laos, who is at risk: the mahouts or their elephants?" Epidemiol Infect 143(5): 922-931.

Tuberculosis (TB) in elephants has the potential to infect humans and is an increasing public health concern. Lao PDR is one of the last countries where elephants are still used for timber extraction and where they live in close contact with their mahouts. There are 500 animals at work in the country, some interacting with wild herds. Although human TB prevalence is known to be high in Laos, studies on elephant TB had yet to be undertaken. From January to July 2012, screening was performed using the ElephantTB Stat-Pak assay on 80 elephants working around the Nam Pouy National Park in Sayaboury Province. This represents more than 18% of the total registered national working elephant population. Here we report that 36% of the elephants were seroreactive to the test. Of these, 31% had contacts with wild individuals, which suggests potential transmission of mycobacteria to the local wild herds. Clinical examination, chest X-rays, sputum microscopy and culture were performed on their 142 mahouts or owners. Despite high TB seroreactivity in elephants, no participant was smear- or culture-positive for Mycobacterium tuberculosis or M. bovis, although atypical mycobacteria were isolated from 4% of participants.

Hlokwe, T. M., P. van Helden and A. L. Michel (2014). "Evidence of increasing intra and inter-species transmission of Mycobacterium bovis in South Africa: Are we losing the battle?" Preventive Veterinary Medicine 115(1-2): 10-17.

Tuberculosis caused by Mycobacterium bovis is recognized worldwide as a significant health risk in domestic cattle, farmed and wild animal species as well as in humans. We carried out spoligotyping and variable number of tandem repeat (VNTR) typing methods to characterize 490 M. bovis isolates from livestock (cattle, n=. 230; pig n=. 1) and wildlife species (. n=. 259) originating from different farms and regions in South Africa, with the aim to further establish the genetic diversity of the isolates, study the population structure of M. bovis and elucidate the extent of interspecies transmission of bovine tuberculosis. A total of ten spoligotype patterns were identified, two of which were novel (SB2199 and SB2200) and reported for the first time in the literature, while VNTR typing revealed a total of 97 VNTR profiles. Our results showed evidence of clonal expansion for some ancestral strains as well as co-infections with two or three M. bovis strains on some of the cattle and game farms, which suggested independent introductions of infected animals from epidemiologically unrelated sources. Five spoligotypes and nine VNTR profiles were shared between cattle and wildlife. Our findings showed that besides cattle, at least 16 different animal species in South Africa are infected with bovine tuberculosis, and highlight a strong evidence of inter and intra-species transmission of M. bovis. Infection of the blue wildebeest (. Connochaetes taurinus) with M. bovis is described for the first time in this report. This update in epidemiological information raises concerns that bovine tuberculosis has increased its spatial distribution in South Africa and is also affecting an increasing number of wildlife species compared to ten years ago. © 2014 Elsevier B.V.

van Sandwyk, J. H., N. C. Bennett, R. Swanepoel and A. D. S. Bastos (2013). "Retrospective genetic characterisation of Encephalomyocarditis viruses from African elephant and swine recovers two distinct lineages in South Africa." Veterinary Microbiology 162(1): 23-31.

Encephalomyocarditis virus (EMCV) outbreaks are rare in southern Africa. Only two have been reported to date from South Africa, both coinciding with rodent irruptions. The first outbreak manifested as acute myocarditis in pigs in 1979, whilst the second, occurring from 1993 to 1994, was linked to the deaths of 64 free-ranging adult African elephants (Loxodonta africana). The P1 genome region, inclusive of the flanking leader (L) and 2A genes, of three South African isolates, one from swine and two from elephants, was characterised by PCR amplification and sequencing of up to 11 overlapping fragments. In addition to the resulting 3329 nucleotide dataset, the 3D region that is widely used in molecular epidemiology studies, was characterised, and three datasets (P1, VP1/3 and 3D), complemented with available homologous EMCV data, were compiled for analyses. Phylogenetic inferences revealed the near-identical elephant outbreak strains to be most closely related to a mengovirus from rhesus macaques (Macaca mulatta) in Uganda, differing from the latter by between 11% (3D) and 15% (VP3/1). The South African pig isolate differed by 4% (3D) and 11% (VP3/1) from available European and Asian pig virus sequences. This study confirms the presence of two genetically distinct EMCV lineages recovered from sporadic outbreaks in wild and domestic hosts in southern Africa, and provides valuable baseline data for future outbreak eventualities in the sub-region. © 2012 Elsevier B.V.

Ong, B. L., Y. F. Ngeow, M. F. Razak, Y. Yakuba, Z. Zakaria, A. R. Mutalib, L. Hassan, H. F. Ng and K. Versahib (2013). "Tuberculosis in captive Asian elephants (Elephas maximus) in peninsular Malaysia." Epidemiol Infect(141): 1481-1487.

A cross-sectional study was conducted from 10 January to 9 April 2012, to determine the seroprevalence of tuberculosis (TB) of all captive Asian elephants and their handlers in six locations in Peninsular Malaysia. In addition, trunk-wash samples were examined for tubercle bacillus by culture and polymerase chain reaction (PCR). For 63 elephants and 149 elephant handlers, TB seroprevalence was estimated at 20·4% and 24·8%, respectively. From 151 trunk-wash samples, 24 acid-fast isolates were obtained, 23 of which were identified by hsp65-based sequencing as non-tuberculous

mycobacteria. The Mycobacterium tuberculosis-specific PCR was positive in the trunk-wash samples from three elephants which were also seropositive. Conversely, the trunk wash from seven seropositive elephants were PCR negative. Hence, there was evidence of active and latent TB in the elephants and the high seroprevalence in the elephants and their handlers suggests frequent, close contact, two-way transmission between animals and humans within confined workplaces.

Obanda, V., J. Poghon, M. Yongo, M. Mulei, M. Ngotho, K. Waititu, J. Makumi, F. Gakuya, P. Osmondi, R. C. Soriguer and S. Alasaad (2013). "First reported case of fatal tuberculosis in a wild African elephant with past human-wildlife contact." Epidemiol Infect 141: 1476-1480.

Tuberculosis is emerging/re-emerging in captive elephant populations, where it causes morbidity and deaths, although no case of TB in wild African elephants has been reported. In this paper we report the first case of fatal TB in an African elephant in the wild. The infection with Mycobacterium tuberculosis was confirmed by post-mortem and histological examinations of a female sub-adult elephant aged >12 years that died in Tsavo East National Park, Kenya, while under treatment. This case is unique in that during its lifetime the elephant had contact with both humans and wild elephants. The source of the infection was unclear because the elephant could have acquired the infection in the orphanage or in the wild. However, our results show that wild elephants can maintain human TB in the wild and that the infection can be fatal.

Miller, M. and F. Olea-Popelka (2013). "One Health in the shrinking world: Experiences with tuberculosis at the human-livestock-wildlife interface." Comparative Immunology Microbiology and Infectious Diseases 36(3): 263-268.

Tuberculosis (TB) is a global anthropozoonotic infection that has raised awareness of the impact of disease at the human-livestock-wildlife interface. There are examples of transmission from livestock resulting in establishment of reservoirs in wildlife populations, and exposures from interactions between humans and wildlife that have resulted in disease outbreaks. A One Health approach is crucial to managing and protecting the health of humans, livestock, wildlife and the environment. Although still in its infancy in many areas of the world, the use of transdisciplinary teams to address wildlife-human-livestock boundary diseases will broaden the scope of options for solutions. This paper reviews some less commonly known examples of threats and outcomes using lessons learned from tuberculosis. (C) 2012 Elsevier Ltd. All rights reserved.

Mikota, S. K., S. Subedi, K. Gairhe, S. Paudel, J. Thapa, B. Vincent and G. Kaufman (2013). Nepal elephant (Elephas maximus) Healthcare and Tuberculosis Surveillance Program Update. American Association of Zoo Veterinarians.

The Nepal Elephant Healthcare and Tuberculosis (TB) Surveillance Program was initiated by Elephant Care International in 2007 following the first comprehensive TB testing of Asian elephants in 2006. Previous reports have described the challenges that TB presents to wildlife, humans, and domestic livestock in Nepal 1-3 and a recent report has demonstrated the risk of transmission to the wild.4

The program is based near Chitwan National Park where a field office and lab are staffed by a full-time veterinarian. Program goals are to 1) mitigate transmission of TB to wild elephants, rhinos and other ungulates by controlling TB at the captive-wild interface, 2) ensure the health of government elephants used for anti-poaching patrols, rhino censuses, and other conservation purposes, 3) safeguard tourism that supports the national parks, 4) build wildlife veterinary capacity, 5) encourage the development of elephant TB control programs other Asian elephant range countries, and 6) advance our knowledge of TB in elephants.

Ninety-three percent of the captive population has been tested using the Elephant TB Stat-Pak® and / or DPP® Vet TB™ assays.a Over 20 elephants have been treated prophylactically or therapeutically for TB based on serology results, culture, and /or exposure history.
The Program has facilitated multiple research projects, involving students and investigators from Tufts University, Michigan State University, Murdoch University, and the Institute of Agriculture and Animal Science (Nepal).

In 2010 the Ministry of Forestry approved the Elephant Tuberculosis Control and Management Action Plan (2011-2015), the first such plan in Asia. The plan is on-line at www.elephantcare.org.

ACKNOWLEDGMENTS

The authors would like to acknowledge the support of the Department of National Parks and Wildlife Conservation of the Government of Nepal, Dr. I.P. Dhakal of the Institute of Agriculture and Animal Science for working with us to establish a fellowship for the first TB Program veterinarian, Dr. Shantraj Jnawali for help in transitioning the TB Program to the National Trust for Nature Conservation, Dr. Christy Williams of WWF-Nepal for construction of a segregation site and mahout TB testing, and Konstantin Lyashchenko of Chembio Diagnostics Systems Inc.a for technical support.

We greatly acknowledge the financial support of the U.S Fish and Wildlife Services Asian Elephant Conservation Fund (Awards 98201-8-G571, 96200-9-G222, and 96200-0-G143), the Mazuri Fund, the Walter J. Ernst Memorial Fund, the Abraham Foundation, Buttonwood Park Zoo, Columbus Zoo, Oklahoma City Zoo, Phoenix Zoo, Busch Gardens Tamps, the Humane Society of the United States, and numerous private donors.

Products Mentioned in the Text: aChembio Diagnostic Systems, Inc, Medford, NY, USA 11763.

LITERATURE CITED

1. Mikota, S.K., G. Kaufman, I.P. Dhakal, and B.D.Pandey. 2009. Tuberculosis in Nepal: elephants, humans, livestock, and wildlife. Proc. Am. Assoc. Zoo Vet. Annual. Conf. Pp. 3-4.
2. Mikota, S.K., M. Miller, G. Dumonceaux, K. Giri, K. Gairhe, K. Hamilton, S. Paudel, K. Lyashchenko, R.S. Larsen, J. Payeur, R. Waters, M.D. Salman, and G. Kaufman, G. 2007. Comparison of four serologic assays and culture to determine tuberculosis infection in captive elephants in Nepal. Proc. Am. Assoc. Zoo Ve.t, Am. Assoc. Wildlife Vet, Am. Zoo and Aquarium Assoc Nutr Adv Group Joint Conf. Pp. 71-72.
3. Mikota, S.K., M. Miller, G. Dumonceaux, K. Giri, K. Gairhe, K. Hamilton, S. Paudel, and B. Vincent. 2006. Elephant tuberculosis diagnosis: implications for elephant management in Asian range countries. Proc. Am. Assoc. Zoo Vet. Annual. Conf. Pp. 142-143.
4. Obanda, V., J. Poghon, M. Yongo, I. Mulei, M. Ngotho, K. Waititu, J. Makumi, F. Gakuya, P. Omondi, R.C. Soriguer, and S. Alasaad. 2013. First report of fatal tuberculosis in a wild African elephant with past human-wildlife contact. Epidemiol. Infect. Pp. 1-5.

Feldman, M., R. Isaza, C. Prins and J. Hernandez (2013). "Point prevalence and incidence of Mycobacterium tuberculosis complex in captive elephants in the United States of America." Veterinary Quarterly 33: 25-29.

Angkawanish, T., D. Morar, P. van Kooten, I. Bontekoning, J. Schreuder, M. Maas, W. Wajjwalku, A. Sirimalaisuwan, A. Michel, E. Tijhaar and V. Rutten (2013). "The elephant interferon gamma assay: a contribution to diagnosis of tuberculosis in elephants." Transbound Emerg Dis 60 Suppl 1: 53-59.

Mycobacterium tuberculosis (M. tb) has been shown to be the main causative agent of tuberculosis in elephants worldwide. M. tb may be transmitted from infected humans to other species including elephants and vice versa, in case of prolonged intensive contact. An accurate diagnostic approach covering all phases of the infection in elephants is required. As M. tb is an intracellular pathogen and cell-mediated immune (CMI) responses are elicited early after infection, the skin test is the CMI assay of choice in humans and cattle. However, this test is not applicable in elephants. The interferon gamma (IFN-gamma) assay is considered a good alternative for the skin test in general, validated for use in cattle and humans. This study was aimed at development of an IFN-gamma assay applicable for diagnosis of tuberculosis in elephants. Recombinant elephant IFN-gamma (rEpIFN-gamma) produced in eukaryotic cells was used to immunize mice and generate the monoclonal antibodies. Hybridomas were screened for IFN-gamma-specific monoclonal antibody production and subcloned, and antibodies were isotyped and affinity purified. Western blot confirmed recognition of the rEpIFN-gamma. The optimal combination of capture and detection antibodies selected was able to detect rEpIFN-gamma in concentrations as low as 1 pg/ml. The assay was shown to be able to detect the native elephant IFN-gamma, elicited in positive-control cultures (pokeweed mitogen (PWM), phorbol myristate acetate plus ionomycin (PMA/I)) of both Asian and African elephant whole-blood cultures (WBC). Preliminary data were generated using WBC from non-infected elephants, a M. tb infection-suspected elephant and a culture-confirmed M. tb-infected elephant. The latter showed measurable production of IFN-gamma after stimulation with ESAT6/CFP10 PPDB and PPDA in concentration ranges as elicited in WBC by Mycobacterium tuberculosis complex (MTBC)-specific antigens in other species. Hence, the IFN-gamma assay presented potential as a diagnostic tool for the detection of elephant tuberculosis. Validation of the assay will require its application in large populations of non-infected and infected elephants.

Verma-Kumar, S., D. Abraham, N. Dendukuri, J. V. Cheeran, R. Sukumar and K. N. Balaji (2012). "Serodiagnosis of tuberculosis in Asian elephants (Elephas maximus) in southern India: a latent class analysis." PLoS ONE 7(11): 1-8.

Lyashchenko, K. P., R. Greenwald, J. Esfandiari, S. Mikota, M. Miller, T. Moller, L. Volgelnest, K. Gairhe, S. Robbe-Austerman, J. Gai and W. R. Waters (2012). "Field application of serodiagnostics to identify elephants with tuberculosis prior to case confirmation by culture." Clinical and Vaccine Immunology 19(8): 1269-1275.

Three serologic methods for antibody detection in elephant tuberculosis (TB), the multiantigen print immunoassay (MAPIA), ElephantTB STAT-PAK kit, and DPP VetTB test, were evaluated using serial serum samples from 14 captive elephants infected with Mycobacterium tuberculosis in 5 countries. In all cases, serological testing was performed prior to the diagnosis of TB by mycobacterial culture of trunk wash or tissue samples collected at necropsy. All elephants produced antibody responses to M.tuberculosis antigens, with 13/14 recognizing ESAT-6 and/or CFP10 proteins. The findings supported the high serodiagnostic test accuracy in detecting infections months to years before M. tuberculosis could be isolated from elephants. The MAPIA and/or DPP VetTB assay demonstrated the potential for monitoring antimycobacterial therapy and predicting TB relapse in treated elephants when continuously used in the posttreatment period. History of exposure to TB and past treatment information should be taken into consideration for proper interpretation of the antibody test results. Data suggest that the more frequent trunk wash culture testing of seropositive elephants may enhance the efficiency of the TB diagnostic algorithm, leading to earlier treatment with improved outcomes.

Yong, H., C. Go-Eun, B. S. Lee, J. Whang and S. J. Shin (2011). "Disseminated infection due to Mycobacterium avium subsp. avium in an Asian elephant (Elephas maximus." Journal of Zoo and Wildlife Medicine 42(4): 743-746.

Witt, C. J., A. L. Richards, P. M. Masuoka, D. H. Foley, A. L. Buczak, L. A. Musila, J. H. Richardson, M. G. Colacicco-Mayhugh, L. M. Rueda, T. A. Klein, A. Anyamba, J. Small, J. A. Pavlin, M. M. Fukuda, J. Gaydos, K. L. Russell, A.-G. P. S. W. Group, R. C. Wilkerson, R. V. Gibbons, R. G. Jarman, K. S. Myint, B. Pendergast, S. Lewis, J. E. Pinzon, K. Collins, M. Smith, E. Pak, C. Tucker, K. Linthicum, T. Myers, M. Mansour, K. Earhart, H. C. Kim, J. Jiang, D. Schnabel, J. W. Clark, R. C. Sang, E. Kioko, D. C. Abuom, J. P. Grieco, E. E. Richards, S. Tobias, M. R. Kasper, J. M. Montgomery, D. Florin, J. P. Chretien and T. L. Philip (2011). "The AFHSC-Division of GEIS Operations Predictive Surveillance Program: a multidisciplinary approach for the early detection and response to disease outbreaks." BMC Public Health 11 Suppl 2: S10.

The Armed Forces Health Surveillance Center, Division of Global Emerging Infections Surveillance and Response System Operations (AFHSC-GEIS) initiated a coordinated, multidisciplinary program to link data sets and information derived from eco-climatic remote sensing activities, ecologic niche modeling, arthropod vector, animal disease-host/reservoir, and human disease surveillance for febrile illnesses, into a predictive surveillance program that generates advisories and alerts on emerging infectious disease outbreaks. The program's ultimate goal is pro-active public health practice through pre-event preparedness, prevention and control, and response decision-making and prioritization. This multidisciplinary program is rooted in over 10 years experience in predictive surveillance for Rift Valley fever outbreaks in Eastern Africa. The AFHSC-GEIS Rift Valley fever project is based on the identification and use of disease-emergence critical detection points as reliable signals for increased outbreak risk. The AFHSC-GEIS predictive surveillance program has formalized the Rift Valley fever project into a structured template for extending predictive surveillance capability to other Department of Defense (DoD)-priority vector- and water-borne, and zoonotic diseases and geographic areas. These include leishmaniasis, malaria, and Crimea-Congo and other viral hemorrhagic fevers in Central Asia and Africa, dengue fever in Asia and the Americas, Japanese encephalitis (JE) and chikungunya fever in Asia, and rickettsial and other tick-borne infections in the U.S., Africa and Asia.

Murphree, R., J. V. Warkentin, J. R. Dunn, W. Schaffner and T. F. Jones (2011). "Elephant-to-human transmission of tuberculosis, 2009." Emerg Infect Dis 17(3): 366-371.

In 2009, the Tennessee Department of Health received reports of 5 tuberculin skin test (TST) conversions among employees of an elephant refuge and isolation of Mycobacterium tuberculosis from a resident elephant. To determine the extent of the outbreak and identify risk factors for TST conversion, we conducted a cohort study and onsite assessment. Risk for conversion was increased for elephant caregivers and administrative employees working in the barn housing the M. tuberculosis-infected elephant or in offices connected to the barn (risk ratio 20.3, 95% confidence interval 2.8-146.7). Indirect exposure to aerosolized M. tuberculosis and delayed or inadequate infection control practices likely contributed to transmission. The following factors are needed to reduce risk for M. tuberculosis transmission in the captive elephant industry: increased knowledge about M. tuberculosis infection in elephants, improved infection control practices, and specific occupational health programs.

Mikota, S. K. and J. N. Maslow (2011). "Tuberculosis at the human-animal interface: an emerging disease of elephants." Tuberculosis (Edinb) 91(3): 208-211.

Over the past 15 years, cases of infection with organisms of the Mycobacterium tuberculosis complex have been diagnosed among captive elephants in the United States and worldwide. Outbreak

investigations have documented that among staff employed at facilities housing infected animals, skin test conversion to purified protein derivative have been documented. Clonal spread among animals in close contact and even inter-species spread between elephant and human has been documented. Detection of actively infected animals relies on samples obtained by trunk wash. Diagnosis has been augmented by the development of a multi-antigen serologic assay with excellent specificity and sensitivity. Treatment regimens are still in development with efficacy largely unknown due to a paucity of both premortem follow-up and necropsy data of treated animals. The epidemiology, diagnosis and treatment of tuberculosis in elephants require additional careful study of clinical data.

Giri, K., G. E. Kaufman and I. P. Dhakal (2011). "The relationship between blood parameter and mycobacterium culture status in captive elephants of Nepal." Nepalese Vet J 30: 1190-1120.

Sang, R., E. Kioko, J. Lutomiah, M. Warigia, C. Ochieng, M. O'Guinn, J. S. Lee, H. Koka, M. Godsey, D. Hoel, H. Hanafi, B. Miller, D. Schnabel, R. F. Breiman and J. Richardson (2010). "Rift Valley fever virus epidemic in Kenya, 2006/2007: the entomologic investigations." Am J Trop Med Hyg 83(2 Suppl): 28-37.

In December 2006, Rift Valley fever (RVF) was diagnosed in humans in Garissa Hospital, Kenya and an outbreak reported affecting 11 districts. Entomologic surveillance was performed in four districts to determine the epidemic/epizootic vectors of RVF virus (RVFV). Approximately 297,000 mosquitoes were collected, 164,626 identified to species, 72,058 sorted into 3,003 pools and tested for RVFV by reverse transcription-polymerase chain reaction. Seventy-seven pools representing 10 species tested positive for RVFV, including Aedes mcintoshi/circumluteolus (26 pools), Aedes ochraceus (23 pools), Mansonia uniformis (15 pools); Culex poicilipes, Culex bitaeniorhynchus (3 pools each); Anopheles squamosus, Mansonia africana (2 pools each); Culex quinquefasciatus, Culex univittatus, Aedes pembaensis (1 pool each). Positive Ae. pembaensis, Cx. univittatus, and Cx. bitaeniorhynchus was a first time observation. Species composition, densities, and infection varied among districts supporting hypothesis that different mosquito species serve as epizootic/epidemic vectors of RVFV in diverse ecologies, creating a complex epidemiologic pattern in East Africa.

Michel, A. L., B. Muller and P. D. van Helden (2010). "Mycobacterium bovis at the animal-human interface: A problem of not?" Veterinary Microbiology 140: 371-381.

Mycobacterium bovis is a pathogen of significant importance in livestock and a wide range of wild animal species worldwide. It is also known to cause tuberculosis disease in humans, a fact which has raised renewed concerns regarding the zoonotic risk for humans, especially those living at the animal-human interface. This review consolidates recent reports in the literature mainly on animal and zoonotic tuberculosis with an emphasis on evolution, epidemiology, treatment and diagnosis. The information presented reveals thefundamental differences in the complexity and level at which the disease affects the economy, ecosystem and human population of regions where animal tuberculosis control is achieved and regions where little or no control is implemented. In conclusion the review suggests that bovine tuberculosis has essentially been reduced to a disease of economic importance in the developed world, while low-income countries are facing a multifaceted impact which potentially affects the health of livestock, humans and ecosystems and which is likely to increase in the presence of debilitating diseases such as HIV/AIDS and other factors which negatively affect human livelihoods.

Landolfi, J. A., S. K. Mikota, J. Chosy, K. P. Lyaschenko, K. Giri, K. Gairhe and K. A. Terio (2010). "Comparison of systemic cytokine levels in Mycobacterium spp seropositive and seronegative Asian elephants (Elephas maximus)." Journal of Zoo and Wildlife Medicine 41(3): 445-455.

Mycobacterium spp. infection is an important health concern for Asian elephant (Elephas maximus) populations worldwide. The disease is of particular concern considering its potential to affect not only the individual animal but also herd and public health. Although elephant tuberculosis susceptibility is poorly understood, immune function alterations are central to disease pathogenesis in other species and probably affect outcome of mycobacterial infections in elephants. Measurement of immune mediator (cytokine) levels within blood samples can provide information regarding immune function that may elucidate disease susceptibility. For this study, mRNA levels of interleukin (IL)-2, IL-4, IL-10, and IL-12; interferon (IFN)-c; tumor necrosis factor (TNF)-a; and transforming growth factor (TGF)-b were measured using elephant-specific, real-time reverse transcriptionpolymerase chain reaction (RT-PCR) assays in RNA-preserved whole blood samples from 106 Asian elephants, 15% of which were Mycobacterium tuberculosis complex seropositive. The Elephant TB STAT-PAKH (Chembio Diagnostics, Inc., Medford, New York 11763, USA), a novel lateral flow antibody detection assay developed for specific use in elephants, was used to determine serologic status for the study. Seropositive animals had higher levels of TNF-a and lower levels of TGF-b than seronegative animals; these differences between groups were statistically significant when levels were analyzed as categorical variables. Trends toward higher levels of IFN-c and IL-4 and slightly lower levels of IL-10 and IL-12 were noted in the seropositive group, although differences between groups were not statistically significant. Presence of other inflammatory conditions was found to be a significant confounding variable in the analysis of the relationship between tuberculosis status and TNF-a levels, necessitating its inclusion in statistical models. Age and sex were not found to significantly affect the relationship between tuberculosis status and any of the cytokines measured. Interleukin-2 levels were below the sensitivity of the realtime RT-PCR assay irrespective of tuberculosis status. These findings provide a foundation for future research intothe immunopathogenesis of elephant tuberculosis.

Ireton, G. C., R. Greenwald, H. Liang, J. Esfandiari, K. P. Lyashchenko and S. G. Reed (2010). "Identification of Mycobacterium tuberculosis antigens of high serodiagnostic value." Clinical and Vaccine Immunology 17(10): 1539-1547.

Tuberculosis (TB) is a chronic infectious disease caused by Mycobacterium tuberculosis, with several million new cases detected each year. Current methods of diagnosis are time-consuming and/or expensive or have a low level of accuracy. Therefore, new diagnostics are urgently needed to address the global tuberculosis burden and to improve control programs. Serological assays remain attractive for use in resource-limited settings because they are simple, rapid, and inexpensive and offer the possibility of detecting cases often missed by routine sputum smear microscopy. The aim of this study was to identify M. tuberculosis seroreactive antigens from a panel of 103 recombinant proteins selected as diagnostic candidates. Initial library screening by protein array analysis and enzyme-linked immunosorbent assay (ELISA) identified 42 antigens with serodiagnostic potential. Among these, 25 were novel proteins. The reactive antigens demonstrated various individual sensitivities, ranging from 12% to 78% (specificities, 76 to 100%). When the antigens were analyzed in combinations, up to 93% of antibody responders could be identified among the TB patients. Selected seroreactive proteins were used to design 3 new polyepitope fusion proteins. Characterization of these antigens by multiantigen print immunoassay (MAPIA) revealed that the vast majority of the TB patients (90%) produced antibody responses. The results confirmed that due to the remarkable variation in immune recognition patterns, an optimal multiantigen cocktail should be designed to cover the heterogeneity of antibody responses and thus achieve the highest possible test sensitivity. 

Angkawanish, T., W. Wajjwalku, A. Sirimalaisuwan, S. Mahasawangkul, T. Kaewsakhorn, K. Boonsri and V. P. M. G. Rutten (2010). "Mycobacterium tuberculosis infection of domesticated Asian elephants, Thailand." Emerg Infect Dis 16(12): 1949-1951.

Alexander, K. A., P. N. Laver, A. L. Michel, M. Williams, P. D. van Helden, R. M. Warren and N. C. G. van Pittius (2010). "Novel mycobacterium tuberculosis complex pathogen, M. Mungi." Emerging Infectious Diseases 16(8): 1296-1299.

Seven outbreaks involving increasing numbers of banded mongoose troops and high death rates have been documented. We identified a Mycobacterium tuberculosis complex pathogen, M. mungi sp. nov., as the causative agent among banded mongooses that live near humans in Chobe District, Botswana. Host spectrum and transmission dynamics remain unknown.

Mikota, S. K., G. Kaufman, I. P. Dhakal and B. D. Pandey (2009). Tuberculosis in Nepal: Elephants, Humans, Livestock, and Wildlife. Proceedings of the American Association of Zoo Veterinarians.

Tuberculosis (TB) is endemic among humans in Nepal. Almost 50% of the > 28 million population are infected and up to 90,000 are active cases (http://www.who-int/infnew/tuber4.htm). Direct observed therapy short-course (DOTS) was instituted in 1996 and now reaches 75% of the population. Implementation of DOTS nation-wide is hampered by the logistics of reaching and servicing remote hill areas. Between 5,000 and 7,000 people die every
year despite DOTS therapy; some of these deaths may be due to multidrug-resistant (MDR) or extensively drug- resistant (XDR) TB. Four drug resistance surveys have been carried out since 2005. MDR-TB rates of 2.9% (1.8%-3.2%) among new cases and 11.7% (7.1%-18.3%) among re-treatment cases were reported at the end of the fourth survey
(http://www.searo.who.int/en/Section10/Section2097/Section2100_14801.htm).

Nepal has a mixed farming system, including over four million buffaloes and almost seven million cattle. Sporadic studies have identified a TB prevalence of 0-24% among cattle and 4.5 to 41% among buffalo. In a recent study Mycobacterium bovis (M. bovis) was isolated from 17% of buffalo and 16% of cattle positive on the single intradermal cervical test.1 There is no formal TB surveillance or control program for cattle or buffalo in Nepal. Although the World Health
Organization recommends test and slaughter to eliminate bovine TB, Nepal is predominantly Hindu and the slaughter of cattle is forbidden.

The prevalence of M. bovis (BTB) infection in humans is unknown as TB diagnostic laboratories in Nepal (as in many countries) report positive culture results as "M. tuberculosis complex" but do not speciate. Risks of TB / BTB transmission from livestock to people exist through direct contact by farmers and slaughterhouse workers and consumption of contaminated meat and unpasteurized milk. Buffalo meat comprises over 64% of the total meat consumed in Nepal. In one study, tuberculosis was diagnosed in 14% of slaughtered buffaloes.2 Intensive livestock production is rare, and human beings live in close association with their farm animals providing increased opportunities for exposure.

Captive elephants in Nepal are cared for by humans, bred by wild elephant bulls, and graze with domestic livestock. Government-owned elephants patrol the Chitwan National Park (and other protected areas) and are essential for rhino counts and other conservation programs. Privately owned elephants used for safaris in the parks generate tourist dollars that support conservation and local businesses.

TB has not yet been diagnosed in wild elephants, rhinos, or other wild mammals in Nepal but poses a significant threat. Controlling TB at the captive elephant interface may decrease transmission to the wild where it would be difficult if not impossible to control. An elephant TB surveillance program was initiated in Nepal in 2006 following the postmortem diagnosis of TB in several captive elephants. To date, 164 captive elephants (79% of the population) have been tested using the ElephantTB STAT-PAK Assay® (Chembio Diagnostic Systems, Inc., 3661 Horseblock Road, Medford, NY 11763, USA). Nineteen elephants are
receiving treatment for TB; one elephant has completed treatment, and one old elephant is under permanent quarantine. Culture-confirmation of TB infection has been unrewarding due to 1) difficulty in performing the trunk wash procedure, 2) sample contamination, and 3) limited laboratory capacity to process elephant samples. Investigation of alternative direct methods for diagnosis are being pursued.3 TB has not been detected in currently employed elephant caretakers tested by the public health system.

Tuberculosis will be a main focus of the newly established One Health-Nepal, spearheaded by the National Trust for Nature Conservation (a Nepal NGO) and the Zoological Society of London. Elephant Care International, the Cummings School of Veterinary Medicine at Tufts University, and the Institute of Agriculture and Animal Science are among the organizations that will collaborate to address cross-species TB issues in Nepal.

ACKNOWLEDGMENTS
The authors gratefully acknowledge the support of the Department of National Parks and Wildlife Conservation, Government of Nepal, and the following partners: National Trust for Nature Conservation, WWF-Nepal, and the Zoological Society of London.

LITERATURE CITED

1. Chandra, V., Y. Morita, M. Dhakal, B. Besnet, T.Sato, A.Nagai, M. Kato, K. Kozawa, S. Yamamoto, and H. Kimura. 2007. Isolation of Mycobacterium spp. from milking buffaloes and cattle in Nepal. J. Vet. Med. Sci. 69(8): 819-825.
2. Joshi, D.D. 1986. Epidemiological situation of tuberculosis in Nepal. J. Inst. Med. 5: 115-128.
3. Wilson, T., D. Akiyoshi, S. Desai, M. Bhandar, S. Paudel, P. Manandhar, S. Manandhar, S. Mikota, J. Mukherjee, and G. Kaufman. 2008. Development of a PCR diagnostic technique for differentiation of Mycobacterium species in elephant trunk wash samples in Nepal. Poster AAZV Annual Conference, Los Angeles, October 12-17, 2008

Mikota, S. K. (2009). Stress, Disease, and Tuberculosis in Elephants. An Elephant in the Room. D. L. Forthman, L. F. Kane, D. Hancocks and P. F. Waldau. North Grafton, Center for Animals and Publci Policy, Cummings School of Veterinary Medicine, Tufts University: 74-84.

Michel, A. L., M. L. Coetzee, D. F. Keet, L. Mare, R. Warren, D. Cooper, R. G. Bengis, K. Kremer and P. van Helden (2009). "Molecular epidemiology of Mycobacterium bovis isolates from free-ranging wildlife in South African game reserves." Vet Microbiol 133: 335-343.

Bovine tuberculosis is endemic in African buffalo and a number of other wildlife species in the Kruger National Park (KNP) and Hluhluwe-iMfolozi Park (HiP) in South Africa. It was thought that the infection had been introduced into the KNP ecosystem through direct contact between cattle and buffalo, a hypothesis which was confirmed in this study by IS6110 and PGRS restriction fragment length polymorphism (RFLP) typing. The molecular

characterisation of 189 Mycobacterium bovis isolates from nine wildlife species in the HiP, including three smaller associated parks, and the Kruger National Park with adjacent areas showed that the respective epidemics were each caused by an infiltration of a single M.bovis genotype. The two M. bovis strains had different genetic profiles, as demonstrated by hybridisation with the IS6110 and PGRS RFLP probes, as well as with regard to evidence of
evolutionary changes to the IS profile. While the M. bovis type in HiP was transmitted between buffaloes and to at least baboon, bushpig and lion without obvious genetic changes in the RFLP patterns, in the KNP a dominant strain was represented in 73% of the M. bovis isolates, whilst the remaining 27% were variants of this strain. No species-specific variants were observed, except for one IS6110 type which was found only in a group of five epidemiologically related greater kudu. This finding was attributed to species-specific behaviour patterns rather than an advanced host-pathogen interaction.

Greenwald, R., O. Lyashchenko, J. Esfandiari, M. Miller, S. Mikota, J. H. Olsen, R. Ball, G. Dumonceaux, D. Schmitt, T. Moller, J. B. Payeur, B. Harris, D. Sofranko, W. R. Waters and K. P. Lyashchenko (2009). "Highly accurate antibody assays for early and rapid detection of tuberculosis in African and Asian elephants." Clin Vaccine Immunol 16(5): 605-612.

Tuberculosis (TB) in elephants is a reemerging zoonotic disease caused primarily by Mycobacterium tuberculosis. Current methods for screening and diagnosis rely on trunk wash culture, which has serious limitations due to low test sensitivity, slow turnaround time, and variable sample quality. Innovative and more efficient diagnostic tools are urgently needed. We describe three novel serologic techniques, the ElephantTB Stat-Pak kit, multiantigen print immunoassay, and dual-path platform VetTB test, for rapid antibody detection in elephants. The study was performed with serum samples from 236 captive African and Asian elephants from 53 different locations in the United States and Europe. The elephants were divided into three groups based on disease status and history of exposure: (i) 26 animals with culture-confirmed TB due to M. tuberculosis or Mycobacterium bovis, (ii) 63 exposed elephants from known-infected herds that had never produced a culture-positive result from trunk wash samples, and (iii) 147 elephants without clinical symptoms suggestive of TB, with consistently negative trunk wash culture results, and with no history of potential exposure to TB in the past 5 years. Elephants with culture-confirmed TB and a proportion of exposed but trunk wash culture-negative elephants produced robust antibody responses to multiple antigens of M. tuberculosis, with seroconversions detectable years before TB-positive cultures were obtained from trunk wash specimens. ESAT-6 and CFP10 proteins were immunodominant antigens recognized by elephant antibodies during disease. The serologic assays demonstrated 100% sensitivity and 95 to 100% specificity. Rapid and accurate antibody tests to identify infected elephants will likely allow earlier and more efficient treatment, thus limiting transmission of infection to other susceptible animals and to humans

Chambers, M. A. (2009). "Review of the diagnosis and study of tuberculosis in non-bovine wildlife species using immunological methods." Transboundary and Emerging Diseases 56: 215-227.

Mikota, S. K. (2008). "Review of tuberculosis in captive elephants and implications for wild populations." Gajah 28: 8-18.

Abraham, D. and J. Davis (2008). "Revised trunk wash collection procedure for captive elephants in a range country setting." Gajah 28: 53-54.

(2008) Guidelines for the control of tuberculosis in elephants.

Une, Y. and T. Mori (2007). "Tuberculosis as a zoonosis from a veterinary perspective." Comp Immunol Microbiol Infect Dis 30: 415-425.

Tuberculosis is an important disease among many zoonoses, because both Mycobacterium tuberculosis and Mycobacterium bovis, which are the major causes of tuberculosis, are highly pathogenic, infect many animal species and thus are likely to be the source of infection in humans. In particular, monkeys are highly susceptible to these bacteria and are important spreaders. Recently, two outbreaks of M. tuberculosis occurred in four different kinds of monkeys and humans were also infected with the disease in Japan. In zoos, tuberculosis was reported not only in monkeys, but also in several different kinds of animals, including elephants. Pets such as dogs and cats are believed to be generally less susceptible to M. tuberculosis, but in this article we introduce a case of infection from man to dog by close contact. Japan is one of the few countries that have been able to control M. bovis infection. In other countries, however, cases of bovine tuberculosis and human M. bovis infection have been reported, and thus further attention is still required in the future.

Mikota, S. K., M. Miller, G. Dumonceaux, K. Giri, K. Gairhe, K. Hamilton, S. Paudel, K. Lyashchenko, R. S. Larsen, J. Payeur, R. Waters, M. D. Salman and G. Kaufman (2007). Comparison of four serological tests and culture to determine tuberculosis infection in captive elephants in Nepal. Proceedings AAZV,AAWV,AZA/NAG Joint Conference.

Hamilton, K., S. K. Mikota, M. Miller, G. Dumonceaux, K. Giri, K. Gairhe, S. Paudel and G. Kaufman (2007). Evaluation of blood chemistry values for possible relationship to tuberculosis infection status in captive elephants in (Elephas maximus) Nepal. Proceedings AAZV,AAWV,AZA/NAG Joint Conference.

One hundred fifteen captive elephants (Elephas maximus) were examined in Nepal as part of a tuberculosis (TB) survey in January 2006. Blood chemistry analysis was performed at Disney's Animal Kingdom laboratory (USA). Trunk wash cultures were performed both in Nepal and in the USA, and serologic TB tests were performed in the USA. Based on culture and serology results, the elephants were grouped as follows: Group 1 (high risk, suggestive or confirmatory for TB infection) and Group 2 (low risk, equivocal or negative for TB infection). Within these groups, subgroups were created based on specific tests results. Blood chemistry results were analyzed to reveal any relationships between these values and TB infection status. Student t-tests were performed on each value between Groups 1 and 2. The only significant difference was a higher BUN/creatinine ratio ($p=0.047$) in Group 1. ANOVA analysis was performed on each value between the various groups. Significant differences were found in the albumin level ($p=0.015$) within the Group 1 subgroups and in the albumin level ($p=0.002$), alpha globulin 1 level ($p=0.030$), and A/G ratio ($p=0.012$) within the Group 2 subgroups.

This study did not reveal an association between certain chemistry values and TB infection. However, this may be due to a variety of age, reproductive statuses, stages of infections, and other possible medical conditions. Future testing of this population will help better define the TB infection status of elephants and may provide additional information to more precisely determine any association between blood chemistry values and tuberculosis infection in Nepal elephants.

ACKNOWLEDGMENTS
The authors gratefully acknowledge the cooperation of the Nepal Department of National Parks and Wildlife Conservation, support from the Abraham Foundation, the Mazuri Fund, the Walter J. Ernst Memorial Fund, the American Veterinary Medical Foundation, and the Dodge Foundation, and

research contributions from Konstantin Lyashchenko, Dr. Scott Larsen, Dr. Janet Payeur, and Dr. Ray Waters.

Rothschild, B. M. and L. D. Martin (2006). "Did ice-age bovids spread tuberculosis?" Naturwissenschaften 93: 565-569.

Pathognomonic metacarpal undermining is a skeletal pathology that has been associated with Mycobacterium tuberculosis in bovids. Postcranial artiodactyl, perissodactyl, and carnivore skeletons were examined in major university and museum collections of North America and Europe for evidence of this and other pathology potentially attributable to tuberculosis. Among nonproboscidean mammals from pre-Holocene North America, bone lesions indicative of tuberculosis were restricted to immigrant bovids from Eurasia. No bone lesions compatible with diagnosis of tuberculosis were found in large samples of other pre-Holocene (164 Oligocene, 397 Miocene, and 1,041 Plio-Pleistocene) North American mammals, including 114 antilocaprids. Given the unchanged frequency of bovid tubercular disease during the Pleistocene, it appears that most did not die from the disease but actually reached an accommodation with it (as did the mastodon) (Rothschild and Laub 2006). Thus, they were sufficiently long-lived to assure greater spread of the disease. The relationships of the proboscidean examples need further study, but present evidence suggests a Holarctic spread of tuberculosis during the Pleistocene, with bovids acting as vectors. While the role of other animals in the transmission of tuberculosis could be considered, the unique accommodation achieved by bovids and mastodons makes them the likely "culprits" in its spread.

Rothschild, B. M. and R. Laub (2006). "Hyperdisease in the late Pleistocene:validation of an early 20th century hypothesis." Naturwissenschaften 93: 557-564.

Peloquin, C. A., J. N. Maslow, S. K. Mikota, A. Forrest, F. Dunker, R. Isaza, L. R. Peddie, J. Peddie and M. Zhu (2006). "Dose selection and pharmacokinetics of rifampin in elephants for the treatment of tuberculosis." Journal of Veterinary Pharmacology and Therapeutics 29(6): 581-585.

Moller, T., B. O. Roken, S. S. Lewerin and K. Lyashchenko (2006). "The elephant Rapid Test (RT) the future diagnostic test for TB (M. tuberculosis) in elephants? Call for a validation study in Europe." Proceedings International Elephant Conservation and Research Symposium: 119-124.

Mikota, S. K., M. Miller, G. Dumonceaux, K. Giri, K. Gairhe, K. Hamilton, S. Paudel and B. Vincent (2006). Elephant tuberculosis diagnosis: implications for elephant management in Asian range countries. 2006 Proceedings American Association of Zoo Veterinarians.

Serologic tests including the ELISA, MAPIA (Multi-Antigen Print Immunoassay), and a rapid test, VetTB StatPak® (Chembio Diagnostic Systems, Inc., Medford, New York 11763 USA) have recently been developed and show great promise for the diagnosis of tuberculosis (TB) in elephants. These serologic tests detect antibodies to antigens of Mycobacterium tuberculosis complex organisms and in some cases have detected infection years in advance of active disease and mycobacterial shedding. The diagnosis of active TB (by culture) or serologic conversion presents management challenges for captive elephants in Asian range countries. Of the 2 billion humans world-wide infected with TB, fewer than 10% will develop active disease. This figure is unknown for elephants. The identification and management of infected elephants has ramifications for elephants and humans alike and issues such as public health and tourism may be impacted. TB is endemic among humans in Asia and where there is intermingling of elephants and humans, both species may act as reservoirs for disease transmission. The various situations in which elephants are kept in Asia (government-owned, privately-owned,

festivals, temples, zoos, etc.) make it difficult to develop a management strategy that will address all circumstances. Other concerns are the cost of treatment for an elephant (~ $50,000 USD) and appropriate monitoring in resource-poor countries. The authors have recently undertaken the screening of 120 elephants in Nepal to further evaluate the above-mentioned (and other) diagnostic tests. To our knowledge, this is the first organized, large-scale initiative to screen Asian elephants within a range country. Preliminary discussions regarding the management of both culture and serologically positive government-owned and privately-owned elephants in Nepal have been initiated and may serve as a starting point for other countries as more elephants are screened within Asia. Basic options for active (culturepositive) cases include (1) treatment, (2) segregation or (3) euthanasia. Options for latent disease (culture-negative, serologically positive) cases include (1) treatment, (2) segregation and monitoring for active disease and (3) euthanasia. The particular ownership/husbandry system, available resources and cultural constraints may dictate final management choices in range countries.

Mikota, S. K., G. Dumonceaux, M. Miller, K. Gairhe, K. Giri, J. V. Cheeran, D. Abraham, K. Lyashchenko, S. Larsen, J. Payeur, R. Waters, G. Kaufman and \ (2006). "Tuberculosis in elephants: An update on diagnosis and treatment; implications for control in range countries." Proceedings International Elephant Conservation and Research Symposium: 109-118.

Michel, A. L., R. G. Bengis, D. F. Keet, M. Hofmeyr, L. M. de Klerk, P. C. Cross, A. E. Jolles, D. Cooper, I. J. Whyte, P. Buss and J. Godfroid (2006). "Wildlife tuberculosis in South African conservation areas:Implications and challenges." Veterinary Microbiology 112: 91-100.

Tuberculosis, caused by Mycobacterium bovis, was first diagnosed in African buffalo in South Africa's Kruger National Park in 1990. Over the past 15 years the disease has spread northwards leaving only the most northern buffalo herds unaffected. Evidence suggests that 10 other small and large mammalian species, including large predators, are spillover hosts. Wildlife tuberculosis has also been diagnosed in several adjacent private game reserves and in the Hluhluwe-iMfolozi Park, the third largest game reserve in South Africa. The tuberculosis epidemic has a number of implications, for which the full effect of some might only be seen in the longterm. Potential negative long-term effects on the population dynamics of certain social animal species and the direct threat for the survival of endangered species pose particular problems for wildlife conservationists. On the other hand, the risk of spillover infection to neighboring communal cattle raises concerns about human health at the wildlife-livestock-human interface, not only along the western boundary of Kruger National Park, but also with regards to the joint development of the Greater Limpopo Transfrontier Conservation Area with Zimbabwe and Mozambique. From an economic point of view, wildlife tuberculosis has resulted in national and international trade restrictions for affected species. The lack of diagnostic tools for most species and the absence of an effective vaccine make it currently impossible to contain and control this disease within an infected free-ranging ecosystem. Veterinary researchers and policy-makers have recognized the need to intensify research on this disease and the need to develop tools for control, initially targeting buffalo and lion.

Lyashchenko, K. P., R. Greenwald, J. Esfandiari, J. H. Olsen, R. Ball, G. Dumonceaux, F. Dunker, C. Buckley, M. Richard, S. Murray, J. B. Payeur, P. Andersen, J. M. Pollock, S. Mikota, M. Miller, D. Sofranko and W. R. Waters (2006). "Tuberculosis in elephants: antibody responses to defined antigens of Mycobacterium tuberculosis, potential for early diagnosis, and monitoring of treatment." Clin. Vaccine Immunol 13(7): 722-732.

Tuberculosis (TB) in elephants is a re-emerging zoonotic disease caused primarily by Mycobacterium tuberculosis. Current diagnosis relies on trunk wash culture, the only officially

recognized test, which has serious limitations. Innovative and efficient diagnostic methods are urgently needed. Rapid identification of infected animals is a crucial prerequisite for more effective control of TB, as early diagnosis allows timely initiation of chemotherapy. Serology has diagnostic potential, although key antigens have not been identified and optimal immunoassay formats are not established. To characterize the humoral responses in elephant TB, we tested 143 serum samples collected from 15 elephants over time. These included 48 samples from five culture-confirmed TB cases, of which four were in Asian elephants infected with M. tuberculosis and one was in an African elephant with Mycobacterium bovis. Multiantigen print immunoassay (MAPIA) employing a panel of 12 defined antigens was used to identify serologic correlates of active disease. ESAT-6 was the immunodominant antigen recognized in elephant TB. Serum immunoglobulin G antibodies to ESAT-6 and other proteins were detected up to 3.5 years prior to culture of M. tuberculosis from trunk washes. Antibody levels to certain antigens gradually decreased in response to antitubercular therapy, suggesting the possibility of treatment monitoring. In addition to MAPIA, serum samples were evaluated with a recently developed rapid test (RT) based on lateral flow technology (ElephantTB STAT-PAK). Similarly to MAPIA, infected elephants were identified using the RT up to 4 years prior to positive culture. These findings demonstrate the potential for TB surveillance and treatment monitoring using the RT and MAPIA, respectively

Dumonceaux, G. and S. Mikota (2006). "Tuberculosis treatment protocols and complications for elephants." Proceedings International Elephant Conservation and Research Symposium: 84-85.

Ball, R. L., G. Dumonceaux, J. H. Olsen, M. S. Burton and K. Lyashchenko (2006). Comparison of trunk wash results matched to multiantigen print immunoassay (MAPIA) in a group of captive Asian elephants (Elephas maximus). 2006 Proceedings American Association of Zoo Veterinarians.

Introduction: Between 1994 and June 2005, there were 34 confirmed cases of tuberculosis in elephants in the U.S. population. Thirty-one Asian (Elephas maximus) and three African (Loxodonta africana) elephants were affected. Mycobacterium tuberculosis was the etiologic agent in 33 cases and M. bovis in one case. Cases of tuberculosis caused by an unusual nontuberculous mycobacteria, M. szulgai have recently occurred as well. Currently, TB in elephants remains a diagnostic dilemma. The sensitivity of trunk wash culture, the currently recommended test for diagnosis, is unknown. False negatives have been documented (trunk wash negative elephants that were subsequently found to be culture positive at necropsy). Other non-culture techniques for TB diagnosis include ELISA, and PCR. A novel technology, MultiAntigen Print ImmunoAssay (MAPIA) and lateral-flow technology (Rapid Test) has been evaluated and used to diagnose tuberculosis in captive elephants with encouraging results. One concern with this serologic testing is the possibility of Mycobacterium other than tuberculosis (MOTT) cross-reacting with the antigen used in the Rapid Test or the MAPIA and leading to a false positive. With numerous MOTT routinely cultured from trunk washes, this is a valid concern. Methods and Materials: A retrospective analysis was done at Busch Gardens Tampa Bay and Chembio, Inc. that matched trunk wash results to serum samples. All serum was collected within 7 days of the trunk wash and analyzed with the Rapid Test and MAPIA. Four Asian elephants with a total of 18 samples met this criteria and had serum submitted for testing. Results and Discussion: Table 1 lists the results and the organisms cultured. While the sampling is limited in this pilot project, it appears that MOTT does not evoke a response when assayed with the Rapid Test or MAPIA. The recent cases of M. szulgai do demonstrate the potential usefulness for this test when a disease develops from MOTT. The usefulness of this new technology, taken in conjunction with other clinical data including trunk washes when indicated, is a valuable tool in the healthcare of captive elephants.

LITERATURE CITED

1 Lacasse, C., K.C. Gamble, K. Terio, L.L. Farina, D.A. Travis, and M.Miller. 2005. Mycobacterium szulgai osteroarthritis and pneumonia in an African elephant (Loxdonta africana). Proc. Am. Assoc. Zoo Vet. Ann. Meet. Pp. 170-172.
2 Larsen, R.S., M.D. Salman, S.K. Mikota, R. Isaza, R.J. Montali, and J. Triantis. 2000. Evaluation of a multiple-antigen enzyme-linked immunosorbent assay for detection of Mycobacterium tuberculosis infection in captive elephants. J. Zoo Wildl. Med. 31:291-302.
3 Lyashchenko, K., et al. 2000. A multiantigen print immunoassay for the serological diagnosis of infectious diseases. J. Immunol. Methods 242:91-100
4 Lyashchenko, K., M. Miller, and W.R. Waters. 2005. Application of multiple antigen print immunoassay and rapid lateral flow technology for tuberculosis testing of elephants. Proc. Am. Assoc. Zoo Vet. Ann. Meet. Pp. 64-65

Zhu, M., J. N. Maslow, S. K. Mikota, R. Isaza, F. Dunker, H. Riddle and C. A. Peloquin (2005). "Population pharmacokinetics of pyrazinamide in elephants." J. Vet. Pharmacol. Ther 28(5): 403-409.

This study was undertaken to characterize the population pharmacokinetics (PK), therapeutic dose, and preferred route of administration for pyrazinamide (PZA) in elephants. Twenty-three African (Loxodonta africana) and Asian (Elephas maximus) elephants infected with or in contact with others culture positive for Mycobacterium tuberculosis were dosed under treatment conditions. PZA was dosed daily at 20-30 mg/kg via oral (fasting or nonfasting state) or rectal (enema or suppository) administration. Blood samples were collected 0-24 h postdose. Population PK was estimated using nonlinear mixed effect modeling. Drug absorption was rapid with T(max) at or before 2 h regardless of the method of drug administration. C(max) at a mean dose of 25.6 (+/-4.6) mg/kg was 19.6 (+/-9.5 microg/mL) for PZA given orally under fasting conditions. Under nonfasting conditions at a mean dose of 26.1 +/- 4.2 mg/kg, C(max) was 25% (4.87 +/- 4.89 microg/mL) and area under concentration curve (AUC) was 30% of the values observed under fasting conditions. Mean rectal dose of 32.6 +/- 15.2 mg/kg yielded C(max) of 12.3 +/- 6.3 microg/mL, but comparable AUC to PZA administered orally while fasting. Both oral and rectal administration of PZA appeared to be acceptable and oral dosing is preferred because of the higher C(max) and lower inter-subject variability. A starting dose of 30 mg/kg is recommended with drug monitoring between 1 and 2 h postdose. Higher doses may be required if the achieved C(max) values are below the recommended 20-50 microg/mL range

Waters, W. R., M. V. Palmer, J. P. Bannantine, R. Greenwald, J. Esfandiari, P. Andersen, J. McNair, J. M. Pollock and K. P. Lyashchenko (2005). "Antibody responses in reindeer (Rangifer tarandus) infected with Mycobacterium bovis." Clinical and Diagnostic Laboratory Immunology 12(6): 727-735.

Despite having a very low incidence of disease, reindeer (Rangifer tarandus) are subject to tuberculosis (TB) testing requirements for interstate shipment and herd accreditation in the United States. Improved TB tests are desperately needed, as many reindeer are falsely classified as reactors by current testing procedures. Sera collected sequentially from 11 (experimentally) Mycobacterium bovis-infected reindeer and 4 noninfected reindeer were evaluated by enzyme-linked immunosorbent assay (ELISA), immunoblotting, and multiantigen print immunoassay (MAPIA) for antibody specific to M. bovis antigens. Specific antibody was detected as early as 4 weeks after challenge with M. bovis. By MAPIA, sera were tested with 12 native and recombinant antigens, which were used to coat nitrocellulose. All M. bovis-infected reindeer developed responses to MPB83 and a fusion protein, Acr1/MPB83, and 9/11 had responses to MPB70. Other antigens less commonly recognized included MPB59, ESAT-6, and CFP10. Administration of purified protein derivatives for skin testing boosted serum antibody responses, as detected by each of the assays. Of the noninfected reindeer, 2/4 had responses that were detectable immediately following skin testing, which correlated with pathological findings (i.e., presence of granulomatous lesions yet the absence of acid-fast bacteria). The levels of

specific antibody produced by infected reindeer appeared to be associated with disease progression but not with cell-mediated immunity. These findings indicate that M. bovis infection of reindeer elicits an antibody response to multiple antigens that can be boosted by skin testing. Serological tests using carefully selected specific antigens have potential for early detection of infections in reindeer.

Pandey, R. and G. K. Khuller (2005). "Antitubercular inhaled therapy: opportunities, progress and challenges." Journal of Antimicrobial Therapy 55: 430-435.

Moller, T., B. Roken, L. Petersson, C. Vitaud and K. Lyashchenko (2005). Preliminary results of a new serological test for detection of TB-infection (Mycobacterium tuberculosis) in elephants (Elephas maximus and Loxodonta africanum) - Swedish Case studies. Verh.ber.Erkrg.Zootiere.

Maslow, J. N., S. K. Mikota, M. Zhu, H. Riddle and C. A. Peloquin (2005). "Pharmacokinetics of ethambutol (EMB) in elephants." J Vet Pharmacol Ther 28(3): 321-323.

Lyashchenko, K., M. Miller and W. R. Waters (2005). Application of MAPIA (Multiple antigen print immunoassay) and rapid lateral flow technology for tuberculosis testing of elephants. 2005 Proceedings AAZV, AAWV, AZA Nutrition Advisory Group.

Tuberculosis (TB) remains a serious re-emerging disease in wildlife and zoo animals. Mycobacterium tuberculosis has been isolated from 30 captive Asian elephant (Elephas maximus within 14 herds in the United States (1994-2004) and Mycobacterium bovis has been isolated from one African elephant (Loxodonta africana) (Mikota, pers. comm.).3 There are several challenges with elephant TB diagnosis. Culture of trunk wash has relatively poor sensitivity and is subject to contamination. Skin test is not validated in elephants and there is little reliability in these results.4 Serologic tests are appealing because samples can be stored for future analysis, archived samples can be analyzed, various assay platforms can be directly compared, and these assays are amenable to serial analysis (e.g., to monitor therapy). There is currently a multiple antigen ELISA test available for experimental use in elephants.1

To improve tuberculosis control, new diagnostic tools should be rapid, accurate, and host species-independent. Two novel serologic methods, MultiAntigen Print ImmunoAssay (MAPIA) and lateral-flow technology (Rapid Test), have been adapted for use in white-tailed deer, European badger, cattle, and Asian and African elephants for the detection of TB-specific antibody. Serologic markers of diagnostic importance have been identified for each host tested so far. With MAPIA, a machine prints specific antigens horizontally on a nitrocellulose membrane which can be cut into strips and used in Western blot.2 Strips are incubated with test serum samples, then an anti-Ig conjugate and color developer. Using this assay, an antibody response to multiple mycobacterial antigens has been observed in sera from M. tb-infected elephants. No antibody response was detected to any antigens in non-infected elephant sera. Additionally, the kinetics of antibody responses by elephants undergoing antibiotic therapy indicates that the MAPIA could be used for monitoring treatment and to determine recrudescence of infection.

Using selected antigens, a lateral-flow test was developed for rapid antibody detection that can be used in multiple species. The Rapid Test can use serum, plasma, or whole blood and provides results within 15 min. These tests are similar to in-clinic tests for FIV/FeLV detection (snap test, IDDEX). If a band is present in the test strip, it indicates a positive reaction (antibody present).
A panel of sera from healthy and TB infected elephants showed good correlation between the MAPIA and the rapid test (Table 1).

In summary, it appears that TB-infected elephants produce a robust antibody response that can be detected in serologic assays. Of special significance is the kinetics of the response, which may permit earlier detection of infection than current diagnostic methods. While initial results are promising, additional studies are required to validate these two assays. A relatively small set of serum samples from documented infected and non-infected elephants was used, and more samples are needed to further validate the tests. MAPIA has been used to optimize antigen selection in order to make the most sensitive and specific Rapid Test. This strategy may also allow for identification of "treatment-sensitive" antigens that could be used in the MAPIA format to monitor TB therapy. While elephants will be used as an initial "proof of concept" species for test development, additional samples from other species will also be evaluated to determine applicability to other species (i.e., a host species-independent test), thus benefiting other groups such as primates, rhinos, cervids, etc.

ACKNOWLEDGMENTS
The authors thank the zoos and individuals that have provided samples and assistance with this research, including Ray Ball, Carol Buckley, Jenifer Chatfield, Genny Dumonceaux, Javan Esfandiary, Rena Greenwald, Scott Larsen, Susan Mikota, Torsten Moller, Dick Montali, Mike Richards, Heidi Riddle, Mo Salman, Scott Terrell, and many others. This research was supported by Chembio Diagnostics, Inc.

LITERATURE CITED

1 Larsen, R.S., M.D. Salman, S.K. Mikota, R. Isaza, R.J. Montali, and J. Triantis. 2000. Evaluation of a multiple-antigen enzyme-linked immunosorbent assay for detection of Mycobacterium tuberculosis infection in captive elephants. J. Zoo Wildl. Med. 31:291-302.

2 Lyashchenko, K., et al. 2000. A multiantigen print immunoassay for the serological diagnosis of infectious diseases. J. Immunol. Methods 242:91-100.

3 Mikota, S.K., and J. Maslow. 2002. Epidemiology and treatment of tuberculosis in elephants: 2002. Proc. Am. Assoc. Zoo Vet. Annu. Meet. Pp. 384-387.

Lewerin, S. S., S. L. Olsson, K. Eld, B. Roken, S. Ghebremichael, T. Koivula, G. Kallenius and G. Bolske (2005). "Outbreak of Mycobacterium tuberculosis infection among captive Asian elephants in a Swedish zoo." Vet. Rec 156(6): 171-175.

Between 2001 and 2003, there was an outbreak of tuberculosis in a Swedish zoo which involved elephants, giraffes, rhinoceroses and buffaloes. Cultures of trunk lavages were used to detect infected elephants, tuberculin testing was used in the giraffes and buffaloes, and tracheal lavage and tuberculin testing were used in the rhinoceroses. The bacteria isolated were investigated by spoligotyping and restriction fragment length polymorphism. Five elephants and one giraffe were found to have been infected by four different strains of Mycobacterium tuberculosis.

Larsen, R. S., M. Kay, J. Triantis and M. D. Salman (2005). Update on serological detection of Mycobacterium tuberculosis infection in Asian elephants. 2005 Proceedings AAZV, AAWV, AZA Nutrition Advisory Group.

Tuberculosis has become an important disease in captive elephants, particularly Asian elephants (Elephas maximus). Diagnosing tuberculosis in elephants has been problematic as many tests have inadequate sensitivity or specificity.2-4 A multiple-antigen enzyme-linked immunosorbent assay (ELISA) was previously investigated for detecting infection in Asian elephants and African elephants (Loxodonta africana); this test had excellent sensitivity and specificity, but needed further evaluation.1 Modifications to the multiple-antigen ELISA panel have since been made. Valuable antigens were retained, other antigens were removed, and new ones were added. This modified ELISA was re-

evaluated, using serum from 68 Asian elephants. Sixteen had M. tuberculosis -positive trunk cultures, while 52 were either culture negative at necropsy or had a history of negative trunk cultures and no contact with infected elephants. Seven elephants were evaluated over time. The test was 100% (95% CI; 95-100%) specific and 94% (95% CI; 79-100%) sensitive using two of the six antigens (M. bovis strain AN5 culture filtrate and M. tuberculosis early secretory antigenic target 6). "Effectively-treated" elephants had decreasing seroreactivity, but those that were culture-positive post-treatment were more consistently seroreactive. Although "effectivelytreated" elephants had declining seroreactivity, they still usually had higher values than animals that had never been infected. Serology continues to show great promise in detecting tuberculosis in elephants, often detecting infection months-to-years sooner than trunk wash culture. Advances in techniques may soon make serology even more practical. While serology should not replace trunk-wash culture, it is a useful adjunct for early detection of infection in elephants and for monitoring treatment.

ACKNOLWEDGMENTS We thank the many veterinarians, owners, caretakers, and managers of elephant-owning institutions that participated in this investigation, as well as Drs. Michele Miller and Susan Mikota for helping to coordinate sample collection. We also thank Kimberly Deines and other laboratory personnel who processed ELISA samples. The study was partially funded by a grant from USDA, CSREES to Colorado State University Program of Economically Important Infectious Animal Diseases.

LITERATURE CITED

1.Larsen, R.S., M.D. Salman, S.K. Mikota, R. Isaza, R.J. Montali, and J. Triantis. 2000. Evaluation of a multiple-antigen enzyme-linked immunosorbent assay for detection of Mycobacterium tuberculosis in captive elephants. J. Zoo Wildl. Med. 31: 291-302.

2. Mikota, S.K., L. Peddie, J. Peddie, R. Isaza, F. Dunker, G. West, W. Lindsay, R.S. Larsen, M.D. Salman, D. Chatterjee, J. Payeur, D. Whipple, C. Thoen, D.S. Davis, R.J. Montali and J. Maslow. 2001. Epidemiology and diagnosis of Mycobacterium tuberculosis in six groups of elephants. J. Zoo Wildl. Med. 32: 1-16.

3. Mikota, S.K., R.S. Larsen, and R.J. Montali. 2000. Tuberculosis in elephants in North America. Zoo Biol. 19: 393-403.

4. U.S. Department of Agriculture. 2003. Guidelines for the control of tuberculosis in elephants. Animal and Plant Health Inspection Service; Animal Care. Washington, D.C. http://www.aphis.usda.gov/ac/TBGuidelines2003.pdf.

Lacasse, C., K. C. Gamble, K. Terio, L. L. Farina, D. A. Travis and M. Miller (2005). Mycobacterium szulgai osteoarthritis and pneumonia in an African elephant (Loxodonta Africana). 2005 Proceedings AAZV, AAWV, AZA Nutrition Advisory Group.

Tuberculosis, particularly Mycobacterium bovis and M. tuberculosis, is an important health issue in zoological collections. Zoos are a particular public health concern because of the close contact between tuberculosis-susceptible animals and humans, specifically animal handlers and visitors.16 Evidence of M. tuberculosis transmission between humans and elephants, confirmed by DNA fingerprinting, has been reported.13 Between 1994 and 2001, M. tuberculosis was isolated from trunk washes of captive elephants from 11 herds in the United States.17 To date, most reported cases of tuberculosis have occurred in captive Asian elephants (Elephas maximus).14 In 1997, the National Tuberculosis Working Group for Zoo and Wildlife Species partnered with the USDA to formulate the "Guidelines for the Control of Tuberculosis in Elephants." 15 This document outlines criteria for the testing, surveillance, and treatment of tuberculosis in elephants. The guidelines recommend annual monitoring of elephants by mycobacterial culture of three direct trunk washes collected over 1 wk. Isolation of Mycobacterium avium and non-tuberculous mycobacteria from elephant trunk wash samples is common, but these organisms have not been associated with clinical disease.14,18 This case

report details clinical disease with fatal complications of an atypical mycobacterial infection in an African elephant (Loxodonta africana). In September 2003, an African elephant presented with acute, severe lameness of the left rear limb with subsequent swelling of the stifle. Diagnostic procedures included aspiration cytology of the swelling, radiographs, and thermographic imaging. The exact location of the injury could not be detected, but a lesion to the stifle or coxofemoral articulation was suspected. After 13 mo of treatment, including pulse therapy with a variety of nonsteroidal anti-inflammatory drugs (NSAIDs), weekly to biweekly injections of polysulfated glycosaminoglycan, and intensive foot care efforts to treat secondary pedal lesions of both rearlimbs, the animal died acutely. Gross necropsy revealed granulomatous osteomyelitis with necrosis/loss of the femoral head and acetabulum and pulmonary granulomas. Both of these lesions contained acid-fast bacteria on cytology. While awaiting confirmatory culture results, quarantine procedures were established for the elephant facility and a program was established to screen all zoo personnel in close contact with the elephant or who participated in the necropsy. All personnel were tested by the Chicago Department of Public Health without documented conversion. Mycobacterium szulgai was ultimately cultured from both coxofemoral and pulmonary lesions. Mycobacterium szulgai is an uncommon nontuberculous mycobacterium that is usually isolated from pathologic lesions in humans.21 This bacterial species was first identified in 1972.11 The lungs are the main locality for pathologic manifestation in humans and several cases have been in patients with acquired immunodeficiency syndrome.9,20,21 Infection due to M. szulgai most frequently produces thin-walled cavities in lungs resembling tuberculosis.4 Other documented sites of infection include the skin, bone, and tendon sheath (causing a carpal tunnel syndrome).2,9,10,12,19,20 Intra-operative contamination from ice water has led to M. szulgai keratitis after laser-assisted ophthalmic surgeries.6 A case of disseminated disease in a previously healthy young human has been reported.5 No evidence of human-to-human transmission of this organism has been documented and human cases are believed to originate from environmental sources.12 The natural habitat of the organism is unknown, but previous reports suggest an association of the bacteria with water of swimming pools and fish tanks.1,21 The organism has been cultured from a snail and tropical fish.1,3 No standard recommendation for the treatment of M. szulgai infection currently exists. In general, triple antibiotic therapies used in standard mycobacterial treatments are reported with a low rate of relapses and sterilization of sputum cultures within a mean of 3 mo.3 Pulmonary lesions in this elephant were chronic; it was not possible to determine when initial infection occurred. Infection could have occurred in captivity or in the wild prior to captivity. Three trunk washes over the past year had been negative for mycobacterial culture. Osteomyelitis in the hip may have developed secondary to hematogenous spread from the lungs with the acute lameness resulting from a pathologic fracture associated with this infection. Alternatively, though considered less likely, a traumatic fracture of the hip could have occurred, with bacterial inoculation and secondary osteomyelitis as a result of increased blood flow to the site. The source of infection for this elephant remains unknown. Prevalence of this organism in the natural habitat or captive environment of the elephants has not been previously documented.

LITERATURE CITED

1 Abalain-Colloc, M.L., D. Guillerm, M. Salaun, S. Gouriou, V. Vincent, and B. Picard. 2003. Mycobacterium szulgai isolated from a patient, a tropical fish, and aquarium water. Eur. J. Clin. Microbiol. Infect. Dis. 22: 768-769.

2.Cross, G.M., M. Guill, and J.K. Aton. 1985. Cutaneous Mycobacterium szulgai infection. Arch. Dermatol. 121: 247-249.

3. Davidson, P.T. 1976. Mycobacterium szulgai: a new pathogen causing infection of the lung. Chest 69: 799- 801.

4. Dylewski, J.S., H.M. Zackon, A.H. Latour, and G.R. Berry. 1987. Mycobacterium szulgai: an unusual pathogen. Rev. Infect. Dis. 9: 578-580.

5. Gur, H., S. Porat, H. Haas, Y. Naparstek, and M. Eliakim. 1984. Disseminated mycobacterial disease caused by Mycobacterium szulgai. Arch. Intern. Med. 144: 1861-1863.
6.Holmes, G.P., G. Bond, R.C. Fader, and S.F. Fulcher. 2002. A cluster of cases of Mycobacterium szulgai keratitis that occurred after laser-assisted in situ keratomileusis. Clin. Infect. Dis. 34: 1039-1046.
7.Horusitzky, A., X. Puechal, D. Dumont, T. Begue, M. Robineau, and M. Boissier. 2000. Carpal tunnel syndrome caused by Mycobacterium szulgai. J. Rheumatol 27: 1299-1302.
8.Hurr, H., and T. Sorg. 1998. Mycobacterium szulgai osteomyelitis. J. Infect. 37: 191-192.
9.Luque, A.E., D. Kaminski, R. Reichman, and D. Hardy. 1998. Mycobacterium szulgai osteomyelitis in an AIDS patient. Scand. J. Infect. Dis. 30: 88-91.
10.Maloney, J.M., C.R. Gregg, D.S. Stephens, F.A. Manian, and D. Rimland. 1987. Infections caused by Mycobacterium szulgai in humans. Rev. Infect. Dis. 9: 1120-1126.
11.Marks, J., P.A. Jenkins, and M. Tsukamura. 1972. Mycobacterium szulgai: a new pathogen. Tubercle 53: 210.
12.Merlet, C., S. Aberrane, F. Chilot, and J. Laroche. 2000. Carpal tunnel syndrome complicating hand flexor tenosynovitis due to Mycobacterium szulgai. Joint Bone Spine 67: 247-248.
13.Michalak, K., C. Austin, S. Diesel, J.M. Bacon, P. Zimmerman, and J. N. Maslow. 1998. Mycobacterium tuberculosis infection as a zoonotic disease: transmission between humans and elephants. Emerg. Infect. Dis. 4: 283-287.
14.Mikota, S.K., R.S. Larsen, and R.J. Montali. 2000. Tuberculosis in elephants in North America. Zoo Biol. 19: 393-403.
15.National Tuberculosis Working Group for Zoo and Wildlife Species. 2000. Guidelines for the control of tuberculosis in elephants. USDA Animal and Plant Health Inspection Services.
16.Oh, P., R. Granich, J. Scott, B. Sun, M. Joseph, C. Stringfield, S. Thisdell, J. Staley, D. Workman-Malcolm, L. Borenstein, E. Lehnkering, P. Ryan, J. Soukup, A. Nitta, and J. Flood. 2002. Human exposure following Mycobacterium tuberculosis infection of multiple animal species in a metropolitan zoo. Emerg. Infect. Dis. 8: 1290-1293.
17.Payeur, J.B., J.L. Jarnagin, J.G. Marquardt, and D.L. Whipple. 2002. Mycobacterial isolations in captive elephants in the United States. Ann. N.Y. Acad. Sci. 969: 256-258.
18.Shojaei, H., J.G. Magee, R. Freeman, M. Yates, N.U. Horadagoda, and M. Goodfellow. 2000. Mycobacterium elephantis sp. nov., a rapidly growing non-chromogenic Mycobacterium isolated from an elephant. Int. J. Syst. Evol. Microbiol. 50: 1817-1820.
19.Stratton, C.W., D.B. Phelps, and L.B. Reller. 1978. Tuberculoid tenosynovitis and carpal tunnel syndrome caused by Mycobacterium szulgai. Am. J. Med. 65: 349-351.
20.Tappe, D., P. Langmann, M. Zilly, H. Klinker, B. Schmausser, and M. Frosch. 2004. Osteomyelitis and skin ulcers caused by Mycobacterium szulgai in an AIDS patient. Scand. J. Infect. Dis. 36: 883-885.
21.Tortoli, E., G. Besozzi, C. Lacchini, V. Penati, M.T. Simonetti, and S. Emler. 1998. Pulmonary infection due to Mycobacterium szulgai, case report and review of the literature. Eur. Respir. J. 11: 975-977.

Cousins, D. V. and N. Florisson (2005). "A review of tests available for use in the diagnosis of tuberculosis in non-bovine species." Rev Sci Tech Off Int Epiz 24(3): 1039-1059.

Bovine tuberculosis is an important disease that has impacts on regional and international trade. The disease can affect both social and economic stability and have a deleterious affect on species diversity. The intradermal tuberculin test has been in use for almost a century and, despite the technological advances of the last two decades, is still the only prescribed test for the diagnosis of tuberculosis in cattle. Many other species of animal, including humans, can be infected with Mycobacterium bovis. This paper reviews the various tests that have been used by researchers for

detecting infection with M. bovis in a variety of animal species, and attempts to prioritise or comment on the importance of having appropriately validated diagnostics for the different species. The difficulties of test validation using small numbers of animals, especially when tuberculosis occurs in only a few instances or the species of animal affected is rare and/or valuable, are discussed.

Stringfield, C. E., P. Oh, R. Granich, J. Scott, B. Sun, M. Joseph, J. Flood and C. J. Sedgwick (2004). Epidemiologic investigation of a Mycobacterium tuberculosis infection of multiple animal species in a metropolitan zoo. 2004 PROCEEDINGS AAZV, AAWV, WDA JOINT CONFERENCE.

From 1997 to 2000, six cases of Mycobacterium tuberculosis (TB) infection were diagnosed in three species of animals at, or recently originating from, the Los Angeles Zoo. Restriction fragment length polymorphism (RFLP) analysis showed that five of six animal isolates shared an identical IS6110 pattern, with the sixth differing only by one additional band. A multiinstitutional epidemiologic investigation was conducted to identify and interrupt possible transmission among the animal cases, and to screen personnel for active TB infection and TB skin-test conversion.

Animal Cases

In April and October of 1994, Asian elephant (Elephas maximus) #1 and Asian elephant #2 arrived at the Los Angeles Zoo from a private elephant facility where they had lived together. They were housed together at the zoo until November of 1996 when elephant #2 was returned to the facility for several months before transfer to another zoo. In the spring of 1997, Elephant #1 (30 yr old) died of salmonellosis, with M. tuberculosis found in granulomatous lymph node lesions from the thoracic and abdominal cavities, and Elephant #2 (30 yr old) was found to have a positive trunk wash culture for M. tuberculosis. In July of 1998, one of a closed herd of three Rocky Mountain goats (Oreamnos americanus) consisting of a sire and two offspring, died of pulmonary M. tuberculosis at 6 yr of age. The goat's asymptomatic herdmates were screened and had negative chest radiographs and tracheal wash cultures, but one of the two goats was positive on tuberculin skin-test. In October of 1998, a clinically normal Black rhinocerus (Diceros bicornis) was diagnosed with Mycobacerium tuberculosis after a positive skin test and nasal wash culture. In the winter of 1998, the two remaining goats were evaluated again with negative chest radiographs and tracheal wash cultures. However, 1 yr later, both were humanely euthanatized at 8 and 12 yr of age due to clinical evidence of tuberculosis on chest radiographs (both animals), and active clinical signs in one (neither were able to be orally treated). In January of 2001, a rhino was humanely euthanatized after a protracted illness that was nonresponsive to aggressive treatment. The rhino was found to have severe multifocal hemosiderosis and atypical mycobacterial infection in her lungs, with no M. tuberculosis cultured. This animal had been treated with oral Isoniazid and Rifampin for 1 yr, cultured routinely, and was never culture positive again.

Epidemiologic Investigation

Investigators examined medical and location histories of the affected animals, animal handling practices, health-care procedures, and performed an infection control assessment of the animal compounds and health-care facilities (including measuring air flow in the compounds by smoke testing). We conducted a review of zoo employee medical records for evidence of TB symptoms, tuberculin skin-test results, and chest radiograph information. A list of current and former employees was cross-matched with reported TB cases in the California state registry from 1985 to 2000. As part of the annual occupational health screening in June of 2000, zoo employees underwent questioning regarding TB symptoms, received tuberculin skin tests, and completed a questionnaire on medical history, job type, and history of contact with the infected animals.

Epidemiologic Findings

No common cross-species contact outside the animal compounds and no contact with an infectious human were found. The distance at which the public was kept from the animals and the distance of the compounds from each other (the elephant compound was 27 meters from the rhino compound and

the goat compound was 90 m from both) suggests that direct transmission was unlikely. No active TB cases in humans were found, and no matches were found in the database of reporte d cases. The RFLP analysis of this strain of M. tuberculosis matched that of three elephants with which #1 and #2 were housed at a private elephant facility from September of 1993-February of 1994.1 We hypothesize that elephants #1 and #2 were infected at the private facility and were shipped with latent M. tuberculosis infection in 1994, subsequently infecting the black rhino and Mountain goats at the Los Angeles Zoo. Of interest, animal caretaking and animal contact were not associated with a positive tuberculin skin-test, while groundskeepers were found to have an increased risk of tuberculin skin-test conversion compared with other job categories. Employees attending the elephant necropsy and employees who trained elephants were more likely to have tuberculin skin-test conversion than those who did not.

Conclusion

This is the first documented human and veterinary epidemiologic investigation of Mycobacterium tuberculosis affecting multiple species in a zoo. 2 No evidence of transmission from humans to animals or active infections in humans were found. Genotyping evidence strongly suggests transmission from one species to another, although no evidence of transmission was discovered. Human tuberculin skin-test conversions associated with the elephants were most likely due to lack of respiratory protection for these employees when the risk of TB infection was not known. The finding that groundskeepers and not animal handlers were associated with a higher risk of tuberculin skin-test conversion was surprising, and we hypothesized that this may have to do with groundskeepers as a group being more likely to have
been born outside of the United States.

Control measures to eliminate the spread of disease to people and animals were undertaken immediately and throughout this outbreak, and no further cases of M. tuberculosis have been diagnosed at the zoo in the past 3 yr despite ongoing surveillance. Four elephants and three rhinos that had direct contact with the infected animals remain TB negative by trunk and nasal wash culture methods as outlined by the USDA for elephant TB surveillance. Methods of indirect transmission in mammalian zoo species and causes of variability in infection and morbidity within and among species warrant further investigation. Ongoing vigilance, occupational health programs and infection control measures in potentially exposed animals are recommended to prevent ongoing transmission of M. tuberculosis in zoo settings.

Acknowledgments

The authors thank the Animal Care and Animal Health staff of the Los Angeles Zoo who cared so well for these animals, and the veterinarians (including consulting pathologists), technicians, and medical records staff who collected, analyzed, and organized the clinical data. We could not have performed this evaluation without Sue Thisdell, Safety Officer at the Los Angeles Zoo; Jothan Staley and Donna Workman-Malcom of the City of Los Angeles Occupational Health Services Division; Lee Borenstein, Elenor Lehnkering, Patrick Ryan, Jeanne Soukup, and Annette Nita of the Los Angeles County Department of Health Services; and Diana Whipple for her RFLP expertise.

LITERATURE CITED

1. Mikota, S.K., L. Peddie, J. Peddie, R. Isaza, F. Dunker, G. West, W. Lindsay, R.S.Larsen, M. D. Salman, D. Chatterjee, J. Payeur, D. Whipple, C. Thoen, D. Davis, C. Sedgwick, R.J. Montali, M. Ziccardi, J. Maslow. 2001. Epidemiology and diagnosis of Mycobacterium tuberculosis in captive asian elephants (Elephas maximus). J. Zoo Wildl. Med. 32: 1-16.

2. Oh, P., R. Granich, J. Scott, B. Sun, M. Joseph, C. Stringfield, S. Thisdell, J. Staley, D. Workman-Malcolm, L. Borenstein, E. Lehnkering, P. Ryan, J. Soukup, A.Nitta, J. Flood. 2002. Human exposure following Mycobacterium tuberculosis infection of multiple animal species in a metropolitan zoo. Emerging Infectious Diseases. 8 (11): 1290-1293.orte

Kaneene, J. B. and C. Thoen (2004). "Tuberculosis." JAVMA 224(5): 685-691.

Janssen, D. L., J. E. Oosterhuis, J. Fuller and K. Williams (2004). Field technique: A method for obtaining trunk wash mycobacterial cultures in anesthetized free-ranging African elephants (Loxodonta africana). 2004 PROCEEDINGS AAZV, AAWV, WDA JOINT CONFERENCE.

The Guidelines for the Control of Tuberculosis in Elephants 2003 (Guidelines) of the National tuberculosis Working Group for Zoo and Wildlife Species were written to protect the health and safety of captive elephants together with their handlers and the viewing public.1 The Guidelines specifically address the display and transport of captive elephants but do not address the unique situation of free-living elephants being imported and subsequently displayed to the public.

Although the Guidelines describe a technique for collecting and handling a trunk wash in a trained, standing, non-anesthetized elephant, it does not describe a similar technique for anesthetized elephants in lateral recumbency. In an attempt to detect active mycobacterial infection in a group of 3 male and 8 female free-ranging African elephants scheduled for import into the United States, a technique was developed for collecting trunk washes in recumbent, anesthetized elephants for mycobacterial culture.

A South African game-capture crew, experienced in translocating elephants, anesthetized elephants in groups via remote drug delivery and from a helicopter. The ground crew accomplished multiple simultaneous procedures including anesthesia maintenance and monitoring, physical and reproductive examinations, collection of general diagnostic and investigative samples, and trunk washes for mycobacterial cultures. This was accomplished while the capture crew was preparing animals for loading into specially designed trailers for transport to a holding boma. Little time was available for any one of procedure with multiple
animals being attended to at one time.

Once an elephant was stable in lateral recumbency, a 3-m foal stomach tube, prepackaged and sterilized, was inserted into the dependent side of the trunk tip. It was then gently fed up the trunk approximately 2.5 m. A 50-ml sample suction trap was attached to the end of the foal tube.The suction trap was then attached to a battery powered, portable aspirator pump designed for emergency medical care. The aspiration pump was activated to collect secretions from the most proximal portion of the trunk. If little or no secretions were collected by this means, the system was disconnected between the sample trap and the foal tube. Then, 100 ml of sterile saline was placed into raised end of the foal tube allowing it to drain toward the tip through gravity. The suction trap and aspiration pump were reattached to collect a sample in the sample trap. Then, the sample trap was replaced with a new trap, and the foal tube was inserted into the oral pharynx for collection of a separate oropharyngeal sample. This same procedure was repeated
with each elephant.

ACKNOWLEDGMENTS
So African veterinarians, Mike Bester, Larry Killmar, Janet Payeur, ARC/OVI, Thomas Hildebrant, Eric Zeehandelar, Kevin Reily, Denise SoFranko.

LITERATURE CITED
1. National tuberculosis Working Group for Zoo and Wildlife Species. 2003. Guidelines for the Control of Tuberculosis in Elephants 2003. USDA-APHIS: http://www.aphis.usda.gov/ac/TBGuidelines2003.pdf

Hirsch, D. C. and E. L. Biberstein (2004). Mycobacterium. Veterinary Microbiology. D. C. Hirsch, N. J. MacLachlan and R. L. Walker. Ames, Iowa, Blackwell: 223-234.

Gerston, K. F., L. Blumberg, V. A. Tshabalala and J. Murray (2004). "Viability of mycobacteria in formalin-fixed lungs." Hum Pathol 35(5): 571-575.

Ziccardi, M., H. N. Wong, L. A. Tell, D. Fritcher, J. Blanchard, A. Kilbourn and H. P. Godfrey (2003). Further optimization and validation of the antigen 85 immunoassay for diagnosing mycobacteriosis in wildlife. Proc Amer Assoc Zoo Vet.

Mycobacteriosis caused by Mycobacterium bovis, M. tuberculosis and M. avium has been a well-documented health problem for zoological collections as long ago as the late 19th century. Prevalence estimation in these captive wildlife populations, however, has been hampered by diagnostic test methods that are oftentimes difficult or impossible to conduct and/or interpret (due to the requirement for multiple immobilizations for measurement of response), the occurrence of non-specific results with methods such as the intradermal skin test, and/or the near-total lack of validation, optimization and standardization of any of the available test methods in the species of interest. Additionally, because intradermal skin testing is the primary screening method for many of these species, the ability to compare exposure in captive wildlife with exposure in free-ranging populations has been limited due to the difficulty with follow-up in free-ranging populations. Lastly, unlike testing methods that use serological techniques, skin testing precludes retrospective studies of banked samples to determine onset of reactivity.

Recently, human tuberculosis researchers working with tuberculosis in humans have developed an immunoassay that detects a serum protein complex (the antigen 85, or Ag85, complex) produced by mycobacteria in the early stages of mycobacterial infections1. Previous work has shown that this method is a promising diagnostic tool in the evaluation of tuberculosis exposure in some primate (including orangutan (Pongo pygmaeus), a species known for non-specific tuberculin responses)2 and captive hoofstock species3. In order to determine the feasibility and applicability of a widespread use of this method for captive and free-ranging wildlife species, we have undertaken a number of pilot studies on different populations of interest, with the goals of optimizing and validating the immunoassay through analysis of serum from known infected and non-infected individuals and through comparisons with other diagnostic methods. Thus far, we have begun evaluating the applicability of the antigen 85 immunoassay in various avian, primate, rhinoceros and hoofstock species for detecting tuberculosis and/or paratuberculosis (Johne's disease) infections. Preliminary results, a summary of which will be presented, indicate that this method may be a valuable adjunct to other testing methods (including gamma interferon and multiple-antigen ELISA) to allow a better evaluation of true mycobacterial status in these species.

LITERATURE CITED

1.Bentley-Hibbert, S. I., X. Quan, T. G. Newman, K. Huygen and H. P. Godfrey. 1999. Pathophysiology of Antigen 85 in patients with active tuberculosis. Infect Immun. 67(2):581-8.
2.Kilbourn, A. M., H. P. Godfrey, R. A. Cook, P. P. Calle, E. J. Bosi, S. I. Bentley-Hibbert, K. Huygen, M. Andau, M. Ziccardi and W. B. Karesh. 2001. Serum Antigen 85 levels in adjunct testing for active mycobacterial infections in orangutans. J. Wildl. Dis. 37(1): 65-71.
3.Mangold, B. J., R. A. Cook, M. R. Cranfield, K. Huygen, and H. P. Godfrey. 1999. Detection of elevated levels of circulating antigen 85 by dot immunobinding assay in captive wild animals with tuberculosis. J. Zoo Wildl. Med. 30(4): 477-483.

Rahman, T. (2003). Infectious and non-infectious disease of elephants. Healthcare, Breeding and Management of Asian Elephants. D. Das. New Delhi, Project Elephant. Govt. of India: 108-118.

Potters, D., M. Seghers, G. Muyldermans, D. Pie´rard, A. Naessens and S. Lauwers (2003). "Recovery of Mycobacterium elephantis from sputum of a patient in belgium." Journal of Clinical Microbiology 41(3): 1344-1344.

Mycobacterium elephantis was isolated from a human respiratory specimen in April 1999, demonstrating its presence in Europe. The biochemical reaction results, antimicrobial susceptibility pattern, and sequence data for this strain are all in agreement with those of M. elephantis strains isolated previously from other continents.

Pavlik, I., W. Y. Ayele, I. Parmova, I. Melicharek, M. Hanzlikova, M. Svejnochova and B. Kormendy (2003). "Mycobacterium tuberculosis in animal and human populations in six Central European countries during 1990-1999." Veterinarni Medicina 48(4): 83-89.

Results of Mycobacterium tuberculosis detection in animals from six Central European countries (Croatia, the Czech Republic, Hungary, Poland, Slovakia and Slovenia) spreading over 610402 km2 with a population of 11.8 million heads of cattle were analysed. In the monitoring period between 1990 and 1999, M. tuberculosis from animals was isolated only in two countries (Poland and Slovak Republic) from 16 animals with tuberculous lesions. These comprise 9 cattle (Bos taurus), 4 domestic pigs (Sus scrofa f. domestica) and three wild animals, an African elephant (Loxodonta africana), agouti (Dasyprocta aguti) and terrestrial tapir (Tapirus terrestris) from a zoological garden Gdansk in Poland. A steady decrease in the incidence of tuberculosis in humans was recorded during the monitoring period in all countries. The human population of the study countries was 68.03 million. In the period monitored, infection caused by M. tuberculosis was identified in a total of 241040 patients with a decreasing incidence of tuberculosis found in all countries. The lowest relative bacteriologically confirmed disease was found in the Czech Republic, Slovak Republic and Slovenia. Given the low number of infected domestic and wild animals, the epidemiological and epizootiological situation may be considered auspicious.

Michel, A. L., L. Venter, I. W. Espie and M. L. Coetzee (2003). "Mycobacterium tuberculosis infections in eight species at the National Zoological Gardens of South Africa, 1991-2001." Journal of Zoo and Wildlife Medicine 34(4): 364-370.

Between 1991 and 2001 a total of 12 cases of Mycobacterium tuberculosis infection in eight different species were recorded in the National Zoological Gardens of South Africa in Pretoria (Tshwane). The genetic relatedness between seven of the M. tuberculosis isolates was determined by IS6110 restriction fragment length polymorphism analysis. For the majority of the isolates that were analyzed, a high degree of polymorphism suggested different sources of infection. Evidence of M. tuberculosis transmission between animals is reported in two chimpanzees (Pan troglodytes) housed together, from which samples were collected for analysis 29 mo apart.

Chakraborty, A. (2003). "Diseases of elephants (Elephas maximus) in India-A Review." Indian Wildlife Year Book 2: 74-82.

(2003) Guidelines for the control of tuberculosis in elephants.

Turenne, C., P. Chedore, J. Wolfe, F. Jamieson, K. May and A. Kabani (2002). "Phenotypic and molecular characterization of clinical isolates of Mycobacterium elephantis from human specimens." J Clin Microbiol 40(4): 1230-1236.

Eleven strains of a rapidly growing mycobacterium were isolated from patient specimens originating from various regions of the province of Ontario, Canada, over a 2-year period. Unique high-performance liquid chromatography (HPLC) and PCR-restriction enzyme pattern analysis (PRA) profiles initially suggested a new Mycobacterium species, while sequencing of the 16S rRNA gene revealed a sequence match with Mycobacterium sp. strain MCRO 17 (GenBank accession no. X93028), an isolate determined to be unique which is to date uncharacterized, and also a close similarity to M. elephantis (GenBank accession no. AJ010747), with six base pair variations. A complete biochemical profile of these isolates revealed
a species of mycobacteria with phenotypic characteristics similar to those of M. flavescens. HPLC, PRA, and 16S rRNA sequencing of strain M. elephantis DSM 44368(T) and result comparisons with the clinical isolates revealed that these strains were in fact M. elephantis, a newly described species isolated from an elephant. All strains were isolated from human samples, 10 from sputum and 1from an axillary lymph node.

Peloquin, C. (2002). "Therapeutic drug monitoring in the treatment of tuberculosis." Drugs 62(15): 2169-2183.

Payeur, J. B., J. L. Jarnagin, J. G. Marquardt and D. L. Whipple (2002). "Mycobacterial isolations in captive elephants in the United States." Ann N Y Acad Sci 969: 256-258.

Interest in tuberculosis in elephants has been increasing over the past several years in the United States. Several techniques have been used to diagnose mammalian tuberculosis. Currently, the test considered most reliable for diagnosis of TB in elephants is based on the culture of respiratory secretions obtained by trunk washes.

Oh, P., R. Granich, J. Scott, B. Sun, M. Joseph, C. Stringfield, S. Thisdell, J. Staley, D. Workman-Malcolm, L. Borenstein, E. Lehnkering, P. Ryan, J. Soukup, A. Nitta and J. Flood (2002). "Human exposure following Mycobacterium tuberculosis infection of multiple animal species in a Metropolitan Zoo." Emerg Infect Dis 8(11): 1290-1293.

From 1997 to 2000, Mycobacterium tuberculosis was diagnosed in two Asian elephants (Elephas maximus), three Rocky Mountain goats (Oreamnos americanus), and one black rhinoceros (Diceros bicornis) in the Los Angeles Zoo. DNA fingerprint patterns suggested recent transmission. An investigation found no active cases of tuberculosis in humans; however, tuberculin skin-test conversions in humans were associated with training elephants and attending an elephant necropsy.

Mikota, S. K. and J. Maslow (2002). Epidemiology and Treatment of Tuberculosis in Elephants: 2002. American Association of Zoo Veterinarians Annual Conference.

Gavier-Widen, D., C. Hard Af Segerstad, B. Roken, T. Moller, G. Bolske and S. Sternberg (2002). Mycobacterium tuberculosis infection in Asian elephants (Elephas maximus) in Sweden. European Association of Zoo and Wildlife Veterinarians 4th Scientific Meeting.

Chandrasekharan, K. (2002). "Specific diseases of Asian elephants." Journal of Indian Veterinary Association Kerala 7(3): 31-34.

The earliest writing describing the diseases of elephants in ancient literature said to be the works on "Gajasastra" (Elephantology) written in Sanskrit by authors like Gautama, Narada,

Mrigacharma, Rajaputra and Vyasa. "Hasthyayurveda" a legendary book in Sanskrit written by a safe Palakapya deals with some diseases, treatment, desirable and undesirable points of selection, management practices and some mythological aspects on the origin of elephants. The earliest book in English dealing with diseases of elephants seems to be that of W. Gilchrist "A practical treatise on the treatment of diseases of elephants" published in 1848. Later Slym (1873), Sanderson (1878), Steel (1885), Evans (1910), Herpburn (1913), Milroy (1922), Ptaff (1940), Ferrier (1947), Utoke Gale (1974), Chandrasekharan (1979) and Panicker (1985) have documented their findings on the incidence, etiology and control of diseases of Asian elephants.

Auclair, B., S. Mikota, C. A. Peloquin, R. Aguilar and J. N. Maslow (2002). "Population pharmacokinetics of antituberculous drugs and treatment of Mycobacterium bovis infection in Bongo Antelope (Tragelaphus eurycrus isaaci )." Journal of Zoo and Wildlife Medicine 33(3): 193-203.

Alexander, K. A., E. Pleydell, M. C. Williams, E. P. Lane, J. F. C. Nyange and A. L. Michel (2002). "Mycobacterium tuberculosis: An Emerging Disease of Free-Ranging Wildlife." Emerging Infectious Diseases 8(6): 598-601.

Expansion of ecotourism-based industries, changes in land-use practices, and escalating competition for resources have increased contact between free-ranging wildlife and humans. Although human presence in wildlife areas may provide an important economic benefit through ecotourism, exposure to human pathogens
may represent a health risk for wildlife. This report is the first to document introduction of a primary human pathogen into free-ranging wildlife. We describe outbreaks of Mycobacterium tuberculosis, a human pathogen, in free-ranging banded mongooses (Mungos mungo) in Botswana and suricates (Suricata suricatta) in South Africa. Wildlife managers and scientists must address the potential threat that humans pose to the health of free-ranging wildlife.

Ratanakorn, P. (2001). Elephant Health Problems and Management in Cambodia, Lao and Thailand. A Research Update on Elephants and Rhinos; Proceedings of the International Elephant and Rhino Research Symposium, Vienna, June 7-11, 2001, Schuling Verlag.

Mikota, S. K., L. Peddie, J. Peddie, R. Isaza, F. Dunker, G. West, W. Lindsay, R. S. Larsen, M. D. Salman, D. Chatterjee, J. Payeur, D. Whipple, C. Thoen, D. S. Davis, C. Sedgwick, R. Montali, M. Ziccardi and J. Maslow (2001). "Epidemiology and diagnosis of Mycobacterium tuberculosis in captive Asian elephants (Elephas maximus)." Journal of Zoo and Wildlife Medicine 32(1): 1-16.

The deaths of two Asian elephants (Elephas maximus) in August 1996 led the United States Department of Agriculture to require the testing and treatment of elephants for tuberculosis. From August 1996 to September 1999. Mycobacterium tuberculosis infection was confirmed by culture in 12 of 118 elephants in six herds. Eight diagnoses were made antemortem on the basis of isolation of M. tuberculosis by culture of trunk wash samples; the remainder (including the initial two) were diagnosed postmortem. We present the case histories, epidemiologic characteristics, diagnostic test results, and therapeutic plans from these six herds. The intradermal tuberculin test, enzyme-linked immunosorbent assay serology, the blood tuberculosis test, and nucleic acid amplification and culture are compared as methods to diagnose M. tuberculosis infection in elephants.

Isaza, R. (2001). The elephant trunk wash - An update. ProcElephant Mangers Association Annual Conference.

Hecht, J. (2001). Telltale bones. New Scientist: 14.

Harr, K., R. Isaza and J. Harvey (2001). Clinicopathological findings in Mycobacterium tuberculosis culture-positive elephants (Elephas maximus) in comparison to clinically normal elephants. Proceedings American Association of Zoo Veterinarians, American Association of Wildlife Veterinarians, Association of Reptilian and Amphibian Veterinarians and the National Association of Zoo and Wildlife Veterinarians Joint Conference 2001, American Association of Zoo Veterinarians.

Davis, M. (2001). "Mycobacterium tuberculosis risk for elephant handlers and veterinarians." Appl Occup Environ Hyg 16(3): 350-353.

Clifton-Hadley, R. S., C. M. Sauter-Louis, I. W. Lugton, R. Jacson, P. A. Durr and J. W. Wilesmith (2001). Mycobacterial diseases. Infectious Diseases of Wild Mammals. E. S. Williams. Ames, Iowa, Iowa State University Press,: 340-361.

Ziccardi, M., S. K. Mikota, R. B. Barbiers and T. M. Norton (2000). Tuberculosis in zoo ungulates:Survey results and surveillance plan. Proc. AAZV and IAAAM Joint Conf.

Shojaei, H., J. G. Magee, R. Freeman, M. Yates, N. U. Horadagoda and M. Goodfellow (2000). "Mycobacterium elephantis sp. nov., a rapidly growing non-chromogenic Mycobacterium isolated from an elephant." International Journal of Systematic and Evolutionary Microbiology 50(5): 1817-1820.

A strain isolated from a lung abscess in an elephant that died from chronic respiratory disease was found to have properties consistent with its classification in the genus Mycobacterium. An almost complete sequence of the 16S rDNA of the strain was determined following the cloning and sequencing of the amplified gene. The sequence was aligned with those available on mycobacteria and phylogenetic trees inferred by using three tree-making algorithms. The organism, which formed a distinct phyletic line within the evolutionary radiation occupied by rapidly growing mycobacteria, was readily distinguished from members of validly described species of rapidly growing mycobacteria on the basis of its mycolic acid pattern and by a number of other phenotypic features, notably its ability to grow at higher temperatures. The type strain is Mycobacterium elephantis DSM 44368T. The EMBL accession number for the 16S rDNA sequence of strain 484T is AJ010747.

Mikota, S. K., R. S. Larsen and R. J. Montali (2000). "Tuberculosis in Elephants in North America." Zoo Biol 19: 393-403.

Within the past 4 years, TB has emerged as a disease of concern in elephants. The population of elephants in North America is declining (Weise,1997), and transmissible diseases such as TB may exacerbate this trend. Guidelines for all elephants for TB, were instituted in 1997 (USDA, 1997, 2000). Between August 1996 and May 2000, Mycobacterium tuberculosis was isolated form 18 of 539 elephants in North America, indicating an estimated prevalence of 3.3%. Isolation of the TB organism by culture is the currently recommended test to establish a diagnosis of TB; however, culture requires 8 weeks. Further research is essential to validate other diagnostic tests and treatment protocols.

Lyashchenko, K., M. Singh, R. Colangeli and M. L. Gennaro (2000). "A multi-antigen print immunoassay for the development of serological diagnosis of infectious disease." Journal of Immunological Methods 242: 91-100.

Larsen, R. S., M. D. Salman, S. K. Mikota, R. Isaza and J. Triantis (2000). Validation and use of a multiple-antigen ELISA for detection of tuberculosis infections in elephants. Proc. AAZV and IAAAM Joint Conf.

Larsen, R. S., M. D. Salman, S. K. Mikota, R. Isaza, R. J. Montali and J. Triantis (2000). "Evaluation of a multiple-antigen enzyme-linked immunosorbent assay for detection of Mycobacterium tuberculosis infection in captive elephants." Journal of Zoo and Wildlife Medicine 31(3): 291-302.

Mycobacterium tuberculosis has become an important agent of disease in the captive elephant population of the United States, although current detection methods appear to be inadequate for effective disease management. This investigation sought to validate a multiple-antigen enzyme-linked immunosorbent assay (ELISA) for screening of M. tuberculosis infection in captive elephants and to document the elephant's serologic response over time using a cross-sectional observational study design. Serum samples were collected from 51 Asian elephants (Elephas maximus) and 26 African elephants (Loxodonta africana) from 16 zoos and circuses throughout the United States from February 1996 to March 1999. Infection status of each animal was determined by mycobacterial culture of trunk washes. Reactivity of each serum sample against six antigens was determined, and the linear combination of antigens that accurately predicted the infection status of the greatest number of animals was determined by discriminant analysis. The resulting classification functions were used to calculate the percentage of animals that were correctly classified (i.e., specificity and sensitivity). Of the 77 elephants sampled, 47 fit the criteria for inclusion in discriminant analysis. Of these, seven Asian elephants were considered infected; 25 Asian elephants and 15 African elephants were considered noninfected. The remaining elephants had been exposed to one or more infected animals. The specificity and sensitivity of the multiple-antigen ELISA were both 100% (91.9-100% and 54.4-100%, respectively) with 95% confidence intervals. M. bovis culture filtrate showed the highest individual antigen specificity (95%; 83.0-100%) and sensitivity (100%; 54.4-100%). Serum samples from 34 elephants were analyzed over time by the response to the culture filtrate antigen; four of these elephants were culture positive and had been used to calculate the discriminant function. Limitations such as sample size, compromised ability to ascertain each animal's true infection status, and absence of known-infected African elephants suggest that much additional research needs to be conducted regarding the use of this ELISA. However, the results indicate that this multiple-antigen ELISA would be a valuable screening test for detecting M. tuberculosis infection in elephant herds.

Boomershine, C. S. and B. S. Zwilling (2000). "Stress and the pathogenesis of tuberculosis." Clinical Microbiology Newsletter 22(23): 177-182.

Mikota, S. K. (1999). "Diseases of the Elephant: A Review." Verh. ber. Erkrg. Zootiere 39: 1-15.

Mangold, B. J., R. A. Cook, M. R. Cranfield, K. Huygen and H. P. Godfrey (1999). "Detection of elevated levels of circulating antigen 85 by dot immunobinding assay in captive wild animals with tuberculosis." Journal of Zoo and Wildlife Medicine 30(4): 477-483.

Antemortem diagnosis of tuberculosis in captive wild animals is often difficult. In addition to the variability of host cellular immune response, which does not always indicate current active infection, reactivity to saprophytic or other mycobacteria is common and may interfere with the interpretation of the intradermal tuberculin skin test. Furthermore, the immobilization required for administrating the test and evaluating skin reactions in these animals may result in unacceptable levels of morbidity and mortality, of particular concern in individuals of rare or endangered species. Proteins of the antigen 85 (Ag85) complex are major secretory products of actively metabolizing mycobacteria in vitro. Production of these proteins by mycobacteria during growth in vivo could result in increases in circulating levels of Ag85 in hosts with active tuberculosis. A dot blot immunoassay has been used to detect and quantify circulating Ag85 in captive wild animals with tuberculosis. Elevated levels of Ag85 were observed in animals with active tuberculosis as compared with uninfected animals. Study populations included a herd of nyala (Tragelaphus angasi) (n=9) with no history of exposure to

Mycobacterium bovis. Serum Ag85 levels ranged from <5 to 15 uU/ml (median, 5 uU/ml). The other group included 11 animals from a mixed collection with a documented history of an M. bovis outbreak. Animals with pulmonary granulomatous lesions (n=3) had serum Ag85 levels ranging from 320 to 1,280 uU/ml (median, 320 uU/ml). Animals with only chronic mediastinal or mesenteric lymphadenitis (n=4) had serum Ag85 levels ranging from <5 to 80 uU/ml (median, <5 uU/ml). This assay could provide an important adjunct to intradermal skin testing for antemortem diagnosis of tuberculosis in nondomestic species.

Isaza, R. and C. J. Ketz (1999). "A Trunk Wash Technique for the Diagnosis of Tuberculosis in Elephants." Verh. ber. Erkrg. Zootiere 39: 121-124.

Biberstein, E. L. and D. C. Hirsch (1999). Mycobacterium species: The agents of animal tuberculosis. Veterinary Microbiology. Maiden, MA, Blackwell Science: 158-172.

Bhat, M. N., R. Manickam and J. Ramkrishna (1999). "Screening of captive wild animals for tuberculosis." Indian Veterinary Journal 76(11): 959-961.

The passive haemagglutination (PHA) test was used to test 109 captive elephants (Elephas maximus), and spotted deer (Cervus axis), blackbuck (Antilope cervicapra) and common langurs (Semnopithecus entellus?) (4 of each) for tuberculosis; 51 of the elephants and the 4 langurs were also assessed by the tuberculin test. PHA titres of 1:16 or 1:32 were found in 4 elephants, 1 deer and 2 langurs, but all were apparently healthy except 1 langur that had clinical signs indicative of tuberculosis. There were 4 positive reactors in the tuberculin tests, all elephants, but these animals did not have significant PHA titres. It is concluded that the procedures and reagents used for the diagnosis of tuberculosis in domestic animals are not reliable for testing wild animals.

Montali, R. J., L. H. Spelman, R. C. Cambre, D. Chattergee and S. K. Mikota (1998). Factors influencing interpretation of indirect testing methods for tuberculosis in elephants. Proceedings AAZV and AAWV Joint Conference.

Serologic and other laboratory tests (such as BTB, ELISA, and gamma interferon) are often used in conjunction with the intradermal tuberculin test to detect tuberculosis (TB) in animals. The skin test is considered the "gold standard" in domestic cattle and humans, and the BTB test has been highly rated for use in cervid species. However, these indirect methods for TB diagnosis have not been proven valid in most exotic species susceptible to Mycobacterium tuberculosis complex (which includes M. bovis) infection. In addition, many of the tuberculin skin testing methods used in exotic species are not uniform in terms of tuberculin type(s) and sites used and interpretation of the end points.

Michalak, K., C. Austin, S. Diesel, M. J. Bacon, P. Zimmerman and J. N. Maslow (1998). "Mycobacterium tuberculosis infection as a zoonotic disease: transmission between humans and elephants." Emerg Infect Dis 4(2): 283-287.

Between 1994 and 1996, three elephants from an exotic animal farm in Illinois died of pulmonary disease due to Mycobacterium tuberculosis. In October 1996, a fourth living elephant was culture-positive for M. tuberculosis. Twenty-two handlers at the farm were screened for tuberculosis (TB); eleven had positive reactions to intradermal injection with purified protein derivative. One had smear-negative, culture-positive active TB. DNA fingerprint comparison by IS6110 and TBN12 typing showed that the isolates from the four elephants and the handler with active TB were the same strain. This investigation indicates transmission of M. tuberculosis between humans and elephants.

Mahato, G., H. Rahman, K. K. Sharma and S. C. Pathak (1998). "Tuberculin testing in captive Indian elephants (Elephas maximus) of a national park." Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases 19(1): 63.

Full text:Tuberculosis, an important zoonotic disease, has been reported in wild African and Asian domestic elephants (Seneviratna and Seneviratna, 1966). Under this communication 25 cative Indian elephants of Kaziranga National Park, Assam, were tested for allergic reaction by injecting 0.1 ml PPD at the base of ear tip. The thickness of skin was measured after 48 and 72 h and an increase of 4 mm or more was taken as positive. Out of 25 elephants tested, 3 adults were found reactors. Base of the ear was found more appropriate site as it remained protected from rubbing against hard object due to irritation caused by the tuberculin and needle. The trunk also could not disturb this inoculation site.

Dunker, F. and M. Rudovsky (1998). Management and treatment of a Mycobacterium tuberculosis positive elephant at the San Francisco Zoo. Proceedings AAZV and AAWV Joint Conference.

Anonymous (1998). "TB in elephants." Communique 18.

Whipple, D. L., R. M. Meyer, D. F. Berry, J. L. Jarnagin and J. B. Payeur (1997
). Molecular epidemiology of tuberculosis in wild white-tailed deer in michigan and elephants. Proceedings One Hundred and First Annual Meeting of the United States Animal Health Association, United States Animal Health Association.

Ryan, C. P. (1997). "Tuberculosis in circus elephants." Pulse Southern California Veterinary Medical Assoc(January): 8.

Peloquin, C. (1997). "Using therapeutic drug monitoring to dose the antimycobacterial drugs." Clinics in Chest Medicine 18: 79-97.

Mikota, S. K. and J. Maslow (1997). Theoretical and technical aspects of diagnostic techniques for mammalian tuberculosis. Proceedings, American Association Zoo Veterinarians.

Maslow, J. (1997). Tuberculosis and other mycobacteria as zoonoses. Proceedings American Association of Zoo Veterinarians.

Mycobacterial infections are common among humans. Of theses, infection with Mycobacterium tuberculosis (TB) is the most common and of greatest concern. Non-tuberculous species of mycobacteria may also cause infections in man, especially among immunosuppressed individuals. Human TB is typically acquired by inhalation of aerosols carrying tubercle bacilli fowwoing exposure to a person with active pulmonary infection; non-tuberculous species of mycobacteria are acquired from environmental sources. Since zoonotic transmission of TB does occur, the identification of acid fast bacilli (AFB) in clinical specimens from animals is a cause of concern, unease, and occasionally misconception for animal care handlers and zoo personnel.

Furley, C. W. (1997). "Tuberculosis in elephants." Lancet British edition 350(9072): 224.

Tests on 171 elephants in zoos and circuses in the USA revealed that 33% were positive to one or more skin tests and 11% were positive by ELISA. As there is no standard procedure for testing elephants caution should be used when interpreting the results.

Essey, M. A. and J. P. Davis (1997). Status of the National cooperative state-federal bovine tuberculosis eradication program fiscal year 1997. Proceedings United States Animal Health Association.

Binkley, M. (1997). Tuberculosis in captive elephants. Proceedings American Association of Zoo Veterinarians.

Sandin, R. L. (1996). "Polymerase chain reaction and other amplification techniques in mycobacteriology." Clinical Mycobacteriology 16(3): 617-639.

Moda, G., C. J. Daborn, J. M. Grange and O. Cosivi (1996). "The zoonotic importance of Mycobacterium bovis." Tubercle and Lung Disease 77: 103-108.

The zoonotic importance of Mycobacterium bovis has been the subject of renewed interest in the wake of the increasing incidence of tuberculosis in the human population. This paper considers some of the conditions under which transmission of M. bovis from animals to humans occurs and reviews current information on the global distribution of the disease. The paper highlights the particular threat posed by this zoonotic disease in developing countries and lists the veterinary and human public health measures that need to be adopted if the disease is to contained. The association of tuberculosis with malnutrition and poverty has long been recognized and the need to address these basic issues as as crucial as specific measures against the disease itself.

Dalovision, J. R., S. Montenegro-James, S. A. Kemmerly, C. F. Genre, R. Chambers, G. A. Pankey, D. M. Failla, K. G. Haydel, L. Hutchinson, M. F. Lindley, A. Praba, K. D. Eisenach and E. S. Cooper (1996). "Comparison of the amplified Mycobacterium tuberculosis (MTB) direct test, aplicor MTB PCR and IS6, 110-PCR for detection of MTB in respiratory specimens." Clin. Infect. Dis 23: 1099-1106.

Chandrasekharan, K., K. Radhakrishnan, J. V. Cheeran, K. N. M. Nair and T. Prabhakaran (1995). Review of the Incidence, Etiology and Control of Common Diseases of Asian Elephants with Special Reference to Kerala. A Week with Elephants; Proceedings of the International Seminar on Asian Elephants. J. C. Daniel. Bombay, India, Bombay Natural History Society; Oxford University Press: 439-449.

Incidence, etiology, symptoms and control of specific and non-specific diseases of captive and wild elephants have been reviewed. Asian elephants have been observed to be susceptible to various parasitic diseases such as helminthiasis, trypanosomiasis and ectoparasitic infestations, bacterial diseases such as tetanus, tuberculosis, haemorrhagic septicemia, salmonellosis and anthrax, viral diseases such as foot and mouth disease, pox and rabies and non-specific diseases like impaction of colon, foot rot and corneal opacity. A detailed study extending over two decades on captive and wild elephants in Kerala, revealed high incidence of helminthiasis (285), ectoparasitic infestation (235), impaction of colon (169) and foot rot (125). Diseases such as trypanosomiasis (21), tetanus (8), tuberculosis (5) pox (2) and anthrax (1) were also encountered. The line of treatment against the diseases mentioned, have been discussed in detail.

(1994). "Treatment of tuberculosis and tuberculosis infection in adults and children." Am J Respir Crit Care Med 149: 1359-1374.

Chandrasekharan, K. (1992). Prevalence of infectious diseases in elephants in Kerala and their treatment. The Asian Elephant: Ecology, Biology, Diseases, Conservation and Management (Proceedings of the National Symposium on the Asian Elephant held at the Kerala Agricultural University, Trichur, India, January 1989). E. G. Silas, M. K. Nair and G. Nirmalan. Trichur, India, Kerala Agricultural University: 148-155.

John, M. C., S. Nedunchelliyan and N. Raghvan (1991). "Tuberculin testing in Indian elephants." Indian Journal of Veterinary Medicine 11(1-2): 48-49.

Fowler, M. E. (1991). Tuberculosis in zoo ungulates. Bovine tuberculosis in cervidae: Proceedings of a symposium, United States Department of Agriculture Miscellaneous Publication No. 1506.

Sabin, J. E. (1990). "Joseph Hersey Pratt's cost-effective class method and its contemporary application." Psychiatry 53: 169-184.

Haagsma, J. and A. Eger (1990). ELISA for diagnosis of tuberculosis and chemotherapy in zoo and wildlife animals.

The aim of this study was to improve the diagnosis of bovine tuberculosis in zoo and wildlife animals, in particular by using an Enzyme-Linked Immunosorbent Assay (ELISA). In addition, suspected cases of tuberculosis (TB) with a positive skin test and /or ELISA were treated with antituberculosis drugs. The diagnosis of TB in animals is based primarily on the intradermal tuberculin test, corresponding with cellular immune response. Although this test has practical disadvantages in zoo animals, the application is still of high value. For this purpose tuberculins with a well controlled high potency and specificity should be used. In order to diagnose hypergic or anergic animals it is recommended to use PPD tuberculin with double strength (2 mg tuberculoprotein per ml) or to double the dose (0.2 ml instead of 0.1 ml), so that about 10,000 I.U. are applied. A strict interpretation scheme can increase the efficacy of the test, in particular in the comparative test. In order to improve the diagnosis, we have studied for some years the use of the ELISA which corresponds with humoral immunity.

Wiegeshaus, E., V. Balasubramanian and D. W. Smith (1989). "Immunity to tuberculosis from the perspective of pathogenesis." Infect Immun 57: 3671-3676.

Thoen, C. O. (1988). "Tuberculosis." J. Am. Vet. Med. Assoc 193(9): 1045-1048.

Arora, B. M. (1986). Tuberculosis in wildlife in India. Summer Institute on Health, Production and Management in Wildlife, Indian Veterinary Institute.

Snider, D. E., Jr., W. D. Jones and R. C. Good (1984). "The usefulness of phage typing Mycobacterium tuberculosis isolates." Am. Rev. Respir. Dis 130: 1095-1099.

Mycobacteriophage typing of Mycobacterium tuberculosis isolates was used as an epidemiologic aid in investigating the transmission of tuberculosis in community, industrial, and institutional outbreaks. The technique was also useful in other situations, e.g., documenting congenital transmission of infection and distinguishing exogenous reinfection from endogenous reactivation. Additional studies are indicated to further explore the value of phage typing for tracking the transmission of tuberculosis in the community

Wallach, J. D. and W. J. Boever (1983). Tuberculosis. Diseases of Exotic Animals.: 791-792.

Saunders, G. (1983). "Pulmonary Mycobacterium tuberculosis infection in a circus elephant." J. Am. Vet. Med. Assoc 183(11): 1311-1312.

Devine, J. E., W. J. Boever and E. Miller (1983). "Isoniazid therapy in an Asiatic elephant (Elephas maximus)." Journal of Zoo and Wildlife Medicine 14: 130-133.

Woodford, M. H. (1982). "Tuberculosis in wildlife in the Ruwenzori National Park, Uganda (Part II)." Trop. Anim. Hlth. Prod 14(3): 155-160.

The results of post-mortem examinations of 90 warthog (Phacochoerus aethiopicus) conducted in the Ruwenzori National Park, Uganda during a survey of tuberculous infection in wildlife are described. Nine per cent of warthog were found to show gross lesions on autopsy and of these organisms which could by typed, Mycobacterium bovis was isolated in 2 of 6 cases and 5 atypical mycobacterial strains were isolated from the remaining 4. The distribution and character of the lesions is described and it is concluded that the route of infection in the warthog is alimentary. A mycobacterial survey of 8 other species of mammals, 7 species of birds, 5 species of fish and 1 species of amphibian is described. None of the mammals (except possibly 1 elephant), birds, fish or amphibia was harbour atypical, probably saprophytic, mycobacterial types. The origin of tuberculosis in buffalo and warthog in the Ruwenzori National Park is discussed and is concluded to have been previous contact with domestic cattle.

Jones, W. D., Jr. and R. C. Good (1982). "Hazel elephant redux (letter)." Am. Rev. Respir. Dis 125(2): 270.

Full text. A recent letter from Greenberg, Jung and Gutter reported the untimely death of Hazel Elephant with Mycobacterium tuberculosis infection. The authors concluded that the animal trainer, who was found to have cavitary tuberculosis, was probably the source of infection. The conclusion was based on data available at the time. The isolates from Hazel Elephant and the animal trainer were submitted to us for further study the state health departments of Louisiana and Florida. Using the methodology and classification scheme previously described, we found that the cultures were of different phage types. The isolate from the elephant was type A0 (7), and the isolate from the trainer was type A1 (7,13,14). The isolates differed by lysis with one major phage (MTPH 5) and two auxiliary phages (MTPH 13 and 14). We have previously used phage typing of M. tuberculosis in several well-defined outbreaks as an adjunct to other epidemiologic procedures. The isolates were typed without the laboratory's knowing epidemiologic relationships between cases. The results indicated that M. tuberculosis transmitted from one individual to another retained the same phage-type characteristics. In the present study, our phage-type results suggest that the animal trainer and the elephant were infected from two different sources and that occurrence of disease in the animal and the trainer was coincidental. We are still evaluating page typing as a procedure for use in tuberculosis epidemiology and can accept selected cultures for phage typing in special situations if we are contacted before the cultures are submitted.

Thoen, C. O. and E. M. Himes (1981). Tuberculosis. Infectious diseases of wild mammals. J. W. Davis, L. H. Karstad and D. O. Trainer. Ames, Iowa, The University of Iowa Press.

Mann, P. C., M. Bush, D. L. Janssen, E. S. Frank and R. J. Montali (1981). "Clinicopathologic correlations of tuberculosis in large zoo mammals." J. Am. Vet. Med. Assoc 179(11): 1123-1129.

In August 1978, a black rhinoceros at the National Zoological Park died with generalized tuberculosis caused by Mycobacterium bovis. A 2nd black rhinoceros was euthanatized 9 months after M bovis was cultured from its lungs. After these 2 deaths, numerous large zoo mammals that had been potentially exposed were subjected to various procedures to ascertain their status regarding tuberculosis. The procedures were: intradermal tuberculin testing, evaluation of delayed hypersensitivity reaction on biopsy specimens, enzyme-linked immunosorbent assay (ELISA) testing,

and culture of various secretions and organs. Several of the animals in this series died during the study. These were necropsied and examined for evidence of mycobacterial infection. The results of tuberculin testing varied from species to species and from site to site within a species. Delayed hypersensitivity responses generally correlated well with the amount of swelling at the tuberculin site. In some cases, however, positive reactions were found without any delayed hypersensitivity response. Results of ELISA testing were confirmatory in tuberculous animals. Several species were judged to be nonspecific reactors, based on positive or suspect tuberculin test results, with negative ELISA results and necropsy findings.

Gutter, A. (1981). Mycobacterium tuberculosis in an Asian elephant. Proc.Am.Assoc.Zoo Vet.

Greenberg, H. B., R. C. Jung and A. E. Gutter (1981). "Hazel Elephant is dead (of tuberculosis) (letter)." Am. Rev. Respir. Dis 124(3): 341.

Full text. Hazel Elephant was only 35 years old (by our estimate) when she died. She was cooperative and trusting to the last. Had we known about her illness sooner, we could have saved her. The Mycobacterium tuberculosis, var hominis that killed Hazel was sensitive to our drugs at the following levels: INH to 0.2mcg/ml; PAS to 2 mcg/ml; R to 1 mcg/ml; and MAB to 5 mcg/ml. Hazel worked and performed for a travelling circus. Ordinarily good-humored and loving, she had been off her feed for weeks. She became listless and apathetic, her eyes lost their sparkle, and she lacked her customary elan. Nonetheless, Hazel continued to perform for the children and do her other chores until she came to New Orleans. When Hazel got to New Orleans, she could barely move. The circus's bosses called for help. The brought her to the hospital at the Audubon Park and Zoological Garden. As soon as we saw Hazel, we admitted her to the isolation ward. We have her fluids, electrolytes, and antibiotics. We got cultures and other clinical laboratory tests. We comforted Hazel and tried to put her at ease. It was too late. She fell to the ground, her rheumy eyes gazed at us pitifully, her respirations failed, and she died. Hazel's postmortem examination took six hours. She was an emaciated Asian elephant whose lungs were filled with caseating granulomata. Since microscopy showed myriads of acid-fast bacilli, we examined everyone who had, or who thought they had, contact with Hazel. We found three persons with positive tuberculin skin test results. None had tuberculous disease. Fortunately, Hazel had been kept away from other animals. Hazel's circus did not wait for the results of our autopsy. It left Louisiana. The U.S. Public Health Service tracked it down and found the man, an animal trainer with cavitary tuberculosis, who probably gave Hazel her fatal disease. Now another health department will have to deal with the circus and its animals.

Thoen, C. O., K. Mills and M. P. Hopkins (1980). "Enzyme linked protein A: An enzyme-linked immunosorbent assay reagent for detecting antibodies in tuberculous exotic animals." Am. J. Vet. Res 41(5): 833-835.

An enzyme-linked immunosorbent assay (ELISA) was developed, using protein A labeled with horseradish peroxidase for detecting antibodies in tuberculous exotic animals (llamas, rhinoceroses, elephants). The modified ELISA provides a rapid procedure for screening several animal species simultaneously for tuberculosis without the production of specific anti-species conjugates. Heat-killed cells of Mycobacterium bovis and M. avium and purified protein-derivative tuberculin of M. bovis were used as antigens for ELISA.

Thoen, C. O. and E. M. Himes (1980). Mycobacterial infections in exotic animals. The comparative pathology of zoo animals. R. J. Montali and G. Migaki. Washington,D.C., Smithsonian Institution Press: 241-245.

Mycobacteria were isolated from 59% of the 826 specimens submitted from exotic animals suspected of having tuberculosis. Mycobacterium bovis and Mycobacterium tuberculosis accounted for 61% of the isolations from nonhuman primates. Mycobacterium bovis was the organism most frequently isolated from hoofed animals and Mycobacterium avium was most commonly isolated from birds. The distribution, pathogenesis, diagnosis, and control of tuberculosis in exotic animals is discussed.

Chandrasekharan, K. (1979). Common diseases of elephants. State Level Workshop on Elephants, College of Veterinary and Animal Sicences, Kerala Agricultural University.

Thoen, C. O., W. D. Richards and J. L. Jarnagin (1977). "Mycobacteria isolated from exotic animals." J. Am. Vet. Med. Assoc 170(9): 987-990.

von Benten, K., H. H. Fiedler, U. Schmidt, L. C. Schultz, G. Hahn and L. Dittrich (1975). "Occurrence of tuberculosis in zoo mammals; a critical evaluation of autopsy material from 1970 to the beginning of 1974." Deutsche Tierarztliche Wochenschrift 82(8): 316-318.

Pinto, M. R. M., M. R. Jainudeen and R. G. Panabokke (1973). "Tuberculosis in a domesticated Asiatic elephant Elephas maximus." Vet. Rec 93(26): 662-664.

A case of tuberculosis in a domesticated Asiatic elephant, Elephas maximus, was diagnosed on post-mortem examination. The causal organism was identified as Mycobacterium tuberculosis var hominis on the basis of cultural, biochemical and virulence studies. Microscopically, the lesions resembled tuberculous lesions as seen in man and other domestic animals, but an important difference was the apparent absence of Langerhan's type giant cells. The problems associated with the clinical diagnosis of tuberculosis in the elephant are discussed.

Gorovitz, C. (1969). "Tuberculosis in an African elephant." Am. Assoc. Zoo Vet. Newsletter January 20.

Seneviratna, P., S. G. Wettimuny and D. Seneviratna (1966). "Fatal tuberculosis pneumonia in an elephant." VM SAC 60: 129-132.

A fatal case of tuberculosis pneumonia with anemia and helminthiasis in a Ceylon elephant is reported. Acid-fast organisms resembling Mycobacterium tuberculosis and tubercular nodules were seen in large numbers in sections of the lung.

Gorovitz, C. (1962). "Tuberculosis in an African elephant." Nord Vet Med 14(Supl 1): 351-352.

Selye, H. (1956). Recent progress in stress research, with reference to tuberculosis. Personality, stress, and tuberculosis. P. J. Sparer. New York, Int. Univ. Press: 45-64.

Holmes, T. H. (1956). Multidiscipline studies of tuberculosis. Personality,stress, and tuberculosis. P. J. Sparer. New York, Int. Univ. Press: 65-125.

Halloran, P. O. (1955). "A bibliography of references to diseases in wild mammals and birds." Am. J. Vet. Res 16(part 2): 161.

Curasson, G. (1942). Traite de pathologie exotique veterinaire et comparee. Paris, Vigot Freres.

Griffith, A. S. (1939). "Infections of wild animals with tubercle and other acid-fast bacilli." Proc. R. Soc. Med 32: 1405-1412.

Winogradradsky, S. (1938). "La microbiologie ecologique ses principes - son procede." Ann. Inst. Pasteur 64(6): 715-730.

Urbain, A. (1938). Tuberculosis in wild animals in captivity. Ann. Inst. Pasteur
Tuberculose chez animaux sauvages en captivite. 61: 705-730.

Iyer, A. K. (1937). "Veterinary science in India, ancient and modern with special reference to tuberculosis." Agric. Livest. India 7: 718-724.

Curasson, G. (1936). Treatise on the pathology of exotic animals. Paris, Vigot Freres,.

Datta, S. C. A. (1934). "Report of the pathology section." Ann. Rep. Imp. Inst. Vet. Research Muktesar: 25-33.

Baldrey, F. S. H. (1930). "Tuberculosis in an elephant." J. R. Army Vet. Corp 1: 252.

Bopayya, A. B. (1928). "Tuberculosis in an elephant." Indian Veterinary Journal 5: 142-145.

Narayanan, R. S. (1925). "A case of tuberculosis in an elephant." J. Comp. Pathol 38: 96-97.

Ishigami, T. (1918). "The influence of psychic acts on the progress of pulmonary tuberculosis." Am. Rev. Tuberc 2: 470-484.

Thieringer, H. (1911). About tuberculosis in an elephant. Berl. Tierarztl. Wschr
Ueber Tuberkulose bei einem Elefanten. 27: 234-235.

Damman and Stedefeder (1909). Tuberculosis diseases in elephants with human type mycobacterium. Deutsche Tierarztliche Wochenschrift
Tuberkulose erkankung elefanten hervorgerufen durch Bazillen des sogenannten typus humanus. 17: 345.

Garrod, A. H. (1875). "Report on the Indian elephant which died in the society's gardens on July 7th, 1875." Proc. Zool. Soc. Lond 1875: 542-543.

# *Mycobacterium tuberculosis* Infection as a Zoonotic Disease: Transmission between Humans and Elephants

**Kathleen Michalak,* Connie Austin,† Sandy Diesel,* J. Maichle Bacon,* Phil Zimmerman,‡ and Joel N. Maslow§**

*McHenry County Department of Health, Woodstock, Illinois, USA; †Illinois Department of Public Health, Springfield, Illinois, USA; ‡University of Illinois, College of Medicine at Rockford, Rockford, Illinois, USA; and §Boston University School of Medicine and the VA Medical Center, Boston, Massachusetts, USA

Between 1994 and 1996, three elephants from an exotic animal farm in Illinois died of pulmonary disease due to *Mycobacterium tuberculosis.* In October 1996, a fourth living elephant was culture-positive for *M. tuberculosis.* Twenty-two handlers at the farm were screened for tuberculosis (TB); eleven had positive reactions to intradermal injection with purified protein derivative. One had smear-negative, culture-positive active TB. DNA fingerprint comparison by IS*6110* and TBN12 typing showed that the isolates from the four elephants and the handler with active TB were the same strain. This investigation indicates transmission of *M. tuberculosis* between humans and elephants.

*Mycobacterium tuberculosis* and *M. bovis*, related organisms of the *M. tuberculosis* complex, infect a wide variety of mammalian species. *M. bovis* is pathogenic for many animal species, especially bovidae, cervidae, and occasionally carnivores. Human disease with *M. bovis* is well described and historically was a common cause of tuberculosis (TB) transmitted by infected dairy products. As a result of milk pasteurization and TB eradication programs in most industrialized countries, zoonotic transmission of *M. bovis* through domestic livestock is now rare. In contrast, a similar eradication program has not been conducted for wild or exotic animals, which therefore remain an uncommon source for *M. bovis* exposure. Zoonotic transmission of *M. bovis* has been reported from seals, rhinoceros, and elk (1-4).

*M. tuberculosis*, the most common species to cause TB, classically causes disease in humans. Animal infection with *M. tuberculosis*, while uncommon, has been described among species (e.g., birds, elephants, and other mammals) with prolonged and close contact with humans (5-10). Transmission of *M. tuberculosis* between animals and humans has not been reported. This paper describes *M. tuberculosis* transmission from elephants to humans.

Address for correspondence: Kathleen Michalak, McHenry County Department of Health, 2200 N. Seminary Avenue, Woodstock, IL 60098, USA; fax: 815-338-7661.

## The Outbreak

In March 1996, five elephants from an exotic animal farm in Illinois were in California as part of a circus act. One elephant (with chronic, unexplained weight loss since October 1995) died under anesthesia on August 3, 1996, during a diagnostic dental work-up. Necropsy showed widespread consolidation of lung tissue with caseous necrosis of the lungs and mediastinal lymph nodes. Short, fat, relatively scant numbers of acid-fast bacilli were observed in necropsy tissues. A presumptive diagnosis of *M. tuberculosis* was made. The remaining four elephants were recalled to the farm in Illinois. A second elephant died en route on August 6, 1996. Necropsy revealed copious respiratory and trunk exudates and caseous necrosis of the lung.

To determine the risk for and possibility of infection among the animal trainers and caretakers, an epidemiologic investigation was initiated. The remaining elephants in the herd and the elephant handlers and trainers who were

still traveling were recalled to the farm and examined for evidence of *M. tuberculosis* infection. All elephants were empirically begun on antituberculous therapy in early December 1996.

## Epidemiologic Investigation

The exotic animal farm was visited on numerous occasions to evaluate the type and degree of contact between elephants and employees. The farm, located in a rural area and surrounded by barbed wire and trees, originally housed 18 Asian and 2 African elephants. Thirteen elephants were tethered on a chain in one large barn, four were housed in a separate large room (two in a common stall), and a baby elephant was in a third room with 5-6 tigers. A separate barn housed approximately 80 tigers.

### TB Screening of Employees

The animal handlers (trainers and caretakers) who had direct contact with the elephants were administered purified protein derivative (PPD) skin tests. Initial screening was performed in August 1996, with subsequent screenings in December 1996 and March, June, and September of 1997. Testing was performed by the McHenry County Department of Health, except in two handlers who had subsequent skin tests performed elsewhere. As part of the screening process, handlers were questioned about their risk factors for TB, including previous bacillus Calmette-Guérin (BCG) vaccination.

Handlers were tested by the two-step method using 5 tuberculin units of PPD (0.1 ml) by intradermal injection in the flexor surface of the forearm. A positive result was defined as an induration of >5 mm. Handlers with positive skin tests were evaluated by a TB health-care worker and had chest radiographs taken. Sputum samples from any handler with a chest radiograph consistent with TB were submitted to the Illinois Department of Public Health Laboratory. Samples were examined by direct microscopy for acid-fast organisms, stained with fluorochrome, and processed for culture by standard methods.

### Examination of Isolates

The human isolate and the four elephant isolates were sent to the National Tuberculosis Genotyping and Surveillance Network at the Michigan Community Public Health Agency for restriction fragment length polymorphism (RFLP) analysis. Southern blots of Pvu II restricted whole chromosomal DNA, resolved in 1% agarose gels, were probed with a DNA fragment corresponding to the right side of IS*6110* and detected by chemiluminescence (11). The number and size of the hybridizing fragments for each isolate were compared in the same gel. Isolates with identical RFLP patterns or with ≤ 2 band differences were considered to represent the same strain. Additionally, Pvu II digested DNA was similarly typed after probing with the repetitive element TBN12.

## Epidemiologic Findings

Elephant handlers worked in very close proximity with the elephants around the clock, whereas tiger handlers had little direct contact with the elephants. Most of the elephant handlers lived on the farm in a separate section of the barn; four lived in trailers on the grounds. The handlers' living quarters had a separate ventilation system from the elephants'; however, the doors between the two quarters were open for unknown periods. Handlers indicated that they held social events in a building connected to the elephant barn.

Necropsies of elephants were performed on the farm and were attended by a number of elephant and tiger handlers (including the handler with the active case). The necropsy of the elephant that died in 1994, also performed on the farm, showed caseous necrosis of the lungs and pleural exudates whose culture yielded *M. tuberculosis*.

In addition to the three elephants that died of *M. tuberculosis* infection, a fourth living elephant was also infected with the mycobacterium; this infection was diagnosed in late December 1996 from a trunk culture obtained in October 1996. Subsequent cultures from this and the other animals have been negative for mycobacteria. Another elephant from this farm died of *M. tuberculosis* infection in 1981 (5), but contact between this elephant and the present herd or any of the handlers could not be established.

Twenty-two handlers at the exotic animal farm had moderate to frequent contact with the infected animals; 12 were elephant handlers and 10 were tiger handlers. Initial PPD testing was performed for 14 handlers in August 1996, 2 in October 1996, and 5 in December 1996. One who was PPD-positive in November 1995 reported receiving BCG more than 10 years before.

Eleven (50%) of 22 handlers were found PPD-

positive as part of this investigation. Eight of the 11 had positive PPD skin test results upon initial testing, with a median induration of 12 mm (range, 10 to 19 mm). Four of the eight were elephant handlers and four were tiger handlers. The skin test reaction of three handlers converted from negative to positive with a median induration of 12 mm (range, 8 to 15 mm). The three PPD converters were initially tested in August 1996; one was positive upon retesting in January 1997, and two tested positive in April 1997 (Table).

Eight of the 11 handlers reported that they had negative skin tests in the past and had not received BCG. The other three reported some type of reaction from a previous skin test in the past but did not know the results. All three also reported receiving BCG more than 10 years before. Eight of the 10 handlers with negative PPD skin tests had at least one negative follow-up test at 3 months; two left the farm and did not receive follow-up testing.

The attack rates were approximately equal for the elephant and tiger handlers. Of the 12 elephant handlers tested, 6 (50%) were PPD-positive with two conversions documented in April 1997; of the 9 tiger handlers, 5 (56%) were PPD-positive, with one conversion documented in January 1997. Overall, a very high rate (52%) of handlers tested positive.

All 12 handlers with positive PPDs (including the one with the known positive PPD) received an evaluation and chest radiograph; one had irregular nodules and interstitial changes in the right apex without retraction of the lungs, consistent with active TB, but no cough, chest pain, fever, night sweats, weight loss, or fatigue.

Three sputum samples were smear-negative for acid-fast bacilli, although one yielded *M. tuberculosis* upon culture. Isoniazid (INH), rifampin, pyrazinamide, and ethambutol treatment was initiated in September 1996, and after 2 months, was reduced to INH and rifampin when the isolate showed no resistance to antituberculous medications. Subsequent chest radiographs revealed improvement or clearing of the initial lesions. Nine of the remaining 11 PPD-positive handlers were prescribed INH prophylaxis; two declined because of the risk for adverse reactions.

Table. TB PPD[a] skin test results of animal handlers, Aug 1996–Sep 1997

| | Positive | Negative |
|---|---|---|
| Previously positive | 1 | |
| Elephant handlers | 4 | 6 |
| Tiger handlers | 4 | 4 |
| Elephant handlers (converted) | 2 | |
| Tiger handlers (converted) | 1 | |
| Total | 12 | 10 |

[a]Tuberculin purified protein derivative.

### Molecular Analysis of Elephant and Human Isolates

The sputum isolate from the handler with active TB was compared with the isolates from the three animals that died and the living elephant whose infection was diagnosed during the investigation. The isolates had identical IS*6110* RFLP pattern, differing by ≤ 2 bands (Figure 1). Additionally, all isolates had the identical TBN12 RFLP pattern, except the isolate from the elephant that died in August 1996, which demonstrated a shift of one band (Figure 2).

## Conclusions

Infection with *M. tuberculosis* or *M. bovis* has not been reported in nondomesticated Asian or African elephants. *M. tuberculosis* infection in domesticated elephants was first reported in 1875 by Garrod and has been recognized in the ancient Ayurvedic literature (10); humans have been considered the source of infection. A trainer with cavitary TB was suspected as the source of infection (8) for one Asian elephant that died of *M. tuberculosis*, although subsequent analysis showed the animal and human isolates to be of two different phage types.

This report describes the first case of zoonotic *M. tuberculosis* transmission. The epidemiologic investigation strongly suggests *M. tuberculosis* transmission between humans and elephants, as evidenced by DNA fingerprinting. RFLP analysis comparing Southern blots of chromosomal DNA probed with IS*6110* and TBN12 indicated that four elephant isolates had identical patterns with the human isolate, differing by ≤ 2 bands. The addition or loss of a single band has been demonstrated in other outbreak settings, and the repetitive element that generates patterns has characteristics of a mobile genetic element (11).

Eleven (50%) of 22 employees screened were skin-test positive, with no difference between tiger and elephant handlers. This is a higher rate of positives than documented in animal handlers exposed to *M. bovis*-infected animals (3,4). Since the handlers had no accurate

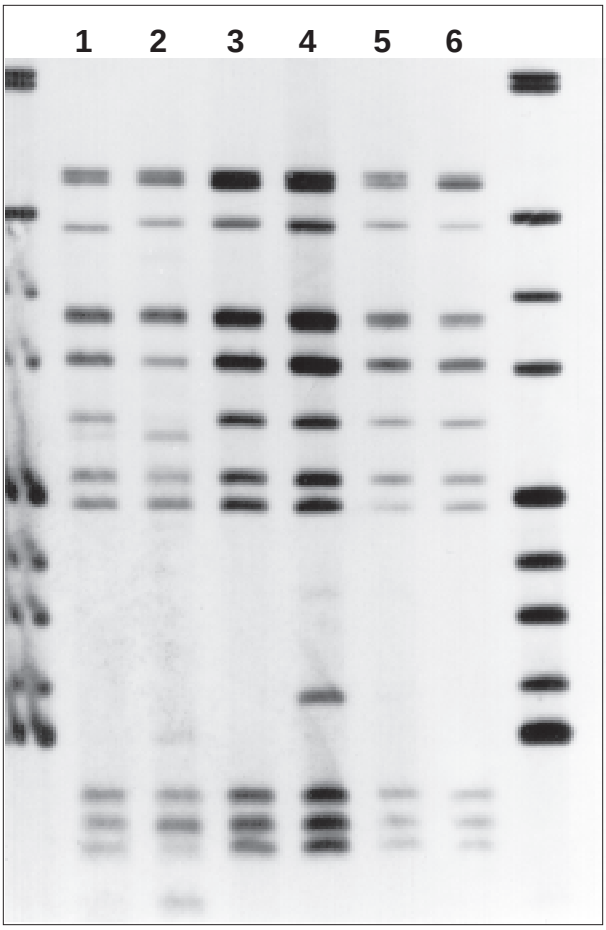



Figure 1. IS*6110* restriction fragment length polymorphism results. Lane1, elephant isolate (died August 6, 1996); Lane 2, elephant isolate (died 1994); Lane 3, living elephant trunk culture (October 1996); Lane 4, elephant lung tissue isolate (died August 3, 1996); Lane 5, elephant lymph node tissue isolate (died August 3, 1996); Lane 6, human sputum isolate (September 1996). Provided by State of Michigan Community Public Health Agency.

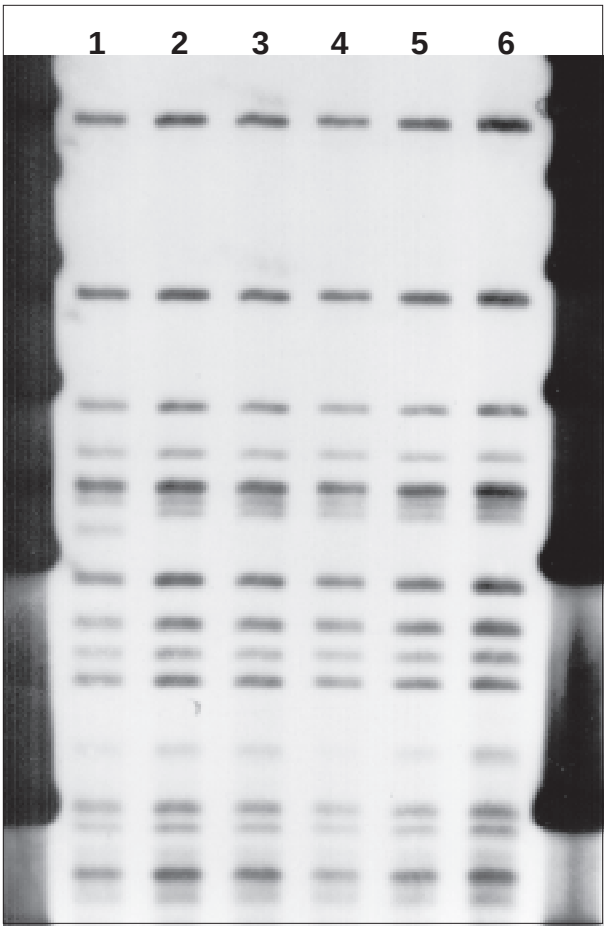



Figure 2. TBN12 restriction fragment length polymorphism results. Lane1, elephant isolate (died August 6, 1996); Lane 2, elephant isolate (died 1994); Lane 3, living elephant trunk culture (October 1996); Lane 4, elephant lung tissue isolate (died August 3, 1996); Lane 5, elephant lymph node tissue isolate (died August 3, 1996); Lane 6, human sputum isolate (September 1996). Provided by State of Michigan Community Public Health Agency.

history of tuberculin skin testing, it was not possible to determine when conversions took place. The original source of infection for both elephants and humans is unknown.

The possible mechanisms of transmission include close contact while handling and training elephants, cleaning the barn, participating in elephant necropsies, and living in close proximity to the elephant barn.

Human-to-human transmission of TB is unlikely because the only handler with active disease did not have cough. Of the three sputum samples initially collected, two were smear- and culture-negative; the third had low numbers of acid-fast bacilli manifested by a negative sputum smear, thus posing a low infectivity risk to others. In contrast, the three elephants that died had evidence of widespread pulmonary disease and, therefore, represented a greater risk for dissemination.

Three handlers converted from negative to positive during the course of the investigation; their relevant exposure is unknown. The source may have been one elephant found antemortem to be culture-positive for M. *tuberculosis*, although this animal did not return to the farm

until November. Contact with this animal is unlikely for handlers whose PPD tests converted in December and unknown for the two handlers whose test results were positive in April (the latter two had not been retested since August).

TB is transmitted through close prolonged contact with a person (or animal) with active TB. The risk for TB transmission from an animal with a case of active TB is higher for daily handlers than for persons with only brief contact, e.g., members of the public viewing a performance or receiving elephant rides. In this outbreak, screening of all persons who had (or thought they had) contact with an elephant that died of *M. tuberculosis* identified three PPD-positive cases but no cases of active TB (8). Because the real risk for transmission to the general public was poorly understood, this case received considerable media attention as well as mention in the medical literature (7,12).

Veterinary practices should be initiated to reduce the risks for exposure to animals infected with *M. tuberculosis.* No data are available on TB incidence among domesticated elephants in the United States. An estimate can be derived from a retrospective study of 379 zoo elephants of which eight (2.3%) had *M. tuberculosis* infection (10).

Reliable diagnosis and prevention of TB in all domesticated and exhibited animals is ideal. Short of this, possible ways to prevent and decrease zoonotic spread of any mycobacterial infection (*M. tuberculosis* or *M. bovis*) include 1) regular skin testing of handlers or keepers; 2) a high index of suspicion of TB in elephants with unexplained weight loss, cough, or rhinorrhea; 3) public health measures of contact tracing and notification; and 4) active and effective treatment of infected personnel and animals (13).

## Acknowledgments

We thank Stephen Dietrich and Laura Mosher, North Central Area TB-RFLP Laboratory, Michigan Department of Public Health; George J. Dizikes, Chief of Molecular Diagnostics, Illinois Department of Public Health, Division of Laboratories; and Janet Payeur, National Veterinary Services Laboratory, U.S. Department of Agriculture, MB Section. This research was supported in part by the Centers for Disease Control and Prevention, National Tuberculosis Genotyping and Surveillance Network cooperative agreement.

Kathleen Michalak is the Director of Nursing for McHenry County Department of Health. She has worked to improve the use of community resources, and improved service to client, providers, and the community as a whole.

## References

1. Thoen CO. Tuberculosis (Revised 1995). Zoonosis updates. Journal of the American Veterinary Medical Association 1995;2:155-8.
2. Thompson PJ, Cousins DV, Gow BL, Collins DM, Willamson BH, Dagnia HT. Seals, seal trainers, and mycobacterial infection. American Review of Respiratory Diseases 1993;147:164-7.
3. Dalovisio JR, Stetter M, Mikota-Wells S. Rhinoceros' rhinorrhea: cause of an outbreak of infection due to airborne *Mycobacterium bovis* in zookeepers. Clin Infect Dis 1992;15:598-600.
4. Fanning A, Edwards S. *Mycobacterium bovis* infection in human beings in contact with elk (*Cervus elaphus*) in Alberta, Canada. Lancet 1991;338:1253-5.
5. Saunders G. Pulmonary *Mycobacterium tuberculosis* infection in a circus elephant. J Am Vet Med Assoc 1983;183:1311-2.
6. Pinto MRM, Jainudeen MR, Panabokke RG. Tuberculosis in a domesticated Asiatic *Elaphas maximus.* Vet Rec 1973;93:662-4.
7. Frankel D. Elephants pack their trunks and leave the circus. Lancet 1997;349:1675.
8. Greenberg AS, Jung RC, Gutter AK. Hazel Elephant is dead (of Tuberculosis). American Review of Respiratory Diseases 1981;124:341.
9. Jones WD, Good RC. Hazel elephant redux. American Review of Respiratory Diseases 1982;125:270.
10. Mikota S, Sargent EL, Ranglack GS. Medical management of the elephant. Tuberculosis and Tuberculin Testing 1994;33-9.
11. Small P, Moss A. Molecular epidemiology and the new tuberculosis. Infect Agents Dis 1993;2:132-8.
12. Furley CW. Tuberculosis in elephants. Lancet 1997;350:224.
13. Centers for Disease Control and Prevention. Guidelines for preventing the transmission of *Mycobacterium tuberculosis* in health-care facilities. MMWR Morb Mortal Wkly Rep 1994;43:63-4.

U.S. NEWS

FEBRUARY 17, 2011 / 7:15 PM / UPDATED 12 YEARS AGO

# Elephant behind TB outbreak at Tennessee sanctuary

By Tim Ghianni

NASHVILLE, Tenn (Reuters) - Liz, an African elephant housed at a sanctuary for the animals, was the source of tuberculosis infections among eight workers at the refuge, an author of a report on the 2009 outbreak said on Thursday.

None of the infected employees at the Hohenwald, Tennessee, sanctuary for old, often abused, elephants, became ill. The workers were given preventive therapy, and 54-year-old Liz is in quarantine and undergoing treatment.

A report by The Centers for Disease Control and Prevention blamed pressure-washing of elephant barns for the spread of the tuberculosis bacteria, which enters through the lungs, said Dr. William Shaffner, who helped write the report and is an expert on infectious diseases at Vanderbilt University in Nashville.

"Elephants can excrete the bacteria through their trunks and even in their feces," which can become an aerosol mist when hit by pressurized water, said Shaffner, who is also president of the sanctuary that is 85 miles South of Nashville.

The mist exposed the workers inside the barn and drifted into an adjacent administrative building where three other employees inhaled it, the report concluded.

An estimated one in eight captive elephants are infected with tuberculosis, he said. There are as many as 600 captive elephants in the United States.

Workers at the sanctuary who deal directly with the elephants now wear more elaborate protective clothing and use lower-pressure hoses to clean the barns, and steps were taken to seal off vulnerable buildings.

The Tennessee sanctuary was created in 1995 and houses 14 African and Asian elephants where they can wander on 2,700 acres.

While elephants can spread the bacteria among themselves and to humans, Shaffner said the first elephant to get tuberculosis likely got it from an infected person.

Editing by Andrew Stern and Greg McCune

*Our Standards: The Thomson Reuters Trust Principles.*

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People.™
Morbidity and Mortality Weekly Report (*MMWR*)

# Diagnosis of Tuberculosis in Three Zoo Elephants and a Human Contact — Oregon, 2013

***Please note:*** *An erratum has been published for this article. To view the erratum, please click here.*

*Weekly*
**January 8, 2016 / 64(52);1398-1402**

Amy Zlot, MPH1; Jennifer Vines, MD1; Laura Nystrom, MPH1; Lindsey Lane, MPH2; Heidi Behm, MPH2; Justin Denny, MD1; Mitch Finnegan, DVM3; Trevor Hostetler4; Gloria Matthews4; Tim Storms, DVM3; Emilio DeBess, DVM2

In 2013, public health officials in Multnomah County, Oregon, started an investigation of a tuberculosis (TB) outbreak among elephants and humans at a local zoo. The investigation ultimately identified three bull elephants with active TB and 118 human contacts of the elephants. Ninety-six (81%) contacts were evaluated, and seven close contacts were found to have latent TB infection. The three bulls were isolated and treated (elephants with TB typically are not euthanized) to prevent infection of other animals and humans, and persons with latent infection were offered treatment. Improved TB screening methods for elephants are needed to prevent exposure of human contacts.

In May 2013, a routine annual culture of a sample from a trunk washing on elephant A, an Asian elephant aged 20 years at a zoo in Oregon's Multnomah County, yielded *Mycobacterium tuberculosis,* indicating active, potentially infectious disease. Bidirectional transmission of *M. tuberculosis* between elephants and humans has been documented (*1*). Assuming that elephant A was not infectious at the time of his previous negative trunk wash sample culture, the infectious period was defined as the 12 months preceding the positive results of the May 2013, trunk wash sample (May 2012–May 2013) (*2*). The Multnomah County Health Department (MCHD) investigated close and casual contacts of elephant A. Close contacts were defined as persons with any presence in the 8,300–square-foot elephant barn or who had been within 15 feet (4.6 m) of any of the eight elephants in the enclosed outdoor area at least weekly during the past 12 months. Casual contacts included zoo employees or volunteers who might have been exposed to elephant trunk secretions or fecal matter (*3*), but who had not had close contact with elephant A. Human contacts were evaluated with either a tuberculin skin test (TST) or interferon gamma release assay (IGRA). For close contacts, TST conversions were defined as indurations of ≥5 mm (rather than ≥10 mm used in TB screening) (*4*) within 2 years of the most recent negative TB screening test, and were considered indicative of infection with *M. tuberculosis*. Historical annual TB screening test results for close contacts were obtained from the zoo's occupational health providers. Historical test results were unavailable for other contacts. TB test results reported for contacts were documented at the initial evaluation and at ≥8 weeks after the last known exposure. Contacts whose first test occurred at least 8 weeks following the last exposure had only one TST or IGRA.

The zoo identified 19 close contacts, all of whom had TSTs at ≥8 weeks after exposure; 13 were negative. Six persons with no previous positive TST and at least one negative TST during the past 2 years had positive TSTs (Figure 1). None of the contacts with positive TSTs had spent time in TB-endemic countries, or had other risk factors for TB, such as a history of homelessness or injection-drug use or diagnosis of human immunodeficiency virus. All had chest radiographs and were evaluated for symptoms; none had active disease. Among close contacts, the number and percentage of conversions from negative TST to positive within 2 years (31.6%) was higher than expected, given the baseline of 4% of the U.S. population having latent infection on the basis of a single ≥10 mm skin test result (*5*).

Because of the positive test results among close contacts, MCHD expanded the investigation to identify 39 casual contacts. A third group of 20 contacts was identified among persons who had attended special events at which elephant A sprayed paint with his trunk onto canvases behind attendees, potentially exposing them to aerosolized *M. tuberculosis*. Among all 59 casual and special event contacts, exposure to elephant A was approximately <30 minutes and at a distance of ≥25 feet. Among the 59 casual and special event contacts identified, 48 (81%) were fully evaluated; none had a positive TST or IGRA (Figure 1).

Before diagnosis of TB in elephant A, elephants were routinely screened for TB by annual cultures of samples collected from trunk washings, with samples collected from each elephant on 3 consecutive days. Following diagnosis of TB in elephant A, the zoo increased the frequency of trunk washings to once a month for infected elephants and once every 3 months for uninfected elephants. Serologic screenings were conducted once or twice a year to identify infected, but culture-negative, elephants. During the course of the investigation, antibodies to *M. tuberculosis* were detected in the serum of elephant A's father (elephant B), aged 51 years. Subsequently, in October 2013, culture of a trunk wash sample from elephant B was positive. The other seven elephants in the herd, including elephant A, had negative trunk washings at that time. Elephant B's close human contacts were identical to those of elephant A, with the exception of one new employee, whose TB screen was negative when he began employment.

In October 2013, another local public health department discovered that patient A, who had completed treatment for culture-confirmed pleural TB in the fall of 2012, had also been a casual contact of elephant A. Upon receiving notification for routine annual TB screening from the zoo, patient A had sought guidance from the health department regarding documentation of TB status. Patient A had worked at the zoo intermittently during 2012, but had limited contact with elephants (1 hour cumulative presence in the elephant barn). Given the pleural (sputum-culture-negative) nature of patient A's disease, patient A was most likely noninfectious.

The Oregon Health Authority had reviewed patient A's *M. tuberculosis* isolate's genotype in 2012, and found no matches in Oregon. When patient A's zoo work history was revealed in October 2013, well into the contact investigation for elephant A, the Oregon Health

Authority reviewed the genotypes of the isolates of patient A and elephant A, and found that they differed by only one locus in the 24-locus mycobacterial interspersed repetitive units (MIRU) pattern (Figure 2). Isolates from patient A and elephant A were analyzed at CDC using whole genome sequencing. Comparison of the assembled genomes from the two isolates identified no differences. Although this result is consistent with transmission, it does not indicate direction of transmission, and does not provide information about how patient A or the elephant contracted TB. Elephant B's isolate was genotyped, and spacer oligonucleotide typing (spoligotype) from this isolate matched those of patient A and elephant A (Figure 2).

In May 2014, a third bull elephant, elephant C, aged 44 years, was found to be infected with *M. tuberculosis* by a positive culture from a trunk washing sample. Elephant C's isolate was not whole genome sequenced; all of this elephant's human contacts were the same as those of elephant B. None of the three elephants had shown signs of illness, although elephant B had experienced temporary weight loss. All three elephants' isolates were susceptible to first-line *M. tuberculosis* drugs. Each bull has received different and changing regimens; treatment is ongoing and guided by drug levels and tolerance.

Because the strain isolated from patient A matched that from elephant A, MCHD personnel searched for an unidentified, common human source and explored the possibility that the elephants might have been previously transmitting TB despite negative trunk washings. During the summer of 2014, the investigation was expanded to include two additional groups: 1) all current and former employees who had worked at the zoo since January 1, 2010, and who met the definition of close contacts, and 2) persons who participated in the same February 2012 zoo orientation as patient A, which was the time when patient A had the most contact with elephants (Figure 1, Figure 3). Among the 28 persons who participated in the 2012 zoo orientation (including patient A), 18 had a negative TST; nine persons no longer worked at the zoo and could not be reached. MCHD concluded that persons who participated in the same orientation as patient A were likely not infected with TB in the course of their orientation. MCHD uncovered no evidence of a previously unidentified human case in the zoo orientation cohort that could have infected other humans or elephant A during this time. As of April 2015, reports from CDC's TB Genotyping Information Management System revealed that the isolates from elephant A and from patient A have unique genotypes (spoligotype + 24-locus MIRU), not matched locally or nationally.

Final results of the investigation of all 31 close contacts since 2010 identified one additional positive TST result from July 2011 (induration = 19 mm); this is close to the zoo's baseline of 0–1 conversions per year (Figure 1). On the basis of these findings, shedding of *M. tuberculosis* by elephants before elephant A's diagnosis was deemed unlikely.

Throughout the investigation, MCHD worked with the zoo and the Oregon Health Authority to ensure the safety of staff members, animals, and the public. Close and prolonged contact, including spending multiple hours indoors with infected elephants, was associated with TB transmission in this investigation. Continuing routine protocols for annual TB screening of humans who work with elephants is warranted, as is a heightened screening recommendation for the closest contacts until summer 2016. In addition to other administrative and environmental controls, all current close contacts wear a fit-tested N-95 respirator or higher level of protection when in the elephant barn or in contact with any potentially infectious elephant. Close contacts will continue to receive a TST every 6 months until summer 2016, at which point the exposure control plan will be reevaluated. Close contacts with previous positive test results will have a periodic TB symptom screen rather than a TST.

Once all elephants complete treatment for active TB, the Oregon Health Authority, MCHD, and the zoo veterinarians will decide whether to modify the exposure control plan. The elephants will continue to be screened at regular intervals according to Department of Agriculture guidelines (*2*). Because of the absence of guidance on determining when an elephant is no longer infectious, the zoo and state and local public health professionals defined an infectious elephant as one that 1) has had *M. tuberculosis* isolated from a culture of a trunk washing sample, 2) has not received at least 2 months of adequate TB treatment, and 3) has not had at least three consecutive negative findings from cultures of monthly trunk washing samples; or that is not responding to treatment, has a worsening serologic picture,* or might otherwise pose a risk to the herd, zoo personnel, or the public. On the basis of the contact investigation results, MCHD has advised that outdoor contact with infectious elephants for <30 minutes and at a distance of ≥25 feet posed minimal risk for TB transmission.

MCHD also worked with zoo veterinarians and the state public health veterinarian to develop guidelines for safe public elephant viewing. Although the contact investigation suggested minimal risk, all infectious elephants were removed from general display and public viewing within 100 feet. Routine indoor and outdoor public viewing of noninfectious elephants is considered safe.

## Discussion

In North America, approximately 5% of captive Asian elephants are infected with *M. tuberculosis*, on the basis of positive cultures of trunk washing samples or necropsy results (*6*). The U.S. Department of Agriculture's Animal and Plant Health Inspection Service has developed guidelines for the screening and diagnosis of TB in captive elephants, including annual trunk wash samples for mycobacterial culture (*2*). However, trunk-wash sample cultures, the standard for diagnosing active TB in elephants, are insensitive, and some cases of TB might be missed. Serologic screening is used in some settings to identify elephants with TB infection (*7*), but is controversial among elephant veterinarians and is subject to false-positive results (*7*).

Although MCHD's investigation did not suggest previously unrecognized shedding of *M. tuberculosis* by the elephants, annual personnel screening is an important component of occupational safety, given the potential risk for TB exposure to staff members as well as the risk to elephants of transmission from humans with undiagnosed TB. Organizations that conduct TB testing for employees should have a mechanism for tracking results and investigating when TST conversions are elevated above the annual baseline. In addition, better understanding of modes of TB transmission between humans, elephants, and other animals might lead to more comprehensive guidelines for prevention of TB transmission in high-risk settings (*8*). Genotyping surveillance, in conjunction with epidemiologic investigation, might also be effective in linking human and non-human TB cases and evaluating unrecognized transmission, especially if the strains are rare. Collaboration between public health, veterinary medicine, and occupational health experts would allow for better understanding of the risks for and prevention of zoonotic transmission of *M. tuberculosis*.

## Acknowledgments

Lauren Cowan, Division of Tuberculosis Elimination, Mycobacteriology Laboratory Branch CDC; Brian Baker, Division of Tuberculosis Elimination, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, CDC; Bob Lee, Oregon Zoo.

1Multnomah County Health Department, Oregon; 2Public Health Division, Oregon Health Authority; 3Oregon Zoo; 4Washington County Department of Health and Human Services, Oregon.

Corresponding author: Amy Zlot, amy.zlot@multco.us, 503-988-3406.

## References

1. Michalak K, Austin C, Diesel S, Bacon MJ, Zimmerman P, Maslow JN. *Mycobacterium tuberculosis* infection as a zoonotic disease: transmission between humans and elephants. Emerg Infect Dis 1998;4:283–7.
2. Animal and Plant Health Inspection Service. Guidelines for the control of tuberculosis in elephants. Washington, DC: US Department of Agriculture, Animal and Plant Health Inspection Service; 2012. Available at http://www.usaha.org/Portals/6/Committees/tuberculosis/TB%20Guidelines%202012%20Draft%20revision%2020April2012.pdf .
3. Murphree R, Warkentin JV, Dunn JR, Schaffner W, Jones TF. Elephant-to-human transmission of tuberculosis, 2009. Emerg Infect Dis 2011;17:366–71.
4. National Tuberculosis Controllers Association; CDC. Guidelines for the investigation of contacts of persons with infectious tuberculosis. Recommendations from the National Tuberculosis Controllers Association and CDC. MMWR Recomm Rep 2005;54(No. RR-15).
5. CDC. Latent tuberculosis infection: a guide for primary health care providers. Atlanta, GA: US Department of Health and Human Services, CDC; 2013. Available at http://www.cdc.gov/tb/publications/ltbi/pdf/targetedltbi.pdf .
6. Feldman M, Isaza R, Prins C, Hernandez J. Point prevalence and incidence of *Mycobacterium tuberculosis* complex in captive elephants in the United States of America. Vet Q 2013;33:25–9.
7. Lyashchenko KP, Greenwald R, Esfandiari J, et al. Tuberculosis in elephants: antibody responses to defined antigens of *Mycobacterium tuberculosis*, potential for early diagnosis, and monitoring of treatment. Clin Vaccine Immunol 2006;13:722–32.
8. Stephens N, Vogelnest L, Lowbridge C, et al. Transmission of *Mycobacterium tuberculosis* from an Asian elephant (*Elephas maximus*) to a chimpanzee (*Pan troglodytes*) and humans in an Australian zoo. Epidemiol Infect 2013;141:1488–97.

* Serologic tests can be used as indicators of active infection in elephants or to assess an elephant's response to infection and treatment.

**Summary**

What is already known on this topic?

In North America, approximately 5% of captive Asian elephants are infected with *Mycobacterium tuberculosis*. Bidirectional spread of *M. tuberculosis* between elephants and humans has been documented.

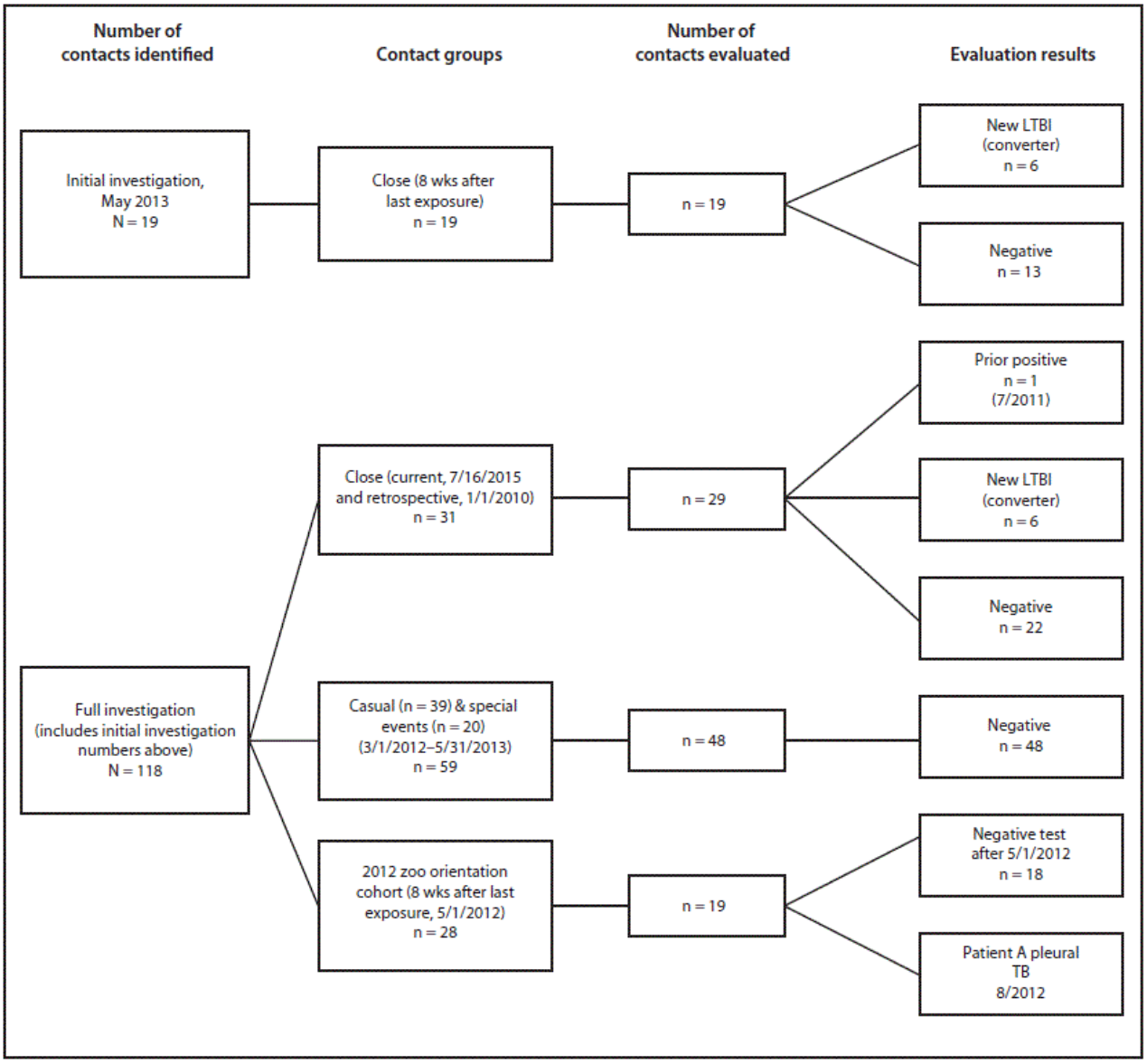
What is added by this report?

Investigation of a tuberculosis (TB) outbreak among three elephants at an Oregon zoo identified multiple close, casual, and spectator contacts. One hundred and eighteen contacts were identified, 96 of these contacts were screened, and seven close contacts (six recent conversions and one earlier positive test) were found to have latent, noninfectious TB. Whole-genome sequencing revealed that one elephant's *M. tuberculosis* isolate identically matched the isolate of a person with pleural TB who attended a zoo orientation in 2012. The lack of guidance about how to manage captive, TB-infected elephants complicated the decision-making process for protection of zoo contacts, other animals at the zoo, and the general public.

What are the implications for public health practice?

Collaboration between public health, veterinary medicine, and occupational health experts could lead to better understanding about associated risks, and could help prevent zoonotic transmission of *M. tuberculosis*. The development of improved TB screening methods for elephants is needed to prevent exposure to humans with close and prolonged contact.

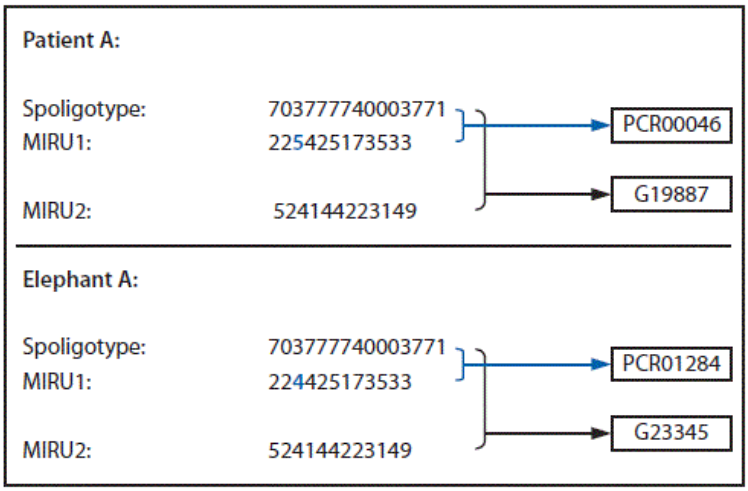
**FIGURE 1. Investigation of contacts of elephants with tuberculosis at a zoo — Oregon, 2013**




**Abbreviations:** LTBI = latent tuberculosis infection; TB = tuberculosis.

**Alternate Text:** The figure above is a diagram showing a contact investigation of elephants with tuberculosis at an Oregon zoo in 2013.

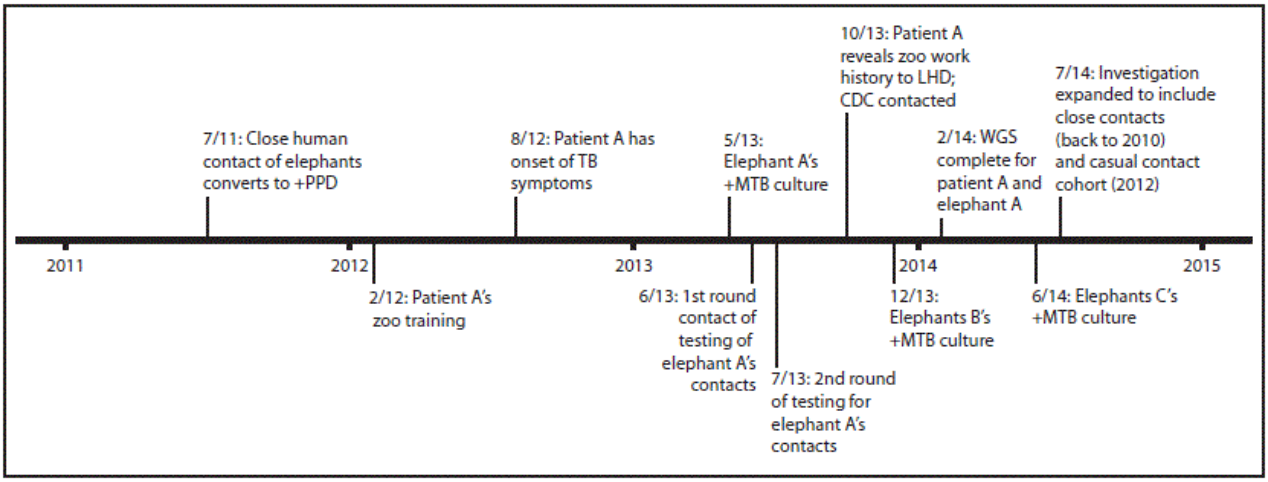
**FIGURE 2. Genotyping analysis of *M. tuberculosis* isolates from patient A and elephant A* — Oregon, 2013**




* Patient A and elephant A have slightly different genotypes (spoligotype+MIRU1+MIRU2), differing by only one locus.

**Alternate Text:** The figure above is a genotyping analysis of *Mycobacterium tuberculosis* isolates from patient A and elephant A from an Oregon zoo tuberculosis outbreak in 2013.

**FIGURE 3. Timeline of tuberculosis diagnoses in three elephants and a casual contact at a zoo — Oregon, 2013***




**Abbreviations:** LHD = local health department; +Mtb = positive for *Mycobacterium tuberculosis*; +PPD = positive purified protein derivative test (tuberculin skin test); TB = tuberculosis; WGS = whole genome sequencing.

* Current contacts (as of May 2013) of Elephant A during March 1, 2012–May 13, 2013 were initially investigated; in July 2014, the investigation was expanded to include close contacts back to January 1, 2012 and a casual (zoo orientation) cohort in February 2012.

**Alternate Text:** The figure above is a timeline of tuberculosis diagnoses in three elephants and a casual contact at an Oregon zoo in 2013.

American Journal of Primatology 76:2–13 (2014)

REVIEW ARTICLE

# The Risk of Tuberculosis Transmission to Free-Ranging Great Apes

TIFFANY M. WOLF[1,2]*, SRINAND SREEVATSAN[3], DOMINIC TRAVIS[3], LAWRENCE MUGISHA[4,5], AND RANDALL S. SINGER[1]

[1]*Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota*
[2]*Minnesota Zoological Gardens, Apple Valley, Minnesota*
[3]*Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota*
[4]*College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Kampala, Uganda*
[5]*Conservation and Ecosystem Health Alliance (CEHA), Kampala, Uganda*

Pathogen exchange between humans and primates has been facilitated by anthropogenic disturbances, such as changing land use patterns, habitat destruction, and poaching, which decrease population sizes and increase levels of primate–human interaction. As a result, human and domestic animal diseases have become a recognized threat to endangered primate populations. Tuberculosis is a major global human and animal health concern, especially in equatorial Africa where many of the remaining free-living great ape populations exist in proximity with exposed and/or infected human populations and their domestic animals. Increased anthropogenic pressure creates an opportunity for the anthropozoonotic spread of this disease. This review examines current evidence of the risk of tuberculosis transmission to great apes, the benefits and limitations of current detection methods, the impact of current great ape conservation and management strategies on this risk, and the need for an ecosystem health-based approach to mitigating the risks of tuberculosis transmission to great apes. Am. J. Primatol. 76:2–13, 2014. 

**Key words: great apes; tuberculosis; anthropozoonotic disease transmission**

## INTRODUCTION

In 2011, there were an estimated 8.7 million new cases of tuberculosis among humans worldwide, with a global prevalence of approximately 170 cases per 100,000 people [WHO, 2012]. This global pandemic is primarily caused by *Mycobacterium tuberculosis*, of which humans are the natural host, although other pathogenic mycobacteria of the *M. tuberculosis* Complex (MTC), such as *Mycobacterium africanum* and *Mycobacterium bovis*, also play a role in human infection [Cosivi et al., 1999; Gagneux, 2012; Kazwala et al., 2001]. Tuberculosis is predominantly a pulmonary disease, spread when bacteria are expelled from the lungs with the onset of active disease, but it may also present as extra-pulmonary disease involving other organs of the body [WHO, 2012]. Among humans infected with *M. tuberculosis*, only about 5–10% develop active disease and become infectious, while the remainder either eliminate infection or remain latently infected and do not transmit infection [Gagneux, 2012; Palomino et al., 2007; WHO, 2012]. However, those co-infected with human immunodeficiency virus (HIV) are much more likely to develop active disease [Cosma et al., 2003; Gagneux, 2012; Palomino et al., 2007; WHO, 2012]. Advances in molecular research are revealing much more genomic heterogeneity of *M. tuberculosis* strains than previously recognized [Cosma et al., 2003; Gagneux, 2012; Hershberg et al., 2008; Sreevatsan et al., 1997]. This genomic diversity has been linked to function and may explain some of the observed differences in infection outcome, disease progression, and transmission among infected humans [De Jong et al., 2008; Gagneux, 2012; Hershberg et al., 2008; Portevin et al., 2011].

Contract grant sponsor: Zoetis/Morris Animal Foundation Veterinary Research Fellowship; contract grant sponsor: Consortium on Law and Values in Health, Environment & the Life Sciences of the University of Minnesota; contract grant sponsor: Minnesota Zoo; contract grant sponsor: USDA-NIFA Specials Grant on Bovine Tuberculosis; contract grant sponsor: Veterinary Population Medicine Department of the University of Minnesota's College of Veterinary Medicine

Conflicts of interest: None

*Correspondence to: Tiffany M. Wolf, 205 Veterinary Sciences Building, University of Minnesota, 1971 Commonwealth Avenue, St. Paul, MN 55108. E-mail: wolfx305@umn.edu

Received 8 December 2012; revised 25 July 2013; revision accepted 29 July 2013

DOI: 10.1002/ajp.22197
Published online 5 September 2013 in Wiley Online Library (wileyonlinelibrary.com).

Until recently, infection with *M. tuberculosis* or any other MTC member has never been detected in free-ranging great ape populations, and many argue that contact between great apes and *M. tuberculosis* infected humans is insufficient for transmission to susceptible free-ranging great apes. However, as tuberculosis remains a major global human health threat and contact rates between humans and great apes increase with habitat encroachment, forest fragmentation, and conservation-driven research and ecotourism, the risk of tuberculosis transmission from humans to great apes must be continuously assessed. Moreover, transmission pathways from humans through other animal hosts whose contact with humans and great apes are high must be closely evaluated. Spillover of MTC infection from domestic animals and possibly humans into free-living monkey populations is well documented and may be an important source of transmission of human or domestic animal tuberculosis infection to great apes [Keet et al., 2000; Sapolsky & Else, 1987; Tarara et al., 1985; Wilbur et al., 2012]. A recent diagnosis of tuberculosis infection in a wild chimpanzee by a novel MTC strain underscores the knowledge gaps on the epidemiology and impact of tuberculosis to primate conservation [Coscolla et al., 2013]. The existence of great ape species in small, isolated populations requires that the long-term impact of tuberculosis transmission on population persistence be considered when characterizing this risk of disease caused by members of the MTC. Here we review reports on disease transmission, great ape conservation strategies, tuberculosis infection in non-human primates, and current methods of detection to demonstrate that tuberculosis transmission is a realistic threat for great ape conservation. Further, we identify specific areas where more research is needed to fully characterize this disease threat for great ape populations and demonstrate the need for an ecosystem health-based approach to mitigate this transmission risk. This review focuses on African populations of great apes, although many of the arguments presented here have application in Asian populations as well.

## DISCUSSION

### Disease Transmission Between Humans and Great Apes

Most extant great ape populations exist in fragmented populations distributed across equatorial Africa. These populations include Eastern (*Gorilla beringei*) and Lowland (*Gorilla gorilla*) gorillas, bonobos or pygmy chimpanzees (*Pan paniscus*), and common chimpanzees (*Pan troglodytes*) [Fruth et al., 2008; Oates et al., 2008; Robbins & Williamson, 2008; Walsh et al., 2008]. Eastern gorillas, of which there are two subspecies, mountain gorillas (*G. b. beringei*) and Eastern lowland gorillas (*G. b. graueri*) can be found in Uganda, Rwanda, and the Democratic Republic of Congo (DRC) [Robbins & Williamson, 2008]. Lowland gorilla (*G. gorilla*) populations, also consisting of two subspecies (*G. g. gorilla* and *G. g. diehli*), exist in forest fragments of several western African countries, such as Angola, Nigeria, Cameroon, Congo, and Gabon [Walsh et al., 2008]. While bonobo populations are limited to DRC, common chimpanzee populations, consisting of four subspecies (*Pan troglodytes verus*, *P. t. ellioti*, *P. t. troglodytes*, and *P. t. schweinfurthii*), are the most widely distributed of the great apes, stretching discontinuously across equatorial Africa from southern Senegal to western Tanzania and Uganda [Fruth et al., 2008; Oates et al., 2008]. All of these great ape populations are declining and are currently listed by the International Union for Conservation of Nature as endangered or critically endangered [Fruth et al., 2008; Oates et al., 2008; Robbins & Williamson, 2008; Walsh et al., 2008]. Each of these species are threatened by infectious diseases such as Ebola and a range of human pathogens, although differences in species behavior and social organization may be influencing exposure to and population impacts associated with certain pathogens [Nunn et al., 2003, 2007].

There is accumulating evidence indicating that great apes are exposed to and, in some cases, suffer disease from human and domestic animal pathogens [Kaur et al., 2008; Köndgen et al., 2008; Palacios et al., 2011; Rwego et al., 2008; Whittier, 2009; Williams et al., 2008]. There have been numerous independent reports of disease outbreaks among great ape populations across Africa in which pathogens have been linked to transmission from humans (Table I). In many of these epidemics, a definitive diagnosis of the etiological agent was not conclusively determined. In these cases, transmission from humans is speculative, based on circumstantial evidence associating animal behavior, clinical disease signs, and contact with local infected humans. However, in recent years molecular epidemiological methods have significantly improved our abilities to more definitively determine the role of human pathogen transmission in the occurrence of infectious disease outbreaks among great apes. For example, several outbreaks of respiratory disease in chimpanzees of Taï National Forest, Côte d'Ivoire were determined by molecular techniques to be caused by human metapneumovirus (HMPV) and respiratory syncytial virus (HRSV) [Köndgen et al., 2008, 2010]. Gene sequencing and phylogenetic analyses of HMPV and HRSV PCR products revealed virus strains to be closely related to those circulating in the human population, providing the first evidence for human disease transmission into a great ape population. Subsequently, HMPV infection has also

**TABLE I. Infectious Disease Epidemics of Free-Ranging Great Ape Populations Linked to Humans**

| Outbreak | Date | Species | Location | Morbidity (%)/mortality[a] | Etiology | Refs. |
|---|---|---|---|---|---|---|
| Paralytic disease | 1966 | Chimpanzees | Gombe National Park, Tanzania | 20/6 | Suspected polio | Williams et al. [2008], Goodall [1986] |
| Respiratory disease | 1968 | Chimpanzees | Gombe National Park, Tanzania | 63/4 | Undiagnosed, *Steptococcus pneumoniae* and *S. pyogenes* (2000 outbreak) | Williams et al. [2008], Mlengeya [2001] |
| | 1987 | | | 40/9 | | |
| | 2000 | | | 75/2 | | |
| Respiratory disease | 1988 | Mountain gorillas | Virunga Volcanoes, Rwanda | 81/3 | Suspected measles and *Mycoplasma pneumoniae* | Sholley [1989], Hastings et al. [1991], Homsy [1999] |
| Respiratory disease | 1990 | Mountain gorillas | Virunga Volcanoes, Rwanda | 61/1 | Suspected paramyxovirus | Homsy [1999] |
| Dermatitis | 1996 | Mountain gorillas | Bwindi Impenetrable National Park, Uganda | 100/1 | Scabies | Kalema-Zikusoka et al. [2002] |
| Respiratory disease | 1999[b] | Chimpanzees | Taï National Forest, Cote d'Ivoire | 100/6 | Human respiratory syncytial virus and metapneumovirus | Köndgen et al. [2008] |
| | 2004[b] | | | 100/8 | | |
| | 2006[b] | | | 92/1 | | |
| Respiratory disease | 2003 | Chimpanzees | Mahale Mountains National Park, Tanzania | 98/4 | Human metapneumovirus | Kaur et al. [2008] |
| | 2005 | | | 52/2 | | |
| | 2006[b] | | | 48/12 | | |
| Respiratory disease | 2009[b] | Mountain gorillas | Virunga Volcanoes, Rwanda | 92/2 | Human metapneumovirus | Palacios et al. [2011] |

[a]Morbidity is represented as the percentage of the total population that demonstrated clinical signs of disease, whereas mortality is the total number of deaths associated with the epidemic.
[b]There is strong molecular evidence that the etiological agents associated with these outbreaks originated from humans.

been associated with separate respiratory outbreaks among chimpanzees of Mahale Mountains National Park, Tanzania and mountain gorillas of Virunga Massif, Rwanda using PCR and phylogenetic analyses [Kaur et al., 2008; Palacios et al., 2011]. These findings demonstrate that sufficient contact between humans and great apes exists which enables the transmission of certain human pathogens, but much remains to be learned about such contact, the dynamics of these transmission events, and if these coupling points between humans and great apes would facilitate the transmission of other human pathogens.

Microbial transmission from humans to free-living great apes and other primates has also been documented beyond the scope of outbreak investigation. Several studies of antimicrobial resistance and genetic relatedness of enteric bacteria have shown that bacterial isolates from primates living in close proximity to humans share similar antimicrobial resistance patterns and are more genetically related to isolates from humans, as opposed to isolates from primates not living in close proximity to humans [Goldberg et al., 2008; Rwego et al., 2008]. These studies highlight the significance of environmental transmission of microorganisms and potential pathogens between humans and great apes.

Besides patterns of contact arising from anthropogenic impacts on the natural environment (e.g., habitat fragmentation and increased human densities surrounding great ape habitat), pathogen transmission has been associated with human habituation of great apes for research and ecotourism [Homsy, 1999; Köndgen et al., 2008]. Human habituation, a tool utilized in the conservation of endangered great apes, entails the conditioning of these animals to close encounters with human observers. The benefits of human habituation to great ape survival have been realized through the reduction of poaching and habitat loss in areas where research and ecotourism exist [Campbell et al., 2011; Köndgen et al., 2008; Pusey et al., 2007]. Thus, to maintain the benefits of habituation and mitigate the disease risks, managers must consider the health of the humans in contact with these animals: tourists, researchers, park workers, and local humans living in proximity or within the parks.

Tourists have been a primary focus in assessing disease risks to great apes given their potential for introducing new pathogens into an ecosystem [Homsy, 1999; Sandbrook & Semple, 2007; Woodford et al., 2002]. However, it is important to note that several disease outbreaks in great ape populations (Table I) have been attributed to transmission from researchers (e.g., HRSV and HMPV outbreaks in Taï National Forest) or the local human population, including park workers (e.g., scabies, measles, polio) [Kalema-Zikusoka et al., 2002; Köndgen et al., 2008; Sholley, 1989; Williams et al., 2008; Woodford et al., 2002]. It has been shown that human behaviors, such as defecating, urinating, poor waste disposal, and aerosol contamination through sneezing and coughing, within and in proximity to mountain gorilla habitat are a health risk to mountain gorilla populations, with local communities posing the greatest risk [Nizeyi et al., 2012]. Thus, as we consider endemic disease risks to habituated great apes, it becomes clear that contact between great apes and local humans may pose a risk for the transmission of *M. tuberculosis* and other pathogenic members of the MTC, pathogens which may have a high prevalence in local African human and domestic animal populations and which may have potentially devastating effects on great ape populations.

## The Risk of Tuberculosis Transmission to Great Apes

With an initial assessment of the risk of tuberculosis transmission from humans to great apes, it may be hypothesized that the risk is fundamentally related to the incidence of active infection in the local human or animal populations with which great apes have contact. In the most basic Susceptible-Infectious-Recovered (SIR) transmission models, contact rate and increasing incidence of infectiousness drive transmission. Thus, in areas where human or domestic animal tuberculosis is higher and there is contact with great apes or other primates, higher transmission risk would be expected. Conversely, in areas where human/domestic animal tuberculosis and/or great ape contact is lower, the risk would inherently be lower. According to the 2012 WHO Global Tuberculosis Control report, among the 8.7 million global incident cases of human tuberculosis, 24% of these occurred in Africa [WHO, 2012]. Furthermore, the geographical distribution of African great ape habitat falls within countries that have some of the world's highest rates of human tuberculosis, ranging from 50 to over 300 incident cases per 100,000 people (Fig. 1) [WHO, 2012]. These statistics as well as the high prevalence of HIV co-infection among humans in this region raises additional concern for transmission risk, as co-infection with HIV generally results in a higher likelihood of active tuberculosis. Furthermore, recent evidence of MTC DNA among populations of free-ranging synanthropic macaques demonstrates that frequent human contact and high tuberculosis prevalence within the human population increases the risk of tuberculosis for non-human primate populations [Wilbur et al., 2012]. Unfortunately, the epidemiology of tuberculosis is not so simple as to be explained by basic SIR models. For instance, most human infections are latent and therefore not infectious, which complicates assessments of risk. Additionally, the contact needed for tuberculosis

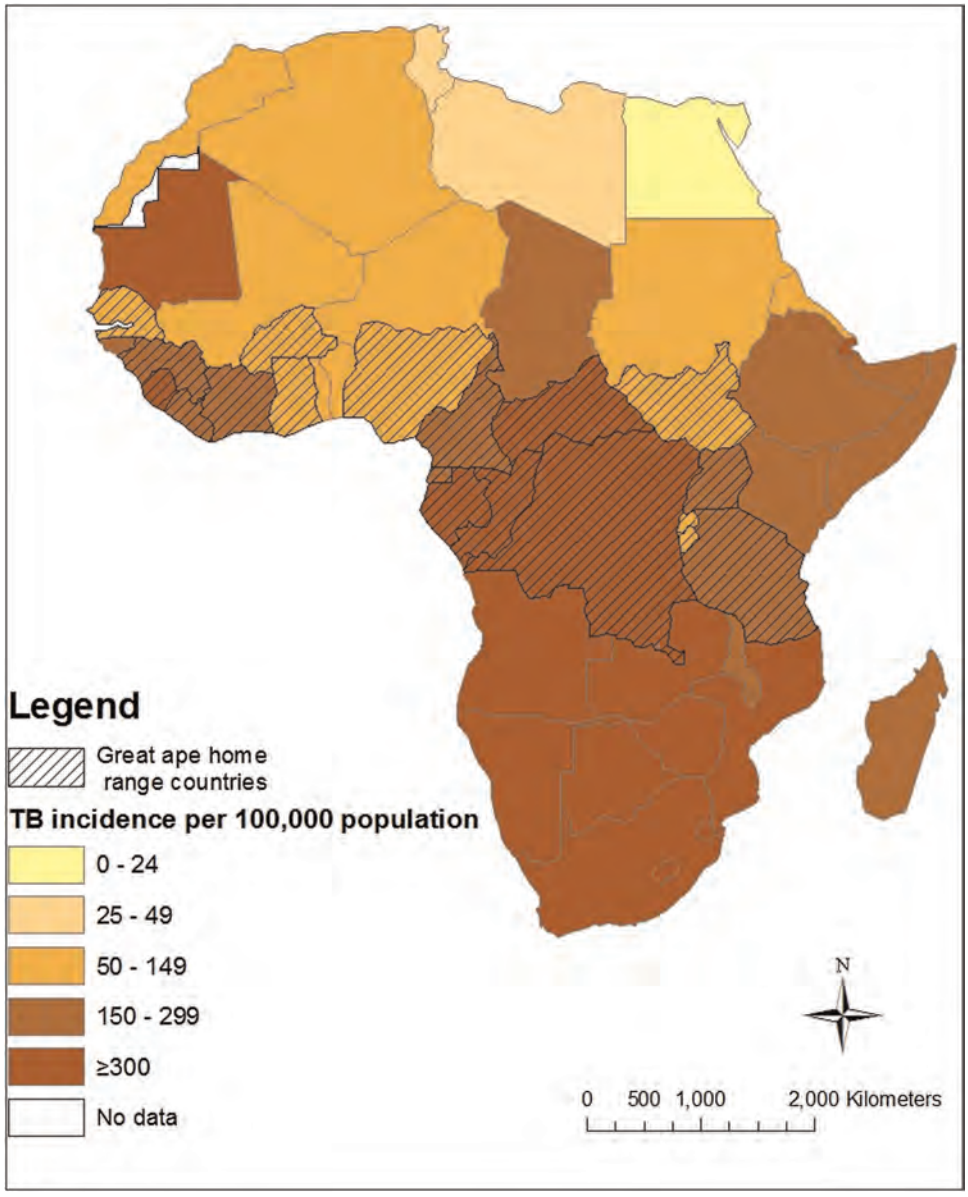



Fig. 1. Geographic distribution of great ape home range countries compared to the incidence of human tuberculosis in Africa. Data contained in this map originated from Robbins & Williamson [2008], Oates et al. [2008], Walsh et al. [2008], and Fruth et al. [2008], and WHO [2012].

transmission among humans is typically close and sustained, which is generally not characteristic of the contact between humans and free-living great apes. Thus, increases in human tuberculosis incidence will not necessarily be linearly related to the tuberculosis risk for great apes, particularly if other hosts are involved in transmission of infection. Moreover, much remains to be understood about the observed variation in susceptibility and transmission of different *M. tuberculosis* strains among humans and the genetic drivers of these events before reasonable predictions can be made about risk to primates [Gagneux, 2012]. However, as basic science and epidemiological research enhances our understanding of this variability among humans, our ability to predict this risk for primate populations will also advance.

A survey conducted in 2000 of local inhabitants of Bwindi Impenetrable Forest National Park, Uganda found that despite a high level of respiratory symptoms in the region, many people were not tested for tuberculosis and infection status was largely unknown [Guerrera et al., 2003]. These data suggest that many cases of tuberculosis may go undetected and untreated. This situation is slowly changing as global efforts and funding for tuberculosis control are increasing, especially in areas of Africa with high HIV prevalence [WHO, 2012]. Since park workers

employed to protect great apes originate from these local communities, updated information on the tuberculosis status and awareness among these communities would be valuable to both public health and great ape conservation. While population managers generally recognize concern for tuberculosis introduction and guidelines for tuberculosis testing among park employees have been developed, employee health and disease screening programs have not yet been widely adopted for park workers [Ali et al., 2004; WCS, 2005].

Many parks have established rules to reduce pathogen transmission from humans to great apes, such as restricting great ape visitation by people who are ill and coughing, or through vaccination [Homsy, 1999; Williamson & Macfie, 2010]. As *M. tuberculosis* is generally transmitted by the aerosolization of infectious particles (through coughing, talking, or sneezing) that can be suspended in the air for hours before being inhaled, such park rules should prevent the transmission of this pathogen to great apes from infectious people with pulmonary tuberculosis simply by eliminating contact [Baker, 1995]. Although this rule may not capture people infected with gastrointestinal tuberculosis, who may be shedding high numbers of organism in their stool, other rules restricting defecation within great ape habitat should reduce such a risk [Rasheed et al., 2007; Sharma & Bhatia, 2004]. However, as habitat use by non-research and non-tourist humans increases, the risks of disease transmission that are mitigated by these rules might be expected to increase. Additionally, in situations where park workers, researchers, or other local humans reside within the park and great apes enter areas of human habitation, restricting visitation by ill humans may not be enough to completely eliminate contact and transmission that may occur within these areas of human habitation. Furthermore, the use of vaccination in humans as a preventative measure may provide a false sense of security, as bacille Calmette-Guérin (BCG), the only vaccine available against tuberculosis, does not reliably protect against pulmonary tuberculosis [Russell et al., 2010].

Indirect routes of transmission such as contamination and pathogen persistence in the environment should also be considered as possible pathways for tuberculosis transmission. Great apes that frequent areas of human habitation, either within or outside of parks, may be at greatest risk for both direct and indirect transmission. For example, *M. tuberculosis* (as well as other respiratory pathogens) may be transmitted via interaction with contaminated objects (i.e., fomites)—such as tissues or handkerchiefs—that capture the attention of curious great apes, which often touch, smell, and potentially consume such novel objects [Wallis & Lee, 1999; Woodford et al., 2002]. In general, *Mycobacterium* species are well adapted to survival in harsh environments, with the lipid-rich, protective cell wall, slow growth rate, and long dormancy [Baker, 1995; Chadwick, 1981; Palomino et al., 2007]. Environmental contamination and fomites have been implicated in the transmission of MTC organisms (e.g., *M. bovis*, *M. mungi*) between wildlife, humans, and domestic animals [Alexander et al., 2010; Courtenay et al., 2006; Tarara et al., 1985]. Further, other primate or wildlife species may serve as a vector for transmission of tuberculosis (human, bovine or other) into great ape populations. The role of environmental, fomite, or vector species transmission in other human respiratory pathogen outbreaks among great apes has not yet been assessed, but should be explored when weighing the risks of tuberculosis transmission into great ape and other primate populations.

Another potential source of human tuberculosis for free-living great ape populations is the reintroduction of rehabilitated great apes by primate sanctuaries. Great apes at these facilities originate from diverse locations throughout Africa in various states of health and have assorted histories of human contact [Mugisha et al., 2011; Schoene & Brend, 2002]. These sanctuaries are challenged with managing injuries and illnesses in the face of limited resources. Crowded conditions and animal stress contribute to efficient disease transmission, and cross-species transmission between animals and human caretakers is a significant concern. This concern was exemplified in a recent study of *Staphylococcus aureus* epidemiology in African sanctuaries where chimpanzees were found infected with a variety of human-associated, multi-drug resistant strains of *S. aureus*, indicating transmission from their human caretakers [Schaumburg et al., 2012b]. Tuberculosis outbreaks have also been diagnosed within primate sanctuaries and are particularly concerning given the challenges of early detection, diagnosis, and management of infected individuals with limited resources [Unwin et al., 2012]. The number of great apes turned over to sanctuaries for medical care and rehabilitation is increasing, as is interest in reintroduction of these animals into their natural habitat. Given the current challenges of disease screening in these settings, rehabilitated animals would pose a significant risk for the introduction of tuberculosis and other human pathogens into presumably naïve free-living populations.

Understanding pathogen transmission across host species within an ecosystem is a complex task, particularly when several closely related pathogens are circulating and causing disease. This is certainly an issue in human medicine, where closely related members of the *Mycobacterium* genus have been responsible for disease in humans. For example, *M. bovis*, the etiologic agent of bovine tuberculosis and close relative of *M. tuberculosis* in the MTC, has been documented in cases of extrapulmonary tuberculosis in rural Tanzania [Kazwala et al., 2001, 2006].

Unfortunately, it is not easily distinguished from *M. tuberculosis* when culture is unavailable, thus its contribution to the tuberculosis epidemic in humans is not fully understood [Cleaveland et al., 2007; Kazwala et al., 2001]. Moreover, despite a growing body of evidence for the zoonotic potential of *M. bovis*, developing countries often lack regulations for control and prevention of infection in livestock, and general knowledge regarding risks of infection are lacking [Cosivi et al., 1999; Michel et al., 2010].

The distinction between *Mycobacterium* species is relevant with regard to the source of transmission and how these pathogens are transmitted between species. Although humans may be infected with and suffer disease from either *M. tuberculosis* or *M. bovis*, a much higher prevalence of *M. tuberculosis* has been documented in humans with tuberculosis [Kazwala et al., 2001, 2006]. Additionally, the transmission of *M. tuberculosis* among humans (e.g., via aerosolized infectious organisms) is generally different than the transmission of *M. bovis* to humans (e.g., via unpasteurized milk and exposure to infected animal tissues) [Baker, 1995; Cosivi et al., 1999]. On the contrary, livestock with tuberculosis are typically infected with *M. bovis* and not *M. tuberculosis*, and are generally infected by *M. bovis* through aerosolized infectious organisms from conspecifics or through exposure to infectious materials such as feces and urine from alternative hosts sharing their environment (as observed with wildlife hosts such as badgers in Britain) [Courtenay et al., 2006; Morris et al., 1994]. This distinction between *Mycobacterium* species becomes important when discussing transmission risk as these organisms are transmitted between species and through the environment by different mechanisms and pathways, which in turn impacts the risk of exposure to these pathogens for primates within their own environment. Therefore, to further consider strategies that might reduce the risk of disease in primate populations, it is important to evaluate *Mycobacterium* species-specific differences (including infected source populations) in transmission that might impact primate exposure.

Given the phylogenetic similarity of humans and great apes, as well as evidence of *M. bovis* infection in free-living baboon populations, it may be presumed that great apes share a similar risk of infection by *M. bovis* [Keet et al., 2000; Sapolsky & Else, 1987; Tarara et al., 1985]. Species such as baboons, whose behavior brings them in frequent contact with humans, livestock, and great apes, might be potential coupling points for disease transmission across some of these populations that might not otherwise come into direct contact [Keet et al., 2000; Müller-Graf et al., 1997; Murray et al., 2000]. Thus, to fully understand the risk of tuberculosis transmission to great apes, the prevalence of *M. bovis* in local livestock as well other wildlife species (e.g., baboons or other monkeys) must also be considered.

## Tuberculosis Infection in Primates

Much of our understanding of naturally acquired tuberculosis infection in great apes and monkeys originates from observations of captive animals [Diniz et al., 1983; Loomis, 2003; Michel et al., 2003; Michel & Huchzermeyer, 1998]. Clinical signs are absent in latent infection, but quite varied with active disease, ranging from nonspecific abnormalities, such as anorexia, lethargy, or weight loss to respiratory signs such as tachypnea or coughing [Diniz et al., 1983; Michel et al., 2003]. Extrapulmonary infection results in changes in health associated with the tissue of infection (e.g., draining abscessation, hemorrhagic diarrhea) [Michel et al., 2003]. Pathologic lesions may be characterized by infiltrates or cavitations of the lungs or other infected tissues, including lymph nodes, bone, kidney, central nervous system, and others. Within the realm of captive management, there is much concern for the transmission of tuberculosis from humans to great apes, due to the recognized susceptibility of great apes to tuberculosis [Loomis, 2003; Michel & Huchzermeyer, 1998]. It is difficult, however, to predict how susceptibility, disease, and transmission of tuberculosis as observed among captive great apes might translate to free-ranging populations. Certainly stress, social interactions, human contact, and activity patterns can strongly influence susceptibility and disease; however, the difficulties in measuring these factors for direct comparison of captive and free-ranging populations challenges our ability to extrapolate from our knowledge of this disease in captivity to estimate the risk of infection and potential impacts on free-ranging populations.

A recent diagnosis of MTC infection in a wild chimpanzee is our first glimpse of tuberculosis infection in free-ranging great apes [Coscolla et al., 2013]. In this report, researchers describe the identification of a genetically distinct MTC strain of tuberculosis, most closely related to Lineage 6 (i.e., *M. africanum* West-Africa type-2), on a routine necropsy of an aged female chimpanzee killed by a leopard in Taï National Forest. Aside from deteriorating body condition over a period of years, the report indicated no other clinical signs associated with the extra-pulmonary tuberculosis infection. The investigators further report that despite extensive necropsies and molecular screens of other chimpanzees in the region, this appears to be a unique finding, and it is yet unknown as to whether this novel strain is a chimpanzee-specific pathogen or one transmitted from another primate or animal host. Although most closely related to human-associated strains of tuberculosis, the results of this investigation do not suggest that infection originated from humans. Undoubtedly, this finding warrants more active investigations into the prevalence of this pathogen and the genetic diversity of tuberculosis infection among free-living

primates to better understand the epidemiology and impact of tuberculosis infection to the conservation of these populations.

There are inherent challenges in positively identifying tuberculosis in great apes. Multiple diagnostic modalities, typically relying on the demonstration of tissue lesions, host immune responses, or culture of the organism, are required for the diagnosis in great apes by standard methods [Lin et al., 2008; Miller, 2008]. Reliance on these traditional tuberculosis test methods makes tuberculosis surveillance impractical given the need for animal handling and anesthesia for collection of the necessary diagnostic specimens. Thus, the detection of tuberculosis in free-ranging species has been mostly limited to post-mortem diagnosis, at which time transmission of tuberculosis may be well advanced through a social primate group. Given these limitations, without systematic monitoring of population health accompanied by recovery and post-mortem examination of all carcasses, a low level of tuberculosis infection among a great ape population might go undetected. To overcome this potential problem, consideration must be given to the application of molecular methods of pathogenic organism detection in the development of non-invasive methods of tuberculosis diagnosis.

Non-invasive sampling refers to the collection of biological samples without the need for animal handling or anesthesia. Such methods have been useful in the screening of saliva, feces and urine for systemic, gastrointestinal, and respiratory pathogens of great ape populations [Gillespie et al., 2010; Kaur et al., 2008; Keele et al., 2009; Köndgen et al., 2010; Liu et al., 2008; Makuwa & Souquiere, 2003; Rudicell et al., 2010; Schaumburg et al., 2012a]. Readers are referred to excellent reviews of infectious diseases of free-living great apes and noninvasive sampling methods for the screening of a variety of pathogens [Calvignac-Spencer et al., 2012; Gillespie et al., 2008; Leendertz et al., 2006]. Accordingly, there are several molecular methods that may be applied to such samples and be useful in the detection of tuberculosis infection (Table II).

The collection of saliva samples of great apes from what is commonly referred to as "wadges," or masticated clumps of forest food, has found use in genetic research of free-living great apes and has more recently been employed in noninvasive disease screening [Inouse et al., 2007; Schaumburg et al., 2012a; Shimada et al., 2004; Smiley et al., 2010]. Saliva samples from animals with clinical signs of disease could be utilized for the detection and genotyping of *M. tuberculosis* through culture and/or commonly employed techniques such as IS*6110* PCR-RFLP, spoligotyping, or mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) genotyping [Sankar et al., 2011; Wilbur et al., 2012]. These techniques are useful in distinguishing *M. tuberculosis* from infection with other MTC strains. In human medicine, mannose-capped lipoarabinomannan (LAM), a cell wall component of pathogenic mycobacteria, has been utilized as a urine biomarker of tuberculosis infection [Hamasur et al., 2001]. The utility of this biomarker in the diagnosis of infection in humans has been limited by low sensitivity and specificity, although it has shown greater accuracy in patients co-infected with HIV [Peter et al., 2010]. The usefulness of LAM in the detection of tuberculosis in non-human primates has yet to be determined. Urine collection is a realistic option for non-invasive sample collection, having been used in other disease surveys; thus, it is reasonable to consider LAM as a possible biomarker for non-invasive tuberculosis detection in great apes [Leendertz et al., 2006]. Fecal samples are the most readily available and easily attainable biological samples of free-ranging great apes. The detection of fecal antibodies against pathogenic organisms has not been widely utilized for disease screening in primates; however, methods for fecal antibody detection have proven successful for the non-invasive detection of Simian Immunodeficiency virus and Simian Foamy virus in wild chimpanzees [Keele et al., 2009; Liu et al., 2008]. Given the development of a detectable humoral immune response to tuberculosis in primates, the detection of anti-tuberculosis antibodies in feces may be a feasible option for diagnosis [Lin et al., 2008; Lyashchenko

**TABLE II. Non-Invasive Sampling and Potential Methods for Tuberculosis Detection**

| Sample | Potential target or detection method | Limitations |
|---|---|---|
| Saliva | Culture and genotyping<br>Mycobacterial PCR<br>Antibodies | Infected individual must be infectious for the detection of organisms by culture or PCR, thus latent infection may go undetected. Possible low sensitivity associated with antibody detection |
| Urine | LAM | Low sensitivity and specificity in humans |
| Feces | Culture and genotyping<br>Mycobacterial PCR<br>Antibodies | Infected individual must be infectious for the detection of organisms by culture or PCR, thus latent infection may go undetected. Possible low sensitivity associated with antibody detection; antibodies present in swallowed sputum may be denatured in the stomach |

LAM, lipoarabinomannan.

et al., 2007]. Hence, exploration into fecal antibody detection may be warranted as another option for non-invasive tuberculosis screening in great apes. Alternatively, fecal culture or molecular detection of mycobacterial DNA in the feces of great apes offers another opportunity for the diagnosis of disease. Recent studies among humans with active pulmonary tuberculosis reveal approximately 50% sensitivity and 100% specificity for the detection of *M. tuberculosis* by stool culture, and even higher sensitivity using molecular detection (e.g., IS*6110* PCR-RFLP) [Cordova et al., 2010; El Khéchine et al., 2009]. Therefore, culture and/or PCR may be a useful approach to pathogen detection in the feces of great apes.

A major limitation to the detection of tuberculosis infection by any of these methods is the latent stage of disease, in which case animals are not infectious and detection of the organism and immune response is often more challenging [Lin et al., 2008]. Alternatively, given the utility of these non-invasively collected specimens for potential disease screening of other pathogens, it is advantageous to move toward the development and validation of such methods. Certainly, as these methods for non-invasive tuberculosis detection improve and become more widely available, a more comprehensive assessment of tuberculosis status among great ape populations (e.g., disease-free or not) can be undertaken through ante-mortem population surveillance or monitoring.

## Directions for Future Research and Mitigation of Tuberculosis Risk

Understanding and/or mitigating the risk of tuberculosis for the conservation of great ape populations requires an ecosystem health approach. *M. tuberculosis* is a human pathogen, and there is evidence of high prevalence among humans residing in close proximity to great ape habitats across their home ranges. Better estimates and understanding of control measures for this disease in local human populations are needed for accurate estimation of risk to great ape populations with which they have contact. Thus, it is essential to develop partnerships among conservation managers and those involved in human health at local and non-governmental levels. Given the evidence of human respiratory diseases in great ape populations, it can be concluded that the necessary contacts already exist between humans and great apes for successful disease transmission. Whether these contacts are sufficient for tuberculosis transmission has yet to be determined. Furthermore, the role of the environment in the transmission of such pathogens remains unknown. Accordingly, epidemiological research into routes of transmission of known human pathogens affecting great ape populations are needed not only for protecting against specific disease, but also in understanding and potentially predicting opportunities for *M. tuberculosis* transmission within these ecosystems. Certainly, the most promising means of protecting great apes from *M. tuberculosis* is by improving the healthcare infrastructure among local human communities, thereby reducing the burden of human tuberculosis in these regions.

*M. tuberculosis* is, unfortunately, not the only mycobacterial pathogen for which great apes may be at risk of infection. *M. bovis*, a known pathogen of domestic livestock and wildlife, not only causes disease in humans, but has also spilled over into free-ranging monkey populations. Thus, understanding of the risk and prevention of *M. bovis* infection in great apes also requires efforts in the area of bovine tuberculosis. There is a significant need for regulation, surveillance, and control of bovine tuberculosis in developing countries, as well as education on the zoonotic potential of this pathogen. Endeavors to meet such objectives could significantly reduce the impact of this disease for humans and their livestock, as has been observed in developed countries, as well as eliminate a disease risk to great ape populations. Until these needs are met, however, estimates of *M. bovis* levels in local livestock populations and potential routes of transmission are necessary to characterize this disease risk to great ape populations.

*M. tuberculosis* is an old pathogen, originating in Africa [Cosma et al., 2003; Gagneux, 2012]. This pathogen's co-evolution with its human host is complex and there is much we are still learning about variability of infection, host response, distribution, and genetic and functional diversity [Cosma et al., 2003; Gagneux, 2012]. Likewise, similar observations of variations in infection and host response among primates have yet to be fully explored. Combined with historical limitations of diagnosing tuberculosis infection in free-ranging primate specie, it cannot be known with certainty that this pathogen is not already present in these populations nor the full extent to which other MTC members (such as "*Chimpanzee bacillus*," reported by Coscolla et al.) infect these populations [Coscolla et al., 2013]. The impact of tuberculosis and the dynamics of co-infection with other diseases (e.g., SIV) on the persistence of free-ranging primate populations cannot be fully assessed without the development and employment of sensitive and reliable means for detecting infection and characterizing the pathogen.

As long as tuberculosis continues as a significant human and livestock disease, there is inherent risk of transmission to remnant great ape populations with which there is human contact. Accordingly, just as protection of these populations against threats of further habitat loss and poaching is ensured through conservation and research activities, we must endeavor to enhance our understanding and mitigate

the risks of tuberculosis and other human and domestic animal pathogens that equally threaten the persistence of these populations.

## ACKNOWLEDGMENTS

Exploration into the risk of tuberculosis for habituated great apes is supported in part by the Zoetis/Morris Animal Foundation Veterinary Research Fellowship, the Consortium on Law and Values in Health, Environment & the Life Sciences of the University of Minnesota, the Ulysses S. Seal Conservation Fund of the Minnesota Zoo, the USDA-NIFA Specials grant on bovine tuberculosis, and the Veterinary Population Medicine Department of the University of Minnesota's College of Veterinary Medicine. The authors also thank the two American Journal of Primatology reviewers who provided insightful and thought-provoking comments and recommendations that contributed to the development of a more complete manuscript. This manuscript adhered to the American Society of Primatologists' Principles for the Ethical Treatment of Primates.

## REFERENCES

Alexander KA, Laver PN, Michel AL, et al. 2010. Novel *Mycobacterium tuberculosis* complex pathogen, *M. mungi*. Emerg Infect Dis 16:1296–1299.

Ali R, Cranfield M, Gaffikin L, et al. 2004. Occupational health and gorilla conservation in Rwanda. Int J Occup Environ Health 10:319–325.

Baker S. 1995. Airborne transmission of respiratory diseases. J Clin Eng 20:401–406.

Calvignac-Spencer S, Leendertz SAJ, Gillespie TR, Leendertz FH. 2012. Wild great apes as sentinels and sources of infectious disease. Clin Microbiol Infect 18:521–527.

Campbell G, Kuehl H, Diarrassouba A, N'Goran P, Boesch C. 2011. Long-term research sites as refugia for threatened and over-harvested species. Biol Lett 7:723–726.

Chadwick M. 1981. Mycobacteria. Boston: Wright/PSG. 114 p.

Cleaveland S, Shaw D, Mfinanga S, et al. 2007. *Mycobacterium bovis* in rural Tanzania: risk factors for infection in human and cattle populations. Tuberculosis 87:30–43.

Cordova J, Shiloh R, Gilman RH, et al. 2010. Evaluation of molecular tools for detection and drug susceptibility testing of *Mycobacterium tuberculosis* in stool specimens from patients with pulmonary tuberculosis. J Clin Microbiol 48:1820–1826.

Coscolla M, Lewin A, Metzger S, et al. 2013. Novel *Mycobacterium tuberculosis* complex isolate from a wild chimpanzee. Emerg Infect Dis. Available online ahead of print: http://dx.doi.org/10.3201/eid 1906.121012

Cosivi O, Grange J, Daborn C, et al. 1999. Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. Emerg Infect Dis 4:59–70.

Cosma CL, Sherman DR, Ramakrishnan L. 2003. The secret lives of the pathogenic mycobacteria. Ann Rev Microbiol 57:641–676.

Courtenay O, Reilly LA, Sweeney FP, et al. 2006. Is *Mycobacterium bovis* in the environment important for the persistence of bovine tuberculosis? Biol Lett 2:460–462.

De Jong BC, Hill PC, Aiken A, et al. 2008. Progression to active tuberculosis, but not transmission, varies by *Mycobacterium tuberculosis* lineage in The Gambia. J Infect Dis 198:1037–1043.

Diniz L, Iwasaki M, Demartin B. 1983. Clinical aspects of tuberculosis in a chimpanzee. Vet Med Small Anim Clin 78:1289–1291.

El Khéchine A, Henry M, Raoult D, Drancourt M. 2009. Detection of *Mycobacterium tuberculosis* complex organisms in the stools of patients with pulmonary tuberculosis. Microbiology 155:2384–2389.

Fruth B, Benishay J, Bila-Isia I, et al. 2008. *Pan paniscus*. IUCN red list of threatened species. Version 2012.2.

Gagneux S. 2012. Host-pathogen coevolution in human tuberculosis. Philos Trans R Soc Lond B Biol Sci 367:850–859.

Gillespie TR, Nunn CL, Leendertz FH. 2008. Integrative approaches to the study of primate infectious disease: implications for biodiversity conservation and global health. Am J Phys Anthropol Suppl 47:53–69.

Gillespie TR, Lonsdorf EV, Canfield EP, et al. 2010. Demographic and ecological effects on patterns of parasitism in eastern chimpanzees (*Pan troglodytes schweinfurthii*) in Gombe National Park, Tanzania. Am J Phys Anthropol 143:534–544.

Goldberg TL, Gillespie TR, Rwego IB, Estoff EL, Chapman CA. 2008. Forest fragmentation as cause of bacterial transmission among nonhuman primates, humans, and livestock, Uganda. Emerg Infect Dis 14:1375–1382.

Goodall J. 1986. The chimpanzees of Gombe: patterns of behavior. Cambridge, MA: Belknap Press of Harvard University Press. 673 p.

Guerrera W, Sleeman J, Jasper S, et al. 2003. Medical survey of the local human population to determine possible health risks to the mountain gorillas of Bwindi Impenetrable Forest National Park, Uganda. Int J Primatol 24:197–207.

Hamasur B, Bruchfeld J, Haile M, et al. 2001. Rapid diagnosis of tuberculosis by detection of mycobacterial lipoarabinomannan in urine. J Microbiol Methods 45:41–52.

Hastings B, Kenny D, Lowenstine LJ, Foster JW. 1991. Mountain gorillas and measles: ontogeny of a wildlife vaccination program. In: Proceedings of the American Association of Zoo Veterinarians annual meeting; September 28–October 3. Calgary, Canada: American Association of Zoo Veterinarians. p 198–205.

Hershberg R, Lipatov M, Small PM, et al. 2008. High functional diversity in *Mycobacterium tuberculosis* driven by genetic drift and human demography. PLoS Biol 6:e311.

Homsy J. 1999. Ape tourism and human diseases: how close should we get? Report of the Consultancy for the International Gorilla Conservation Programme. 86 p. Available online at: http://wildpro.twycrosszoo.org/000ADOBES/D133 Homsy_rev.pdf [accessed May 8, 2012].

Inoue E, Inoue-Murayama M, Takenaka O, Nishida T. 2007. Wild chimpanzee infant urine and saliva sampled noninvasively usable for DNA analyses. J Primatol 48:156–159.

Kalema-Zikusoka G, Kock R, Macfie E. 2002. Scabies in free-ranging mountain gorillas (*Gorilla beringei beringei*) in Bwindi Impenetrable National Park, Uganda. Vet Rec 150:12–15.

Kaur T, Singh J, Tong S, et al. 2008. Descriptive epidemiology of fatal respiratory outbreaks and detection of a human-related metapneumovirus in wild chimpanzees (*Pan troglodytes*) at Mahale Mountains National Park, Western Tanzania. Am J Primatol 70:755–765.

Kazwala R, Daborn C, Sharp J, et al. 2001. Isolation of *Mycobacterium bovis* from human cases of cervical adenitis in Tanzania: a cause for concern? Int J Tuberc Lung Dis 5:87–91.

Kazwala RR, Kusiluka LJM, Sinclair K, Sharp JM, Daborn CJ. 2006. The molecular epidemiology of *Mycobacterium bovis* infections in Tanzania. Vet Microbiol 112:201–210.

Keele BF, Jones JH, Terio KA, et al. 2009. Increased mortality and AIDS-like immunopathology in wild chimpanzees infected with SIVcpz. Nature 460:515–519.
Keet D, Kriek N, Bengis R, Grobler D, Michel A. 2000. The rise and fall of tuberculosis in a free-ranging chacma baboon troop in the Kruger National Park. Onderstepoort J Vet Res 67:115–122.
Köndgen S, Kühl H, N'Goran PK, et al. 2008. Pandemic human viruses cause decline of endangered great apes. Curr Biol 18:260–264.
Köndgen S, Schenk S, Pauli G, Hoesch C, Leendertz F. 2010. Noninvasive monitoring of respiratory viruses in wild chimpanzees. EcoHealth 7:332–341.
Leendertz FH, Pauli G, Maetz-Rensing K, et al. 2006. Pathogens as drivers of population declines: the importance of systematic monitoring in great apes and other threatened mammals. Biol Conserv 131:325–337.
Lin PL, Yee J, Klein E, Lerche NW. 2008. Immunological concepts in tuberculosis diagnostics for non-human primates: a review. J Med Primatol 37:44–51.
Liu W, Worobey M, Li Y, et al. 2008. Molecular ecology and natural history of simian foamy virus infection in wild-living chimpanzees. PLoS Pathog 4:e1000097.
Loomis M. 2003. Great apes. In: Fowler M, Miller R, editors. Zoo and wildlife medicine, current therapy, 5th edition. St. Louis: Saunders. p 381–396.
Lyashchenko KP, Greenwald R, Esfandiari J, et al. 2007. PrimaTB STAT-PAK assay, a novel, rapid lateral-flow test for tuberculosis in nonhuman primates. Clin Vaccine Immunol 14:1158–1164.
Makuwa M, Souquiere S. 2003. Occurrence of hepatitis viruses in wild-born non-human primates: a 3 year (1998–2001) epidemiological survey in Gabon. J Med Primatol 32:307–314.
Michel A, Huchzermeyer H. 1998. The zoonotic importance of *Mycobacterium tuberculosis*: transmission from human to monkey. J S Afr Vet Med Assoc 69:64–65.
Michel A, Venter L, Espie I, Coetzee M. 2003. *Mycobacterium tuberculosis* infections in eight species at the National Zoological Gardens of South Africa, 1991–2001. J Zoo Wildl Med 34:364–370.
Michel AL, Müller B, Van Helden PD. 2010. *Mycobacterium bovis* at the animal-human interface: a problem, or not? Vet Microbiol 140:371–381.
Miller M. 2008. Current diagnostic methods for tuberculosis in zoo animals. In: Fowler M, Miller R, editors. Zoo and wild animal medicine, current therapy 6, 6th edition. St. Louis: Saunders Elsevier. p 10–19.
Mlengeya T. 2001. Respiratory disease outbreak in the chimpanzee population of Gombe National Park. TANAPA Veterinary Department Annual Report 2000/2001.
Morris RS, Pfeiffer DU, Jackson R. 1994. The epidemiology of *Mycobacterium bovis* infections. Vet Microbiol 40:153–177.
Mugisha L, Kücherer C, Ellerbrok H, et al. 2011. Multiple viral infections in confiscated wild born semi-captive chimpanzees (*Pan troglodytes schweinfurthii*) in a sanctuary in Uganda: implications for sanctuary management and conservation. In: Proceedings of the American Association of Zoo Veterinarians annual meeting. Kansas City: American Association of Zoo Veterinarians. p 190–195.
Müller-Graf C, Collins D, Packer C, Woolhouse M. 1997. *Schistosoma mansoni* infection in a natural population of olive baboons (*Papio cynocephalus anubis*) in Gombe Stream National Park, Tanzania. Parasitology 115:621–627.
Murray S, Stem C, Boudreau B, Goodall J. 2000. Intestinal parasites of baboons (*Papio cynocephalus anubis*) and chimpanzees (*Pan troglodytes*) in Gombe National Park. J Zoo Wildl Med 31:176–178.
Nizeyi J, Nabambejja S, Mugisha L, Majalija S, Cranfield R. 2012. Risk assessment of human behaviours that may impact on the health of the Mountain Gorillas around Bwindi Impenetrable National Park, Western Uganda. Afr J Anim Biomed Sci 7:102–113.
Nunn CL, Altizer S, Jones KE, Sechrest W. 2003. Comparative tests of parasite species richness in primates. Am Nat 162:597–614.
Nunn CL, Thrall PH, Stewart K, Harcourt AH. 2007. Emerging infectious diseases and animal social systems. Evol Ecol 22:519–543.
Oates J, Tutin C, Humle T, et al. 2008. *Pan troglodytes*. IUCN Red List of Threatened Species. Version 2012. 2.
Palacios G, Lowenstine L, Cranfield MR, et al. 2011. Human metapneumovirus infection in wild mountain gorillas, Rwanda. Emerg Infect Dis 17:711–713.
Palomino J, Leao S, Ritacco V. 2007. Tuberculosis 2007: from basic science to patient care. Available online at: http://dspace.itg.be/handle/10390/2116 [accessed February 28, 2011].
Peter J, Green C, Hoelscher M, et al. 2010. Urine for the diagnosis of tuberculosis: current approaches, clinical applicability, and new developments. Curr Opin Pulm Med 16:262–270.
Portevin D, Gagneux S, Comas I, Young D. 2011. Human macrophage responses to clinical isolates from the *Mycobacterium tuberculosis* complex discriminate between ancient and modern lineages. PLoS Pathog 7:e1001307.
Pusey AE, Pintea L, Wilson ML, Kamenya S, Goodall J. 2007. The contribution of long-term research at Gombe National Park to chimpanzee conservation. Conserv Biol 21:623–634.
Rasheed S, Zinicola R, Watson D, Bajwa A, McDonald PJ. 2007. Intra-abdominal and gastrointestinal tuberculosis. Colorectal Dis 9:773–783.
Robbins M, Williamson L. 2008. *Gorilla beringei*. IUCN red list of threatened species Version 2012.2.
Rudicell RS, Holland Jones J, Wroblewski EE, et al. 2010. Impact of simian immunodeficiency virus infection on chimpanzee population dynamics. PLoS Pathog 6:e1001116.
Russell DG, Barry CE, Flynn JL. 2010. Tuberculosis: what we don't know can, and does, hurt us. Science 328:852–856.
Rwego IB, Isabirye-Basuta G, Gillespie TR, Goldberg TL. 2008. Gastrointestinal bacterial transmission among humans, mountain gorillas, and livestock in Bwindi Impenetrable National Park, Uganda. Conserv Biol 22:1600–1607.
Sandbrook C, Semple S. 2007. The rules and the reality of mountain gorilla *Gorilla beringei beringei* tracking: how close do tourists get? Oryx 40:428–433.
Sankar S, Ramamurthy M, Nandagopal B, Sridharan G. 2011. An appraisal of PCR-based technology in the detection of *Mycobacterium tuberculosis*. Mol Diagn Ther 15:1–11.
Sapolsky R, Else J. 1987. Bovine tuberculosis in a wild baboon population: epidemiological aspects. J Med Primatol 16:229–235.
Schaumburg F, Alabi A, Köck S, et al. 2012a. Highly divergent *Staphylococcus aureus* isolates from African non-human primates. Environ Microbiol Rep 4:141–146.
Schaumburg F, Mugisha L, Peck B, et al. 2012b. Drug-resistant human *Staphylococcus aureus* in sanctuary apes pose a threat to endangered wild ape populations. Am J Primatol 74:1071–1075.
Schoene CUR, Brend SA. 2002. Primate sanctuaries—a delicate conservation approach. S Afr J Wildl Res 32:109–113.
Sharma M, Bhatia V. 2004. Abdominal tuberculosis. Indian J Med Res 120:305–315.
Shimada MK, Hayakawa S, Humle T, et al. 2004. Mitochondrial DNA genealogy of chimpanzees in the Nimba mountains and Bossou, West Africa. Am J Primatol 64:261–275.
Sholley C. 1989. Mountain gorilla update. Oryx 23:57–58.
Smiley T, Spelman L, Lukasik-Baum M, et al. 2010. Noninvasive saliva collection techniques for free-ranging mountain gorillas and captive eastern gorillas. J Zoo Wildl Med 41:201–209.

Sreevatsan S, Pan X, Stockbauer KE, et al. 1997. Restricted structural gene polymorphism in the *Mycobacterium tuberculosis* complex indicates evolutionarily recent global dissemination. Proc Natl Acad Sci USA 94:9869–9874.

Tarara R, Suleman M, Sapolsky R, Wabomba M, Else J. 1985. Tuberculosis in wild olive baboons, *Papio cynocephalus anubis* (Lesson), in Kenya. J Wildl Dis 21:137–140.

Unwin S, Colin C, Nente C, et al. 2012. Stat-pak® blood assays in *M. tuberculosis* complex surveillance testing in populations of chimpanzees (*Pan* sp) and orang-utans (*Pongo* sp) in range countries. In: Szentiks, Schumann, editors. 736 Proceedings of the EAZWV/IZW international conference on diseases of zoo and wild animals. Bussolengo, Italy: European Association of Zoo and Wildlife Vets/Leibniz Institute for Zoo and Wildlife Research, p 109.

Wallis J, Lee D., 1999. Primate conservation: the prevention of disease transmission. Int J Primatol 20:803–826.

Walsh P, Tutin C, Oates J, et al. 2008. *Gorilla gorilla*. IUCN Red List of Threatened Species. Version 2012. 2.

WCS. 2005. Consensus document outlining practical considerations for reducing health risks to African great apes and conservation employees through an occupational health program. 13 p. Available online at: http://www.wcs-ahead.org/workinggrps_greatapes.html [accessed May 16, 2013].

Whittier C. 2009. Diagnostics and epidemiology of infectious agents in Mountain gorillas [dissertation]. Raleigh, NC: North Carolina State University. 280 p. Available online at: http://repository.lib.ncsu.edu/ir/bitstream/1840.16/6215/1/etd.pdf [accessed June 5, 2012].

WHO. 2012. Global Tuberculosis Report 2012. Geneva, Switzerland. Available online at: http://www.who.int/tb/publications/global_report/en/ [accessed February 4, 2013].

Wilbur AK, Engel GA, Rompis AA, et al. 2012. From the mouths of monkeys: detection of *Mycobacterium tuberculosis* complex DNA from buccal swabs of synanthropic macaques. Am J Primatol 74:676–686.

Williams J, Lonsdorf E, Wilson M, et al. 2008. Causes of death in the Kasekela chimpanzees of Gombe National Park, Tanzania. Am J Primatol 70:766–777.

Williamson E, Macfie E. 2010. Best practice guidelines for great ape tourism. Gland, Switzerland: IUCN/SSC Primate Specialist Group. 78 p.

Woodford MH, Butynski TM, Karesh WB. 2002. Habituating the great apes: the disease risks. Oryx 36:153–160.

# A natural asymptomatic herpes B virus infection in a colony of laboratory brown capuchin monkeys (*Cebus apella*)

**C. Coulibaly[1], R. Hack[2], J. Seidl[1], M. Chudy[1], G. Itter[2] & R. Plesker[1]**

[1]Paul Ehrlich Institute, Federal Agency for Sera and Vaccines, Langen, Germany and [2]Aventis Pharma Deutschland GmbH, Frankfurt, Germany

## Summary

Herpes B virus (BV) infection of macaques persists in the natural host, but is mainly asymptomatic. However, BV can cause fatal disease in humans and in several non-macaque species such as capuchin monkeys (*Cebus apella*). The BV infection described here in a colony of capuchin monkeys was persistent but asymptomatic. Initially the infection was detected serologically in five out of seven animals. However, using polymerase chain reaction (PCR) developed specifically for BV, we found the virus in all seven clinically healthy animals. It is probable that the infection was transferred from BV-infected macaques housed in different cages but in the same room for several years. We have no evidence to indicate that similar asymptomatic infections may occur in other New World species but the possibility should not be discounted. We recommend that the housing of capuchin monkeys in close proximity to macaques should be avoided and that greater caution should be used when handling capuchin monkeys and possibly other New World species that have been in contact with macaques. All may act as a source of BV infection in humans, hence routine, repeated testing of all primates is essential.

**Keywords** Herpes B virus; asymptomatic; infection; capuchin monkeys, *Cebus*; polymerase chain reaction

*Herpesvirus simiae* (herpes B virus, BV) is enzootic in Old World monkeys, particularly in macaques (genus *Macaca*). Antibody studies have shown that 70% to 90% of adult macaques are infected (Palmer 1987, Weigler 1992, Holmes *et al.* 1995). BV infection of the natural host resembles *Herpes simplex* infection in humans. Usually it is latent and asymptomatic or causes only minor illness. In some cases, however, it is fatal in Old World monkeys (Meredith *et al.* 1993, Carlson *et al.* 1997).

*Correspondence to: Dr Cheick Coulibaly, Paul Ehrlich Institute, Paul-Ehrlich-Str. 51–59, D-63225 Langen, Germany*
*E-mail: couch@pei.de*

Recrudescence, which can be asymptomatic, increases the risk of transmission. BV infection in humans can result in serious clinical symptoms and is often lethal (Holmes *et al.* 1995). In addition, it also causes fatal disease in several non-macaque species such as patas monkeys (*Erythrocebus patas*), black and white colobus (*Colobus abyssinicus*), capuchin monkeys (*Cebus apella*), common marmosets (*Callithrix jacchus*) and debrazza's monkeys (*Cercopithecus neglectus*) (Gay & Holden 1933, Loomis *et al.* 1981, Wilson *et al.* 1990, Weigler 1992).

This report describes detection of a persistent but asymptomatic BV infection

Accepted 11 February 2004
 

in a colony of New World monkeys (*Cebus apella*). It is likely that the infection was contracted through indirect contact with BV-infected macaques housed in the same room but in different cages.

## Materials and methods

*Animals and housing conditions*

*Colony history* The capuchin monkey colony (four males and three females) was part of a collection of several non-human primate species in the research facility of Aventis Pharma in Frankfurt, Germany. The colony was set up in 1977 with wild caught animals from Costa Rica and was never restocked with external animals. The only additions were animals bred in-house.

In April 1997, the collection consisted of 27 rhesus macaques (*Macaca mulatta*), four cynomolgus monkeys (*Macaca fascicularis*), three stump-tailed macaques (*Macaca arctoides*), 11 squirrel monkeys (*Saimiri sciureus*) and eight capuchin monkeys (*Cebus apella*), including one suckling offspring that was too young to test.

Since most of the animals were kept over a very long period in the research department in different buildings, and in various breeding and housing groups, it was not possible to follow their detailed housing history over the years.

After 1985 when the last rhesus and 1992 the last cynomolgus monkeys were brought to the colony no external monkeys were introduced until September 1993 when two rhesus were added. In February 1994 one rhesus and in April 1994 another five rhesus entered the colony.

In all species the housing was changed from single housing to group housing in custom-made enclosures of various sizes. Some of the macaques' enclosures had an outside run. All enclosures exceeded by far the dimensions recommended by the relevant guidelines and were equipped with various enrichment devices. The macaque species were kept in several buildings with frequent direct intra-species contact either for breeding and/or during experiments. Since different macaque species were housed in the same area but in different enclosures, indirect inter-species contact via caretakers at feeding or cleaning is highly probable. The capuchin group was kept in the same room with one rhesus group. This capuchin enclosure, however, was physically separated thereby preventing direct contact between rhesus and capuchins. Indirect contact through aerosol, dust or via caretakers cannot be excluded. In the period between 1994 and 1997 the capuchins were moved out of contact with the rhesus. During this time the capuchins shared a room with the squirrel monkeys, again with only indirect contact via the caretakers.

The monkeys were investigated regularly for salmonellosis and for tuberculosis. In March 1994 the first serological test for BV was performed by Charles River Laboratories, Wilmington, USA in all monkeys. All samples were tested negative.

In late December 1996 one rhesus showed clinical lesions of the genital and oral mucosa which could be interpreted as signs of BV infection. A clinical differential diagnosis of *Herpes genitalis* and/or *buccalis* was made. As a consequence of the clinical case, in February 1997 all monkeys (excluding the squirrel monkeys) were serologically tested at the Simian Diagnostic Laboratory, Virus Reference Laboratory in San Antonio, USA, and resulted in positive findings for BV. The test was repeated with new samples and the positive results were confirmed. Samples were also sent to the Public Health Laboratory Services (PHLS, London, UK) and the American results were essentially confirmed. The results of the investigations showed seropositivity in four of 25 tested rhesus, two of three stump-tailed macaques, none of four cynomolgus and five of eight capuchin monkeys (one of the five capuchins was equivocal in the COMPRIA test and slightly positive in the neutralization test).

All seropositive rhesus and all stump-tailed macaques were euthanized and necropsied at Paul Ehrlich Institute (PEI, Langen, Germany) without any further findings pointing to a BV infection. The rhesus that had shown clinical signs was seropositive but did not show any relevant changes at necropsy.

From the seropositive findings of the capuchin monkeys it was not possible to determine if these antibodies were homologous to BV or raised against heterologous, antigenetically similar *alpha-herpesviruses* which have been isolated from other South American species (*Herpesvirus saimiri-1*, *Herpesvirus ateles-1* in squirrel and spider monkey, respectively). The PHLS speculated on the possibility of this being an antibody to a capuchin monkey *alpha-herpesvirus* as, based on prevailing information, the capuchins would normally have succumbed to BV. It was concluded that the capuchins were not BV infected. We were however advised by PHLS that these monkeys be assumed to have been infected with a potential non-BV human pathogen based on the cross-species transmission data with primate *alpha-herpesviruses*. Therefore, we decided to keep the capuchins but introduced suitable isolation procedures.

In conclusion, the somewhat incomplete colony history indicated that the capuchins became seropositive between 1994, the time of the first negative BV test and 1997, when positive BV results were found and the species were separated.

All remaining rhesus monkeys were concentrated and separated off in one single barrier unit with several enclosures in pair or group housing. The access was limited and people had to change into protective clothes including goggles and gloves, which were discarded on leaving the barrier. The whole capuchin group was allowed to survive and housed in a second barrier unit with less stringent access restrictions. The squirrel monkeys had been given to a zoo and the cynomolgus monkeys were given to another research institute.

In April 1999 a retest at PHLS of the remaining 22 rhesus monkeys resulted in entirely negative findings.

Following a management decision to close the primate facility at Aventis Pharma in Frankfurt, all rhesus were transferred to other research institutions by 2001. However, any attempt to find a research facility willing to take the capuchin monkeys failed. Before giving the animals to a private zoo, they were retested for BV at the PHLS in February 2001. The results showed that five of seven animals tested were seropositive and it was considered too risky to give potentially BV-infected animals to private care. At the PEI, parallel attempts were made to check the unexpected results of PHLS. The positive results were confirmed by polymerase chain reaction (PCR) and serology and, after consultation with the responsible authorities, the animals were euthanized and investigated further.

### *Samples*

*In vivo* sampling and euthanasia were carried out at the Animal Facilities, Aventis Pharma, Frankfurt. Samples for serological testing and BV PCR were collected from the capuchin monkeys after anaesthesia with a mixture of 2.5 mg xylazine hydrochloride (Rompun® TS, Bayer, Leverkusen, Germany) and 5.0 mg ketamine hydrochloride (Ketamin 10%, WDT, Garbsen, Germany) per kg body weight injected i.m. into the thigh. The animals were assumed to be anaesthetized when they became immobile and the eye reflexes stopped. Blood samples were collected from the femoral vein using the vacutainer system (Becton and Dickinson GmbH, Heidelberg, Germany).

For oral sampling, the mouth mucosa was gently wiped thoroughly with a sterile swab (Heiland Vet. GmbH and Co. KG, Hamburg, Germany). For ocular sampling, the surface of the eyeball and the conjunctiva were wiped with a swab moistened with sterile PBS. The swab samples were placed into tubes with 2 ml of PBS and stored at –70°C.

After sampling, the animals were euthanized with 5 ml of T61 (Intervet Deutschland GmbH, Unterschleißheim, Germany) given intravenously. The carcasses were transported immediately to the PEI in Langen for necropsy where there were suitable facilities for handling potentially infected materials.

### *Necropsy, histopathology and immunohistology*

Necropsy was performed approximately one hour after euthanasia (transport time).

Organs (brain, nerve, ganglia, liver, kidney, heart, etc.) were fixed either with 4% formaldehyde solution or methanol (100%) for at least 24 h. Tissue samples were embedded in paraffin wax, 4 μm slides were prepared and haematoxylin–eosin staining was performed according to standard procedures. In addition, immunohistology was performed with a monoclonal antibody specific for *Herpes simplex* virus (Biodesign, Asbach, Germany) according to standard methods.

### *Serological assays*

Serological BV testing was done at the PHLS using BV specific monoclonal antibodies in a competitive radioimmunoassay (Norcott & Brown 1993). For comparison the same samples were retested at PEI using the Enzygnost® Anti-HSV/IgG Test Kit (DADE Chiron, Marburg, Germany). The test was optimized by using peroxidase conjugates with anti-monkey IgG (Nordic Immunological Laboratories, Tilburg, The Netherlands) instead of anti-human IgG for detection of anti-herpesvirus IgG antibodies in monkey serum samples.

### *Polymerase chain reaction*

The PCR method here described is based on a previously unpublished method developed for the specific diagnosis of BV in rhesus using the primers shown in Table 1.

NP40 inactivated BV propagated in Vero cells (kindly provided by Professor Schmitt, Bernhard Nocht Institute, Hamburg) and a cloned 3.7 kb fragment of the glycoprotein C (gC) region (kindly provided by Dr Huemer, University of Innsbruck) were used as positive controls. DNA from all samples was extracted using the QIAamp Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

The final incubation volume was 50 μl, containing the following components: 10 μl of the sample DNA, 50 mM KCl, 2 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 200 μM (each) of dNTP, 15 pMol sense and antisense primer, 1 M betaine, and 1.25 units AmpliTaq Gold DNA polymerase (PE Applied Biosystems, Weiterstadt, Germany). The reaction was run with one cycle at 95°C for 10 min, 60°C for 30 s, and 72°C for 40 s, followed by 39 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 40 s, and a final elongation step of 7 min at 72°C. The nested PCR was performed with 2 μl of the first reaction product in a 50 μl reaction mixture containing 50 mM KCl, 2 mM $MgCl_2$, 10 mM Tris HCl (pH 8.3), 200 μM (each) of dNTP, 1 μM of each internal primer, 1 M betaine, and 1.25 units AmpliTaq Gold DNA polymerase. The cycle programme was identical to the first PCR run, except the annealling temperature of 56°C. The amplified DNA was separated on an agarose gel and visualized by ethidium bromide staining on an UV illuminator. Procedures designed to avoid contamination were strictly applied throughout the studies (Kwok & Higuchi 1989). For DNA sequencing an aliquot of the amplified PCR product was directly cloned into the pCR2.1 vector with TA cloning system under the conditions suggested by the manufacturer (Invitrogen BV, Leek, The Netherlands). Plasmid DNA with an insert of the expected size was sequenced with the inner sense primer by using the 373 DNA Sequencer Stretch Line (PE Applied Biosystems, Weiterstadt, Germany).

**Table 1 Primer selection for amplification and detection of Herpes BV gC region**

| Primer position | Primer sequence | PCR |
|---|---|---|
| 1352 | 5'CGA GAT GGA GTT CGG GAG CGG CGA3' | Outer forward primer |
| 1646 | 5'GGT CAC CTG CTG GCC CAC GGG GTC3' | Outer reverse primer |
| 1410 | 5'GTG GAG CTG CAG TGG CTG CT3' | Internal forward primer |
| 1558 | 5'AGC CGG CAG GTG TAC TCG CT 3' | Internal reverse primer |

## Results

*Clinical symptoms*

The capuchin monkeys did not show any clinical signs of disease or lesions in the mucosa of the mouth or genitals at the time of swab sampling.

*Serological and PCR survey*

The results of the serology and PCR testing are shown in Table 2. The obtained sequences of the amplification products were identical to the published BV sequence of the gC region in GenBank (Accession No: AJ012474). Each animal was BV positive in at least one of the tests in 2001. PCR detected BV in each animal.

*Pathology*

Macroscopically, no evidence of a BV infection could be detected. Histologically, one animal (No. 29) showed a clear perivascular lymphoid infiltration around one vessel in the brain cortex. However, immunohistology specific for *Herpes simplex* virus did not demonstrate any specific reaction.

## Discussion

BV is usually highly pathogenic in non-macaque primates including humans. The BV infection cases reported for those vulnerable species occurred mostly by either occupational (Weigler 1992, Holmes *et al.* 1995) or housing contact with macaques (Loomis *et al.* 1981, Wilson *et al.* 1990, Weigler *et al.* 1993). In this report, direct contact between macaques and capuchin monkeys allowing bites or scratches was neither noticed nor likely, but cannot be excluded.

Indirect contact via contaminated protective clothes of personnel or via aerosolized excretions seems probable. It is noteworthy that the caretakers did not change their protective clothing during the daily care of both monkey species. The rhesus monkeys housed in the same room were found to be BV positive 5 years before and were euthanized immediately due to the high infection risk for the caretakers. At the same time, five of the capuchin monkeys were also BV seropositive. However, there were some doubts about the specificity of the serological assay in respect to cross-reaction with a presumed (at that time unidentified) capuchin monkey *alpha-herpesvirus*, which, had it existed, would be closely related to BV. Therefore, the animals were considered to be a potential risk to the caretakers and were kept under heightened isolation conditions. Based on prevailing advice and information there was not enough justification for euthanasia of these animals for safety reasons.

Enzygnost® Anti-HSV/IgG is an enzyme immunoassay to detect human IgG antibodies against *Herpes simplex* virus (HSV). The anti-human IgG/horse radish peroxidase

**Table 2 Results of the serological and PCR testing of capuchin monkeys for herpes B virus infection**

| | | Serology | | PCR | | | |
|---|---|---|---|---|---|---|---|
| Identity ID No. | Birth | PHLS 1997 | PHLS/PEI 2001 | Oral swab | Conjunctival swab | Serum | Sequencing |
| 1-M | 1977 | Pos. | Pos. | Pos. | Neg. | Pos. | Pos. |
| 21-M | 1977 | Neg. | Pos. | Pos. | Pos. | Neg. | Pos. |
| 23-M | 1977 | Pos. | Pos. | Pos. | Neg. | Neg. | Pos. |
| 25-M | 1980 | Pos. | Pos. | Neg. | Pos. | Pos. | Pos. |
| 27-F | 1988 | Pos. | Pos. | Neg. | Pos. | Pos. | Pos. |
| 29-F | 1993 | Neg. | Neg. | Pos. | Neg. | Neg. | nd |
| 30-F | 1997 | nd | Neg. | Pos. | Neg. | Pos. | nd |

M = male, F = female, Pos. = positive, Neg. = negative, nd = not done
Animal 16 (not shown): equivocal results in 1997 then euthanized for ethical reasons

conjugates used to detect antibodies to HSV are known to also react with monkey IgG. However, we optimized the test using anti-rhesus IgG/horse radish peroxidase conjugates. Although the modified test did not specifically detect antibodies to BV, it always confirmed the BV results at the PHLS for the same samples. Therefore we normally used this test to detect antibodies to BV in the PEI non-human primate colony.

The present BV serological testing confirmed the results of all but one animal (Table 2, Animal 21M) tested 5 years ago. At that time this animal was BV seronegative and seroconverted in the meantime, suggesting that the animal was either already infected without seroconversion or BV spread within the colony in the past 5 years. We cannot exclude a false seronegative result at the time, however the PHLS result from 1997 was clearly negative.

Rapid diagnosis of BV and consequent antiviral treatment is essential for the human patient's survival. For this purpose, specific PCR is a reliable diagnostic tool which needs to be standardized and used in laboratories involved in routine BV diagnosis.

By means of PCR, BV DNA was detected in each animal regardless of its serological status. Consequently, all capuchin monkeys were indeed shown to be infected with BV, even though no clinical signs were seen at any time. It is of importance that seronegative (below the detection limit) animals can harbour and shed BV. If these animals are implicated in an injury to humans, PCR testing should be performed at once. Since BV virus was detectable in only the oral swab of Animal 29, PCR testing should necessarily include ocular and oral swabs as well as blood samples. Similar results were reported by Huff *et al.* (2003) who used real-time PCR to detect BV in mucosal swabs of rhesus macaques.

Unfortunately, no samples for PCR testing were taken from the BV-infected macaques at the time of necropsy at PEI in 1997. Unlike Ohsawa *et al.* (2002), we could therefore not compare the sequences of BV DNA found in the capuchins with those of the rhesus to investigate whether they were indeed the source of the BV infection. Unlike Ohsawa *et al.* (2002), as we detected BV in swabs and/or serum, it was not necessary for us to assay the trigeminal nerve, which is tested only when other samples give negative or poor results. The comparison of the obtained BV sequences with those sequences published by Ohsawa *et al.* (2002) is not possible due to the amplification of different BV regions.

Although some authors (Weir *et al.* 1993) using traditional virus isolation methods reported that the shedding of BV even from seropositive animals is uncommon, PCR based virus detection was successful when the classical virus isolation failed (Slomka *et al.* 1997).

Despite the shortcomings expressed in our study, i.e. lack of comparison data with the original macaques and the fact that our PCR is designed for rhesus BV, our results indicate that greater precautions should be taken during contact with capuchin monkeys that have been in contact with rhesus as they may act as a source of BV infection for humans. We recommend that the housing of capuchins in close proximity to macaques should be avoided in zoos and in other facilities. With hindsight, we must admit that we have been fortunate in not having had a human case bearing in mind the close contact between our caretakers and capuchins. Testing captive primates for serious pathogens is essential to ensure adequate health and safety provision and protect other species.

## References

Carlson CS, O'Sullivan MG, Joyo MJ, Anderson DK, Harber ES, Jerome WG, Bullock BC, Heberling RL (1997) Fatal disseminated cercopithecine *Herpesvirus 1* (herpes B infection in cynomolgus monkeys (*Macaca fascicularis*). *Veterinary Pathology* **34,** 405–14

Gay FP, Holden M (1933) The herpes encephalitis problem. *Journal of Infectious Diseases* **171**, 287–303

Holmes GP, Chapman LE, Stewart JA, Straus SE, Hilliard JK, Davenport DS (1995) Guidelines for the prevention and treatment of B-virus infections in exposed persons. *Clinical Infectious Diseases* **20**, 421–39

Huff JL, Eberle R, Capitanio J, Zhou SS, Barry PA (2003) Differential detection of B virus and rhesus cytomegalovirus in rhesus macaques. *Journal of General Virology* **84**, 83–92

Kwok S, Higuchi R (1989) Avoiding false positives with PCR. *Nature* **339**, 227

Loomis MR, O'Neill T, Bush M, Montali RJ (1981) Fatal *Herpesvirus* infection in patas monkeys and a black and white colobus monkey. *Journal of American Veterinary Medicine Association* **179**, 1236–9

Meredith AS, Muthiah DD, David LP, Norwal WK, Douglas JR (1993) Disseminated B virus infection in a cynomolgus monkey. *Laboratory Animal Science* **3**, 545–50

Norcott JP, Brown DW (1993) Competitive radioimmunoassay to detect antibodies to herpes B virus and SA8 virus. *Journal of Clinical Microbiology* **31**, 931–5

Ohsawa K, Black DH, Torii R, Sato H, Eberle R (2002) Detection of a unique genotype of monkey B virus (*Cercopithecine herpesvirus 1*) indigenous to native Japanese macaque (*Macaca fuscata*). *Comparative Medicine* **52**, 555–9

Palmer AE (1987) B virus, *Herpesvirus simiae*: historical perspective. *Journal of Medical Primatologie* **16**, 99–130

Slomka MJ, Brown DW, Clewley JP, Bennett AM, Harrington L, Kelly DC (1997) Polymerase chain reaction for detection of *Herpesvirus simiae* (B virus) in clinical specimens. *Archives of Virology* **131**, 89–99

Weigler BJ (1992) Biology of B virus in macaques and human host: a review. *Clinical Infectious Diseases* **14**, 555–67

Weigler BJ, Hird DW, Hilliard JK (1993) Epidemiology of cercopithecine *Herpesvirus1* infection and shedding in a large cohort of rhesus macaques. *Journal of Infectious Diseases* **167**, 257–63

Weir EC, Bhatt PN, Jacoby RO, Hilliard JK, Morgenstern S (1993) Infrequent shedding and transmission of *Herpesvirus simiae* from seropositive macaques. *Laboratory Animal Science* **43**, 541–4

Wilson RB, Holscher MA, Chang T, Hodges JR (1990) Fatal *Herpesvirus simiae* B (B virus) infection in a patas monkey (*Erythrocebus patas*). *Journal of Veterinary Diagnostical Investigation* **2**, 242–4

Article

# A review of captive exotic animal-linked zoonoses.

January 2012 · Journal of Environmental Health 12:9-24

Phillip Arena



## Abstract

Captive exotic animal-linked zoonoses are part of a major global emerging disease problem. Exotic animals are notably represented in the pet trade, zoos, and to a far lesser extent in circuses, with exotic pets being the primary concern. Combined, in the UK there may be approximately 42 million exotic pets (including fishes) in private homes, an unknown number in zoos, and in circuses less than 40 individuals. A wide range of species is involved, and a large and expanding array of potentially pathogenic agents. Sixty-one percent of human diseases have a potentially zoonotic origin and 75% of global emerging human diseases have a wild animal link. Exotic pets in particular may represent a source of largely unrecognised and unrecorded microbes and macroparasites in the domestic environment. Pet markets constitute an especially high risk of infection and these risks are fundamentally uncontrollable. Future guidance may include advising against keeping exotic animals as pets unless excellent monitoring for diseases and essential husbandry practices are pursued. Zoos and circuses also involve zoonotic risks but may be relatively low because public visits and exposure are infrequent. The prevalence of exotic animal-linked zoonoses in the UK is unknown. Many cases of zoonotic disease are probably misdiagnosed as other conditions and under-reporting in general is a likely major factor in under-ascertainment of cases. In addition, border and domestic biosecurity is lacking. New guidance on

zoonoses monitoring, prevention and control is included as well as upgraded public health

... Read more

Public Full-texts

We'll let you know when the authors upload this research.

Full-text requested

USDA
Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

# Animal Care Tech Note

## Guidance for Zoos and Captive Wildlife Facilities: Protecting Susceptible Animals From SARS-CoV-2 Infection

While SARS-CoV-2 (the virus that causes COVID-19) spreads mainly from person to person, it can also infect certain susceptible animals. This includes dogs and cats, nondomestic big cats, nonhuman primates, ferrets, and mink, among others. Natural infections have occurred in captive gorillas, Asian small-clawed otters, several big cat species at zoos and sanctuaries, and in farmed mink after being exposed to animal caretakers with COVID-19. Other animals (other nonhuman primates, as well as ferrets, deer mice, white-tailed deer, raccoon dogs, and tree shrews) have shown they are susceptible to infection under laboratory conditions. In addition, SARS-CoV-2 antibodies have been found in some populations of free-ranging white-tailed deer. As research progresses, we continue learning more about if and how SARS-CoV-2 affects different species.

The following guidance is intended as a general aid for zoos and captive wildlife facilities that house susceptible animals.

### Standard Practices and Procedures

Preventing infection—among facility staff and between facility staff and animals—is important. Facilities can establish policies and procedures that reduce the risk of disease spread. Examples include:

- Non-punitive sick leave policies for people with COVID-19 symptoms
- Minimizing contact with susceptible animals
- Standard operating procedures for disinfecting enclosures and utensils used to feed animals
- Training staff on proper use of personal protective equipment (PPE)

For more examples and information on this topic, visit the Centers for Disease Control and Prevention online at www.cdc.gov/coronavirus/2019-ncov/your-health/wildlife.html.

### Preventing SARS-CoV-2 Spread Between the Public and Animals

Animals may be best protected by:

- Asking the public to wear a face mask at the facility
- Ensuring that members of the public cannot come within 6 feet of nonhuman primates, nondomestic big cats, and all species of mustelids (e.g., ferrets, mink, otters)
- Suspending hands-on encounters with any of the SARS-CoV-2-susceptible animals

While there's no current evidence that contact with animal fur can spread SARS-CoV-2, we are still learning about this virus and the COVID-19 disease.

### Learn More

If you have questions about protecting animals at your facility from SARS-CoV-2, contact our Animal Care staff at (970) 494-7478 or animalcare@usda.gov. For more information on SARS-CoV-2 and animals, including the latest research on susceptible species, go to:

- www.cdc.gov/coronavirus/2019-ncov/daily-life-coping/animals.html
- www.aphis.usda.gov/aphis/ourfocus/animalhealth/SA_One_Health/sars-cov-2-animals-us





APHIS 41-35-078 ▪ Issued April 2021 ▪ Slightly revised December 2021

(n) *Training.* (1) A program must train all governing body, policy council, management, and staff who determine eligibility on applicable Federal regulations and program policies and procedures. Training must, at a minimum:

(i) Include methods on how to collect complete and accurate eligibility information from families and third party sources;

(ii) Incorporate strategies for treating families with dignity and respect and for dealing with possible issues of domestic violence, stigma, and privacy; and,

(iii) Explain program policies and procedures that describe actions taken against staff, families, or participants who intentionally attempt to provide or provide false information.

(2) A program must train management and staff members who make eligibility determinations within 90 days following the effective date of this rule, and as soon as possible, but within 90 days of hiring new staff after the initial training has been conducted.

(3) A program must train all governing body and policy council members within 180 days following the effective date of this rule, and within 180 days of the beginning of the term of a new governing body or policy council member after the initial training has been conducted.

(4) A program must develop policies on how often training will be provided after the initial training.

[FR Doc. 2015–02491 Filed 2–9–15; 8:45 am]

**BILLING CODE P**

## DEPARTMENT OF COMMERCE

### National Oceanic and Atmospheric Administration

### 50 CFR Part 224

**[Docket No. 130321272–5109–03]**

**RIN 0648–XC589**

## Listing Endangered or Threatened Species: Amendment to the Endangered Species Act Listing of the Southern Resident Killer Whale Distinct Population Segment

**AGENCY:** National Marine Fisheries Service (NMFS), National Oceanic and Atmospheric Administration (NOAA), Commerce.

**ACTION:** Final rule.

**SUMMARY:** On January 25, 2013, we, NMFS, received a petition submitted by the People for the Ethical Treatment of Animals Foundation to remove the exclusion of captive animals from the endangered species listing of Southern Resident killer whale DPS, as well as, recognize the captive killer whale (*Orcinus orca*) ''Lolita'' as a protected member of the endangered Southern Resident killer whale Distinct Population Segment (DPS). We completed a status review and published a proposed rule, and we are now amending the regulatory language of the Endangered Species Act (ESA) listing of the DPS by removing the exclusion for captive members of the population. We have further determined that Lolita, a female killer whale captured from the Southern Resident killer whale population in 1970 who resides at the Miami Seaquarium in Miami, Florida, is not excluded from the Southern Resident killer whale DPS due to her captive status.

We proposed to amend the regulatory language of the ESA listing to remove the exclusion for captive whales from the Southern Resident killer whale DPS on January 27, 2014. Additionally, we solicited scientific and commercial information pertaining to the proposed rule and also conducted a peer review of the status review information on Lolita that informed the proposed rule. We have determined that captive members of the Southern Resident killer whale population should be included in the listed Southern Resident killer whale DPS. This rule amends the regulatory language of the listing to remove the exclusion for captive members of the DPS.

**DATES:** This final rule becomes effective on May 11, 2015.

**ADDRESSES:** Information supporting this final rule can be found on our Web site at: *http://www.westcoast.fisheries.noaa.gov/protected_species/marine_mammals/killer_whale/lolita_petition.html.*

Or in our office at:

• Protected Resources Division, NMFS, Northwest Region, Protected Resources Division, 7600 Sand Point Way NE., Attention Lynne Barre, Branch Chief.

**FOR FURTHER INFORMATION CONTACT:** Lynne Barre, NMFS Northwest Region, (206) 526–4745; Marta Nammack, NMFS Office of Protected Resources, (301) 427–8469.

**SUPPLEMENTARY INFORMATION:**

### ESA Statutory Provisions and Policy Considerations

On January 25, 2013, we received a petition submitted by the People for the Ethical Treatment of Animals Foundation on behalf of the Animal Legal Defense Fund, Orca Network, Howard Garrett, Shelby Proie, Karen Munro, and Patricia Sykes to remove the exclusion of captive whales from the SRKW DPS ESA listing and to include the killer whale known as Lolita in the ESA listing of the Southern Resident killer whales. Lolita is a female killer whale captured from the Southern Resident population in 1970, who currently resides at the Miami Seaquarium in Miami, Florida. Copies of the petition are available upon request (see **ADDRESSES**, above).

In accordance with section 4(b)(3)(A) of the ESA, to the maximum extent practicable within 90 days of receipt of a petition to list, reclassify, or delist a species, the Secretary of Commerce is required to make a finding on whether that petition presents substantial scientific or commercial information indicating that the petitioned action may be warranted, and to promptly publish such finding in the **Federal Register** (16 U.S.C. 1533(b)(3)(A)). The Secretary of Commerce has delegated this duty to NMFS. If we find that the petition presents substantial information indicating that the petitioned action may be warranted, we must commence a review of the status of the species concerned, during which we will conduct a comprehensive review of the best available scientific and commercial information. On April 29, 2013 we made a finding (78 FR 25044) that there was sufficient information indicating that the petitioned action may be warranted and requested comments to inform a status review.

After accepting a petition and initiating a status review, within 12 months of receipt of the petition we must conclude the review with a determination that the petitioned action is not warranted, or a proposed determination that the action is warranted. Under specific facts, we may also issue a determination that the action is warranted but precluded. On January 27, 2014 we made a finding (79 FR 4313) that the petitioned action to remove the exclusion of captive killer whales from the ESA listing of the Southern Resident killer whale DPS and to include captive killer whales in the ESA listing of the Southern Resident killer whale DPS was warranted and proposed to amend the regulatory language describing the DPS by removing the current exclusion for captive whales. Within 12 months of issuing a proposed rule on a listing determination, we must publish a final regulation to implement the determination or publish a notice extending the 12-month period. This notice is a final rule to implement our

determination that the petitioned action is warranted and to amend the language describing the endangered listing of the Southern Resident killer whale DPS by removing the exclusion for captive whales.

Under the ESA, the term ''species'' means a species, a subspecies, or a DPS of a vertebrate species (16 U.S.C. 1532(16)). A joint NMFS-U.S. Fish and Wildlife (USFWS) policy clarifies the Services' interpretation of the phrase ''Distinct Population Segment,'' or DPS (61 FR 4722; February 7, 1996). The DPS Policy requires the consideration of two elements when evaluating whether a vertebrate population segment qualifies as a DPS under the ESA: (1) Discreteness of the population segment in relation to the remainder of the species/taxon, and, if discrete; (2) the significance of the population segment to the species/taxon.

A species is ''endangered'' if it is in danger of extinction throughout all or a significant portion of its range, and ''threatened'' if it is likely to become endangered within the foreseeable future throughout all or a significant portion of its range (ESA sections 3(6) and 3(20), respectively, 16 U.S.C. 1532(6) and (20)). Thus, we interpret an ''endangered species'' to be one that is presently in danger of extinction. A ''threatened species,'' on the other hand, is not presently in danger of extinction, but is likely to become so in the foreseeable future (that is, at a later time). In other words, the primary statutory difference between a threatened species and an endangered species is the timing of when a species may be in danger of extinction, either presently (endangered) or in the foreseeable future (threatened). Pursuant to the ESA and our implementing regulations, we determine whether a species is threatened or endangered based on any one or a combination of the following section 4(a)(1) factors: the present or threatened destruction, modification, or curtailment of habitat or range; overutilization for commercial, recreational, scientific, or educational purposes; disease or predation; inadequacy of existing regulatory mechanisms; and any other natural or manmade factors affecting the species' existence (16 U.S.C. 1533(a)(1), 50 CFR 424.11(c)).

We make listing determinations based on the best available scientific and commercial data available after conducting a review of the status of the species and after taking into account efforts being made by any State or foreign nation or political subdivision thereof to protect the species.

**Background**

Three distinct forms or ecotypes of killer whales, termed residents, transients, and offshores, are recognized in the northeastern Pacific Ocean. Resident killer whales in U.S. waters are distributed from Alaska to California, with four distinct populations: Southern, Northern, Southern Alaska, and Western Alaska (Krahn *et al.,* 2002; 2004). Resident killer whales are fish eaters and live in stable matrilineal pods. The West Coast transient killer whales have a different social structure, are found in smaller groups, and eat marine mammals. Offshore killer whales are found in large groups, and their diet is presumed to consist primarily of fish, including sharks. While the ranges of the different ecotypes of whales overlap in the northeastern Pacific Ocean, available genetic data indicate that there is a high degree of reproductive isolation among residents, transients, and offshores (Krahn *et al.,* 2004; NMFS, 2013).

The Southern Resident killer whale population consists of three pods, identified as J, K, and L pods, that reside for part of the year in the inland waterways of Washington State and British Columbia (Strait of Georgia, Strait of Juan de Fuca, and Puget Sound), principally during the late spring, summer, and fall (NMFS, 2008). Pods visit coastal sites off Washington and Vancouver Island, and travel as far south as central California and as far north as Southeast Alaska (Ford *et al.,* 2000; NMFS, 2008; Department of Fisheries and Oceans, unpublished data).

In 2001 we received a petition to list the Southern Resident killer whale population as threatened or endangered under the ESA (CBD, 2001) and we formed a Biological Review Team (BRT) to assist with a status review (NMFS, 2002). After conducting the status review, we determined that listing the Southern Resident killer whale population as a threatened or endangered species was not warranted because the science at that time did not support identifying the Southern Resident killer whale population as a DPS as defined by the ESA (67 FR 44133; July 1, 2002). Because of the uncertainties regarding killer whale taxonomy (*i.e.,* whether killer whales globally should be considered as one species or as multiple species and/or subspecies), we announced that we would reconsider the taxonomy of killer whales within 4 years. Following the determination, the Center for Biological Diversity and other plaintiffs challenged our ''not warranted'' finding under the ESA in U.S. District Court. The U.S. District Court for the Western District of Washington issued an order on December 17, 2003, which set aside our ''not warranted'' finding and remanded the matter to us for redetermination of whether the Southern Resident killer whale population should be listed under the ESA (*Center for Biological Diversity* v. *Lohn,* 296 F. Supp. 2d. 1223 (W.D. Wash. 2003)). The court found that where there is ''compelling evidence that the global *Orcinus orca* taxon is inaccurate,'' the agency may not rely on ''a lack of consensus in the field of taxonomy regarding the precise, formal taxonomic redefinition of killer whales.'' As a result of the court's order, we co-sponsored a Cetacean Taxonomy workshop in 2004, which included a special session on killer whales, and reconvened a BRT to prepare an updated status review document for Southern Resident killer whales (NMFS, 2004).

The BRT agreed that the Southern Resident killer whale population likely belongs to an unnamed subspecies of resident killer whales in the North Pacific, which includes the Southern and Northern Residents, as well as the resident killer whales of Southeast Alaska, Prince William Sound, Kodiak Island, the Bering Sea and Russia (but not transients or offshores). The BRT concluded that the Southern Resident killer whale population is discrete from other populations within the North Pacific Resident taxon and significant with respect to the North Pacific Resident taxon and therefore should be considered a DPS. In addition, the BRT conducted a population viability analysis, which modeled the probability of species extinction under a range of assumptions. Based on the findings of the status review and an evaluation of the factors affecting the DPS, we published a proposed rule to list the Southern Resident killer whale DPS as threatened on December 22, 2004 (69 FR 76673). After considering public comments on the proposed rule and other available information, we reconsidered the status of the Southern Resident killer whale DPS and issued a final rule to list the Southern Resident killer whale DPS as endangered on November 18, 2005 (70 FR 69903). The regulatory language in the listing limited the DPS to whales from J, K and L pods, wherever they are found in the wild, and not including Southern Resident killer whales placed in captivity prior to listing or their captive born progeny.

Following the listing, we designated critical habitat, completed a recovery plan, and conducted a 5-year review for

the Southern Resident killer whale DPS. We issued a final rule designating critical habitat for the Southern Resident killer whale DPS on November 29, 2006 (71 FR 69055). After engaging stakeholders and providing multiple drafts for public comment, we announced the Final Recovery Plan for the Southern Resident killer whale DPS on January 24, 2008 (73 FR 4176). We have continued working with partners to implement actions in the recovery plan. In March 2011, we completed a 5-year review of the ESA status of the Southern Resident killer whale DPS, concluding that no change was needed in its listing status and that the Southern Resident killer whale DPS would remain listed as endangered (NMFS, 2011). The 5-year review also noted that there was no relevant new information for this species regarding the application of the DPS policy.

On August 2, 2012, we received a petition submitted by the Pacific Legal Foundation on behalf of the Center for Environmental Science Accuracy and Reliability, Empresas Del Bosque, and Coburn Ranch to delist the endangered Southern Resident killer whale DPS under the ESA. We made a 90-day finding accepting the petition and soliciting information to inform a status review (77 FR 70733; November 27, 2012). Based on a review of the scientific information (NWFSC, 2013) and our full status review, we issued a 12-month finding on August 5, 2013, that the petitioned action was not warranted and the Southern Resident killer whale DPS remains listed as endangered (78 FR 47277).

**Lolita Petition**

On January 25, 2013, we received a petition submitted by the People for the Ethical Treatment of Animals Foundation on behalf of the Animal Legal Defense Fund, Orca Network, Howard Garrett, Shelby Proie, Karen Munro, and Patricia Sykes to remove the exclusion of captive killer whales from the ESA listing of the Southern Resident Killer Whale DPS and to include the killer whale known as Lolita in the ESA listing of the Southern Resident killer whales. The petition described Lolita, a female killer whale captured from the Southern Resident population in 1970, who currently resides at the Miami Seaquarium in Miami, Florida, as the only remaining member of the Southern Residents alive in captivity. The petitioners presented information about Lolita's origin and contended that Lolita is a member of the endangered Southern Resident DPS and should be included within the ESA listing. In addition, they provided a legal argument that ''the ESA applies to captive members of listed species'' and asserted that ''NMFS has a non-discretionary duty to include Lolita in the listing of the Southern Resident killer whales under the ESA.'' The petition also included information about how each of the five section 4(a)(1) factors applies with respect to Lolita. Lastly, the petitioners contended that including Lolita in the ESA listing will contribute to conservation of the wild Southern Resident killer whale population.

On April 29, 2013, we found that the information contained in the petition, viewed in the context of information readily available in our files, presented substantial scientific information that would lead a reasonable person to believe the petitioned action may be warranted (78 FR 25044). We noted that the information on Lolita's genetic heritage and consideration of captive individuals under the ESA provided a basis for us to accept the petition. The petition included an assessment of how listing Lolita would help conserve the wild Southern Resident population and also a review of the 4(a)(1) factors described earlier and considered in listing determinations. Our 90-day finding accepting the petition, however, was based on the biological information regarding Lolita's genetic heritage and consideration of the applicability of the ESA to captive members of endangered species. Our review of Lolita's status with respect to the Southern Resident killer whale DPS similarly focused on these two aspects and did not include a review of the Section 4(a)(1) factors for Lolita or the wild population. Our status review considered the best available information including information received through the public comment period, a review of scientific information conducted by our Northwest Fisheries Science Center, including published peer-reviewed journal articles and unpublished scientific reports, and information in the petition.

Upon publishing our 90-day finding accepting the petition, we initiated a status review update and solicited information from the public to help us gather any additional information to inform our review of Lolita's relationship to the Southern Resident killer whale DPS. Based on the information informing the 90-day finding, the status review update, and the public comments on the 90-day finding, we published a proposed rule on January 27, 2014 (79 FR 4313), proposing to amend the regulatory language of the ESA listing of the DPS by removing the exclusion for captive members of the population and requesting comments.

During the public comment period for the proposed rule, which closed on March 28, 2014, we received over 17,000 comments from citizens, researchers, non-profit organizations, and the public display industry; comments came from the United States and around the world. While we solicited information concerning the proposal to amend the regulatory language describing the listing of the Southern Resident killer whale DPS by removing the exclusion of captive whales and Lolita's genetic heritage and status, the vast majority of individual commenters simply stated their support for the proposal to include Lolita as a member of the Southern Resident killer whale DPS. Along with support for the proposed rule or as a stand-alone comment, many commenters suggested that Lolita be freed from her captivity and returned to her native waters of the Pacific Northwest. Commenters also expressed concern over Lolita's current care at the Miami Seaquarium under the purview of the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) under the Animal Welfare Act (AWA). The AWA captive care requirements are not under NMFS jurisdiction and are beyond the scope of our response to the petition; thus, comments pertaining to AWA compliance are not addressed in this final rule.

In addition to a very large number of brief comments in support of the proposed rule, we received over 60 detailed comments raising substantive issues. The majority of these comments provided substantive support for recognition of Lolita as a member of the listed DPS. Several substantive comments, primarily submitted by groups or individuals associated with the public display industry, opposed the proposed rule, with several also opposing any relocation of Lolita.

In addition to public review, we solicited peer review of information about Lolita's heritage supporting our conclusion in the proposed rule that Lolita originated from the Southern Resident killer whale population. On July 1, 1994, the NMFS and USFWS published a series of policies regarding listings under the ESA, including a policy for peer review of the scientific data (59 FR 34270). The intent of the peer review policy is to ensure that listings are based on the best scientific and commercial data available. Pursuant to our 1994 policy on peer review, the Data Quality Act, and the Office of Management and Budget (OMB) Peer Review Bulletin (OMB 2004), we

solicited technical review from four qualified specialists of specific information regarding Lolita's heritage and our conclusion that she originated from the Southern Resident killer whale population as described in our status review update (NMFS, 2013). A status review of biological information and our DPS determination was conducted by the NMFS Northwest Fisheries Science Center in response to the petition to delist the Southern Resident killer whale DPS and included a review of information specific to Lolita's genetic heritage (NMFS, 2013). The peer review request focused on the specific paragraph regarding Lolita in the status review update (NMFS, 2013) that informed the proposed rule, and we received reviews from two independent experts. We received one comment on the peer review plan and peer review charge statement and provided that comment letter to the peer reviewers. We made the peer review charge, comments received on the peer review charge, and ultimate peer review report available online at: *http://www.cio.noaa.gov/services_programs/prplans/ID261.html.* The peer reviewer comments and conclusions and our responses to public comments are included in the summary below.

**Summary of Peer Review and Public Comments Received**

Below we summarize and address the substantive public comments that were received during the public comment period for the proposed rule. In addition, information from the peer reviews is presented in both comment summaries and responses. Substantive comments and our responses are organized by relevant topics.

*Biological Information on Lolita's Origin*

*Comment 1:* Several commenters and the two peer reviewers noted that the best available scientific information indicates that Lolita is most likely a member of the Southern Resident population. Many commenters cited the acoustic and genetic evidence provided in the proposed rule as proof that Lolita is a member of the Southern Resident community. Commenters cited the references in the status review update, including Hoelzel *et al.* (2007), Hoelzel (personal communication), Ford (1987), Candice Emmons (personal communication), and Pilot *et al.* (2010) (also referred to as Pilot (2009) in some comments). Commenters cited Pilot *et al.* (2010) as evidence that Lolita is related to Southern Residents using one genetic method, while others referenced the same paper noting that three other genetic methods did not indicate a relationship with Southern Residents. One commenter addressed the sample assigned to Lolita in Pilot *et al.* (2010), referenced personal communications with the lead author of the paper, and noted that results from the tests are insufficient to conclude that Lolita was a Southern Resident killer whale. In addition to the papers listed above, the peer reviewers also provided additional references to support their conclusions that Lolita is most likely a member of the Southern Resident population. One peer reviewer noted that our summary in the status review update (NMFS, 2013) was overly simplistic. The comments on the peer review plan focused on individual data points and the uncertainties for individual genetic tests and requested additional information be provided to the peer reviewers.

*Response:* We considered the best available information regarding Lolita's origin, including genetic test results from multiple papers, the peer reviews, and other lines of evidence in making our conclusions. In addition to the original peer review request, we also provided comments on the peer review plan and additional information for the reviewers to consider. The peer reviewers stated that mitochondrial DNA (mtDNA) tests are very likely diagnostic of natal populations. The mtDNA control region sequence is fixed for a single haplotype within most killer whale populations in the North Pacific. Lolita has the haplotype for Southern Residents, and the haplotype is distinct from the haplotypes found in transient, offshore and Northern Resident communities (including SE Alaska and Bering Sea). Based on sample sizes in studies to date, it is extremely unlikely that transient or Northern Residents have a Southern Resident haplotype that has gone undetected due to chance. Due to smaller sample sizes for offshores, it is harder to rule out that offshores might contain the Southern Resident haplotype in a small fraction of the population (*i.e.,* 10 percent), but it has yet to be detected. The Southern Resident haplotype is shared with whales sampled off the Kamchatka Peninsula in Russia and from Prince William Sound in Alaska (Barrett-Lennard, 2000; Parsons *et al.,* 2013); however, additional data can be used to rule out the possibility that Lolita originated from these other populations. Using microsatellite analysis, researchers assigned Lolita to populations using different programs with varying probabilities and assessed kinship (Hoelzel *et al.,* 2007; Pilot *et al.,* 2010). In Pilot *et al.* (2010), Lolita was assigned to the Southern Resident population with the highest probability (0.464) and with low probability to Kamchatka (0.016) or SE Alaska residents (0.004). Tests for kinship using microsatellite data found a presumed match between Lolita and a member of the Southern Resident L pod based on one of four tests, but it was not a close relationship (*e.g.,* parent, offspring, or full sibling). Lolita did not show potential kinship with individuals of any other population. Using a different analysis, Pilot *et al.* (2010) also assigned Lolita to a Southern Resident cluster and not to the Kamchatka cluster. The microsatellite data do not appear to provide conclusive evidence on their own to identify Lolita's population of origin, but the data support the finding that she is a Southern Resident.

The peer reviews concluded that the summary of our findings regarding Lolita in our status review update (NMFS, 2013) likely correctly concluded that Lolita is a Southern Resident and that, taken together, the mtDNA and microsatellite DNA provide a strong case for the assignment of Lolita to the Southern Resident population. While some comments focused on individual test results to form conclusions, we relied on all of the best available information in the petition, public comments on the 90-day finding and the proposed rule, peer review, peer reviewed journal articles, unpublished science reports, and the recovery plan (NMFS, 2008), taken together, to inform our internal review and conclusions. Based on the best available information regarding the location of capture and genetic information, we are confident that Lolita originated from the Southern Resident population.

*Comment 2:* One commenter provided information from her study of the specific acoustic call type produced by Lolita, matching Lolita's calls to Southern Resident specific call types. The commenter suggested that further identification of Lolita's calls could be matched with specific matrilines. Other commenters noted that there is no statistically significant or peer reviewed data or analysis that the calls recorded opportunistically from Lolita match L pod calls. In addition, commenters noted that the Ford (1987) paper cited in the status review did not include specific information about Lolita and her calls. One peer reviewer noted that additional information about the timing of the recording of Lolita's calls and the origin of the whale sharing Lolita's tank would shed light on whether Lolita was an L pod whale or if she could have learned L pod calls from another whale.

*Response:* In the status review update (NMFS, 2013), the Ford (1987) paper was cited to demonstrate that calls can be identified to population and also to pod, and we acknowledge that it does not include specific information about Lolita's calls. While the acoustic information about Lolita's calls is not published in a peer reviewed article, the personal communication by Candice Emmons does lend an additional line of evidence that is consistent with Lolita originating from the Southern Resident killer whale population. The study provided by a commenter is also not a peer reviewed published article. In addition, the peer review comments also raised uncertainty about identifying Lolita by her acoustic calls based on the personal communication. While we considered the anecdotal and unpublished information on Lolita's acoustic calls, noting the uncertainty surrounding them, we relied on the genetic data and capture location as the primary support for Lolita's status as a member of the Southern Resident killer whale population.

*Comment 3:* In addition to genetic and acoustic information, Lolita's capture history was also mentioned by commenters and peer reviewers as evidence that she came from the Southern Resident population. One commenter noted photographs from the capture operation were identified as Southern Residents and that members of different communities have never been observed associating, concluding that all of the whales captured at Penn Cove were members of the Southern Resident community. One commenter, however, noted that the capture history raised questions about Lolita's origin, mentioning that the total number of whales in the area was too high to account for only the Southern Residents and that L pod whales were photographed near the operation but not in the net. The peer reviewers referenced the sighting history of killer whales in the capture area as support for Lolita's identification as a Southern Resident.

*Response:* We did not receive any photo-identification quality photographs of the capture and have no specific documentation of the captures beyond the information summarized in the Recovery Plan for Southern Resident Killer Whales (NMFS, 2008) that attributes captures from Penn Cove, Washington, to the Southern Resident population. One peer reviewer noted the location of capture does not rule out that she is a transient (but mtDNA makes this highly unlikely), and that the capture location makes it highly unlikely that she is a Northern Resident, offshore, Western Pacific, Alaska Resident or from a distant, poorly known population. A review of the information raised in public comments, the peer reviews, comments on the peer review plan, and other available information finds this information continues to find the capture information regarding Lolita consistent with her membership as a Southern Resident. That review (Ford, 2014) notes that based on what is known about the ranges of North Pacific killer whales, the Penn Cove, WA capture location limits the possible populations of origin to Southern Residents or transients which are commonly seen, or far less likely to Northern Residents (only seen a handful of times in U.S. waters of the Salish Sea) or offshores (only sighted six times in 30 years of observations and never south of Admiralty Inlet) (Krahn et al., 2004; Ford, 2006; Dahlheim *et al.,* 2008). Regular observations in the Salish Sea have occurred since the mid-1970s, several years after the capture in question, and it seems highly unlikely that the distributions and habits of these populations would change dramatically over that short period of time (Ford, 2014).

*Comment 4:* Several commenters noted that, morphologically, Lolita's saddle patch patterns do not readily match the majority of saddle patch patterns of the Southern Resident DPS, but they are more similar to saddle patches of the Alaska and Bering Strait residents. One peer reviewer suggested saddle patch and dorsal fin shape could be used to further address Lolita's origin.

*Response:* Bain (1988) found differences between Northern and Southern Resident saddle shapes and Baird and Stacey (1988) reported different distributions of saddle shapes among residents and transients. Baird and Stacey (1988) identified five different patterns, with all five patterns present in resident killer whales. Lolita's saddle shape appears to be consistent with the ''horizontal notch'' type. While this saddle patch type is seen in Alaska Residents, it is more common in Southern Residents (Baird and Stacey, 1988). The information above regarding sighting records and the capture location includes an assessment by a peer reviewer, noting that it is highly unlikely that Lolita is an Alaska Resident.

*Comment 5:* Several commenters reviewed the ESA section 4(a)(1) factors and identified how they applied to Lolita. Other commenters noted that none of the threats identified in the listing of the Southern Resident killer whale DPS (*i.e.,* food scarcity, vessels, contaminants) apply to Lolita.

*Response:* In March 2011, we completed a 5-year review of the ESA status of the Southern Resident killer whale DPS, concluding that no change was needed in its listing status and that the Southern Resident killer whale DPS would remain listed as endangered (NMFS, 2011). The endangered status of the DPS is not the subject of the petitioned action. The petition requests we include Lolita in the ESA listing of Southern Residents and notes that an analysis of the five ESA section 4(a)(1) factors is not required to justify Lolita's inclusion in the DPS and that Lolita's genetic heritage is sufficient to support her inclusion in the listing. We agree that biological information regarding Lolita's origin and consideration of the applicability of the ESA to captive members of endangered species provide a sufficient basis for our determination and, therefore, do not include a review of the section 4(a)(1) factors for Lolita or the wild population in this notice.

*Captivity and Release*

*Comment 6:* One commenter questioned why the ESA applied to Lolita at all, considering she was held in captivity prior to December 28, 1973, and the date of the listing of the Southern Resident killer whales.

*Response:* The commenter presumably refers to section 9(b) of the ESA, 16 U.S.C. 1538(b)(1), which provides certain exemptions for animals already held in captivity or a controlled environment on either December 28, 1973, or the date of publication in the **Federal Register** of the final regulation adding such species to the list of endangered species, provided that such holding and any subsequent holding of the animal is not in the course of a commercial activity.

In fact, this section is not a blanket exemption from the ESA for any animal so held; rather, it only lifts the ban on two very specific activities enumerated in subsections (a)(1)(A) and (G) of section 9: import or export of such species, and violation of any regulation pertaining to such species or to any threatened species. In other words, all of the other prohibitions of section 9 apply to animals that were held in captivity pre-ESA or pre-listing, including the prohibitions on take as well as on interstate or foreign commerce. Any import or export of Lolita that might be proposed in the future is beyond the scope of this rulemaking. For additional discussion of ESA section 9(b), see *American Society for the Prevention of Cruelty to Animals* v. *Ringling Brothers*

*and Barnum and Bailey Circus,* 502 F.Supp. 2d 103 (2007).

*Comment 7:* Several commenters noted that the ESA does not allow for the exclusion of captive members from a listed species based on their captive status and referenced court cases (*Safari Club International* v. *Jewell and Alsea Valley Alliance* v. *Evans,* cited below in response) and recent USFWS notices regarding antelopes and chimpanzees that were referenced in the proposed rule. In addition, commenters noted that if Lolita is included in the listing, the ESA prohibitions on export, take, and interstate commerce will apply to her.

*Response:* As the commenters note, several courts have held, and NMFS agrees, that the ESA does not allow for captive held animals to be assigned separate legal status from their wild counterparts on the basis of their captive status or through designation as a separate DPS (*Safari Club International* v. *Jewell,* 960 F.Supp. 2d 17 (D.D.C. 2013); *Alsea Valley Alliance* v. *Evans,* 161 F. Supp. 2d 1154 (D.Or. 2001). As noted in this final rule, as well as in recent regulations addressing captive antelopes (78 FR 33790; June 5, 2013) and a proposed rule for chimpanzees (78 FR 35201; June 12, 2013), captive members of a species have the same legal status as the species as a whole. Finally, as the commenters note, captive members of a listed species are also subject to the relevant provisions of section 9 of the ESA as warranted.

*Comment 8:* One commenter expressed concern that including Lolita in the ESA listing would result in a violation of the Fifth Amendment, denying the property owners their rights without satisfying the Constitution's public use and just compensation requirements. One commenter supported their opposition to including Lolita in the ESA listing by citing examples of how extending regulations to privately owned members of a listed species could undermine private efforts to avoid extinction and recover species through private governance. Commenters also noted that financial considerations should not be considered in listing decisions.

*Response:* First, section 4(b)(1)(A) of the ESA and its legislative history provides that listing decisions be based ''solely'' on the best scientific and commercial data available without reference to economic costs or private party impacts (*H.R. Rep. No. 97–567, at 12, 1982 U.S.C.C.A.N. 2807, 2812).* Second, to the extent there are concerns about specific activities (including acts supporting conservation) associated with listed species, these issues are better evaluated in the context of a specific permit request and through the section 10 permit process, which provides an avenue for defining, evaluating, and authorizing specific activities (50 CFR 222.301 *et seq.).* Accordingly, speculating about whether there are activities that property owners may wish to take is beyond the scope of this rule.

*Comment 9:* One commenter took issue with our assertion that if Lolita was included in the ESA listing, we would not seek to amend critical habitat to include consideration of her or her captive environment. The commenter cited the requirement to designate critical habitat with the listing of a species in section 4(a)(3)(A) of the ESA.

*Response:* NMFS designated critical habitat for the Southern Resident killer whale DPS on November 29, 2006 (71 FR 69054). NMFS interprets critical habitat to comprise the habitat used by the species in the wild, not the artificial surroundings of a particular species member in captivity, because those areas do not include relevant primary constituent elements of critical habitat (70 FR 52630; September 2, 2005). Accordingly, we do not intend to amend the existing critical habitat designation for Southern Resident killer whales with respect to Lolita.

*Comment 10:* We received many comments addressing the type and scope of activities that might trigger section 9 concerns and/or warrant consideration for a section 10 permit. These comments took varying positions on the scope of activities that might fall within the category of allowable captive care activities.

*Response:* In the proposed rule, we said that, depending on the circumstances, we would likely not find continued possession, care, and maintenance of a captive animal to be a violation of ESA section 9 (and therefore, such activities would not require a section 10 permit). Our discussion in the proposed rule was intended to be a general indication of our views, not factual findings on Lolita's actual circumstances or any proposals for future activities. Such findings are beyond the scope of this listing rule.

We appreciate the concerns raised by the many comments regarding how the ESA section 9 prohibitions might apply to Lolita's particular circumstances. We believe these comments demonstrate the need for a more focused evaluation of these factors, which is more appropriately performed as part of a permit application process as opposed to this listing rule. Should the Miami Seaquarium apply for an ESA section 10 permit, the process would involve a **Federal Register** notice of receipt followed by a public comment period.

*Comment 11:* Commenters raised questions about the Miami Seaquarium conducting commercial activity with Lolita, stating their belief that section 9(b) of the ESA allows for captives to remain in captivity so long as they are not held or used for purposes of commercial activity. Other commenters stated that there is nothing illegal about exhibiting endangered animals for a fee.

*Response:* Some commenters may have misinterpreted section 9(b) in this regard. As noted above, section 9(b) is a very limited exclusion from the prohibition on import and export, as well as certain regulatory requirements not applicable here. Any future proposal to import or export Lolita is beyond the scope of this rule, and so we need not further address the 9(b) exemption, including its clause regarding commercial activity, at this time.

*Comment 12:* One commenter urged us to acknowledge that interstate movement of Lolita or any other captive listed species merely for display or as part of an animal exhibition would not require a permit under the ESA, citing U.S.C. 1538(a)(E) and 50 CFR 17.3.

*Response:* At this time, the Miami Seaquarium has not presented any proposal to move Lolita, regardless of purpose, so we will not address this further in this listing rule, other than to note that the cited CFR provision is a regulation promulgated by the USFWS, and is therefore applicable to species under their jurisdiction.

*Comment 13:* Commenters expressed concern over captivity of killer whales in general and about Lolita's current care at the Miami Seaquarium under the purview of APHIS under AWA. Other commenters noted the high level of care provided to Lolita at the Miami Seaquarium.

*Response:* As noted above, Lolita's current captive care requirements are regulated by APHIS under the AWA and are currently the subject of ongoing litigation (*Animal Legal Defense Fund et al.* v. *Elizabeth Goldentyer, USDA and Marine Exhibition Corporation* No. 14–12260 (11th Circuit Court of Appeals 2014)). Specific AWA captive care requirements are not under NMFS jurisdiction and are beyond the scope of our response to the petition. Therefore, comments regarding AWA compliance are not addressed in this final rule.

*Comment 14:* Many comments supported Lolita's transfer to a sea pen or release from captivity into her home waters. Some commenters, while in favor of Lolita's ultimate release, argued that any decision on this issue in the

absence of a specific proposal is premature. Comments on whether there would be any conservation benefit to the conservation of wild killer whales from Lolita's release were mixed. Some comments identified benefits to Lolita and to the wild Southern Resident killer whale population, such as her ability to aid in the care of young whales (*i.e.,* alloparenting). Others were against any relocation efforts, claiming that there would be no conservation benefits to wild whales and noting Lolita currently has a high level of care, contributes to educating the public, and there are risks to Lolita and the wild population associated with transport and release. One commenter noted that regulations regarding marine mammal rehabilitation under the MMPA declare that a marine mammal that has been in human care for 2 or more years is presumptively non-releaseable.

*Response:* As noted above, the Miami Seaquarium has not presented any proposal to move (or release) Lolita. As for any future proposal to release her, we indicated in the proposed rule that there were certain activities that we believe could result in violations of section 9 of the ESA, specifically including ''releasing a captive animal into the wild.'' 79 FR at 4318 (January 27, 2014). We based this on our proposed rule listing five species of sturgeon (since finalized at 79 FR 31222, June 2, 2014). After taking into account the numerous comments on this topic, and examining our existing regulations, policies and practices, we have decided to elaborate on our views in this final rule. Releasing captive marine mammals to the wild is not without risk. Issues of concern include: disease transmission and/or unwanted genetic exchange between released animals and wild stocks; the ability of released animals to adequately forage and defend themselves from predators; and any behavioral patterns developed in captivity that could affect the social behavior of wild animals, as well as the social integration of the released animals.

In fact, as one commenter noted, NMFS' MMPA regulations address a presumption of non-releasability, as well as dictate legal requirements under the MMPA for any proposal to release a captive animal. First, 50 CFR 216.27(a)(1)(iii), addressing stranded marine mammals, states that the animal's potential for survival in the wild must be evaluated at 6-month intervals, ''until 24 months from capture or import, at which time there will be a rebuttable presumption that release to the wild is not feasible.'' Second, 50 CFR 216.35(e) states: ''Captive marine mammals shall not be released into the wild unless specifically authorized by the Office Director under a scientific research or enhancement permit.''

The issues surrounding any release of Lolita to the wild are numerous and complex and are not ripe for analysis in this listing rule. Such issues would be more appropriately evaluated in the context of a specific section 10 permit application. Any such process would include rigorous review by the scientific community, the Marine Mammal Commission, and the public, and be subject to an associated NEPA analysis, prior to action being taken.

**Changes From the Proposed Rule**

There are no changes from the proposed amendment to the ESA listing of the Southern Resident killer whale DPS in this final rule. This final rule implements the amendment to the listing language, removing the exclusion for captive whales from the regulatory description of the Southern Resident killer whale DPS. The public comments provided opposing positions on this approach, as well as Lolita's status as a member of the Southern Resident killer whale population. The peer reviews supported Lolita's status as a member of the Southern Resident killer whale population. See the Summary of Peer Review and Public Comments Received section above and the Final Determination and Amendment to Listing section below for information on the additional data that support the conclusion that captive members should be included in the listing and the determination that best available science supports Lolita's status as a member of the Southern Resident killer whale population and therefore the ESA-listed DPS.

**Determination of Taxon and DPS**

Based on the best information available, we previously concluded, with advice from the 2004 BRT (Krahn *et al.,* 2004), that the Southern Resident killer whale population (J, K, and L pods) met the two criteria of the DPS policy (discreteness and significance) and constituted a DPS of the North Pacific Resident subspecies. A detailed analysis of (1) the reference taxon for consideration under the DPS policy, (2) the discreteness of the Southern Resident population from other populations within that taxon, and (3) the significance of the Southern Resident population to that taxon was included in our 12-month determination that the petition to delist was not warranted (78 FR 47277; August 5, 2013) and is summarized below. Based on our recent status review and in response to a petition to delist the Southern Resident killer whale DPS, we concluded that the best available scientific information indicates that, similar to our 2005 rulemaking when we listed the Southern Resident DPS, the North Pacific Resident subspecies is the appropriate reference taxon for considering whether the Southern Resident killer whale population is discrete and significant. In our 2005 rulemaking we concluded there was strong evidence that the Southern Resident killer whale population is discrete from other North Pacific Resident killer whale populations as defined by the 1996 DPS policy. The new information subsequent to 2004, such as recent genetic studies, is consistent with and generally strengthens the conclusion that the Southern Resident killer whale population is a discrete population within the North Pacific Resident taxon. As in 2004, all the available information clearly indicates that the Southern Resident population is discrete from other populations in the North Pacific resident subspecies. In addition, we concluded that the new information on genetics and behavioral and cultural diversity available since 2004 was consistent with or strengthens the 2004 BRT's conclusion that the Southern Resident killer whale population meets the significance criterion of the DPS policy. In summary, in our 12-month finding that delisting was not warranted, we concluded that members of the Southern Resident killer whale population are discrete from other populations within the North Pacific Resident killer whale taxon and significant with respect to the North Pacific Resident killer whale taxon and therefore comprise a valid DPS which remains listed as endangered (78 FR 47277; August 5, 2013).

**Final Determination and Amendment to Listing**

The petition maintains that Lolita is a member of the Southern Resident killer whale population and states that she must, therefore, be included in the listed DPS. As summarized above, our consideration of the petitioned action focuses on biological information regarding Lolita's genetic heritage and the application of the ESA to captive members of a listed species or DPS. The petitioners contend that Lolita was taken from L pod during captures on August 8, 1970, in Penn Cove, approximately 50 miles (80 km) north of Seattle, Washington. The peer reviewers referenced the capture location and sighting history of different populations, in addition to other information (*i.e.,*

genetics), to support their conclusions that Lolita most likely came from the Southern Resident population. The petition notes that Lolita's mother is believed to be L25, an adult female Southern Resident killer whale who remains in the wild, and that Lolita makes the unique calls of the L25 subpod. In our recent status review update (NMFS, 2013), we cited genetic analysis completed since the original 2005 listing, that indicates Lolita has a genotype consistent with a Southern Resident origin (Hoelzel *et al.,* 2007; Hoelzel, personal communication), and we noted that Lolita's acoustic calls are typical of L pod (Ford, 1987; Candice Emmons, personal communication). The status review update (NWFSC, 2013) also cites information in Pilot *et al.* (2010). As described above, in support of the DPS determination for Southern Resident killer whales, recent genetic studies all indicate that the Southern Resident population is significantly differentiated and there is a high degree of reproductive isolation from other resident populations that comprise the North Pacific Resident subspecies.

As described above in the response to comments, the peer reviewers identified that mtDNA tests are very likely diagnostic of natal populations. The mtDNA control region sequence is fixed for a single haplotype within most killer whale populations in the North Pacific. Lolita has the haplotype for Southern Residents, which is distinct from the haplotypes found in transient, offshore, and Northern Resident communities (including SE Alaska and Bering Sea). Based on sample sizes in studies to date, it is extremely unlikely that transient or Northern Residents have a Southern Resident haplotype that has gone undetected due to chance. Due to smaller sample sizes for offshores, it is harder to rule out that offshores might contain the Southern Resident haplotype in a small fraction of the population (*i.e.,* 10 percent), but it has yet to be detected. The Southern Resident haplotype is shared with whales sampled off the Kamchatka Peninsula in Russia and from Prince William Sound in Alaska (Barrett-Lennard, 2000; Parsons *et al.,* 2013), but additional data can be used to rule out the possibility that Lolita originated from these other populations. Using microsatellite analysis, researchers assigned Lolita to population using different programs with varying probabilities and assessed kinship (Hoelzel *et al.,* 2007; Pilot *et al.,* 2010). In Pilot *et al.* (2010), Lolita was assigned to the Southern Resident population with the highest probability (0.464) and with low probability to Kamchatka (0.016) or SE Alaska residents (0.004). Tests for kinship found a putative match between Lolita and a member of the Southern Resident L pod based on one of four tests, but it was not a close relationship (*e.g.,* parent, offspring, or full sibling). Lolita did not show potential kinship with individuals of any other population. Using a different analysis, Pilot *et al.* (2010) also assigned Lolita to a Southern Resident cluster and not to the Kamchatka cluster. The microsatellite data do not appear to provide conclusive evidence on their own to identify Lolita's population of origin, but they are consistent with her being a Southern Resident.

The peer review conclusions were that our status review update (NMFS, 2013) was overly simplistic, but likely correctly concluded that Lolita is a Southern Resident and that, taken together, the mtDNA and microsatellite DNA data provide a strong case for the assignment of Lolita to the Southern Resident population. As described above, we relied on information in the petition, public comments on the 90-day finding and the proposed rule, peer review and best available information, including peer reviewed journal articles and unpublished science reports and the recovery plan (NMFS, 2008) to inform our internal review and conclusions. Similar to the peer reviews and as raised in public comments, we acknowledge the uncertainty inherent in individual test results and observations; however, based on all of the best available scientific information, taken together, including results from multiple genetic studies, as well as other lines of evidence regarding capture and sighting history, we can be confident that Lolita originated from the Southern Resident population (Ford, 2014). Differences in acoustic behavior between populations of resident killer whales also support the conclusion that Southern Resident killer whales are discrete and significant and, therefore, qualify as a DPS. Ford (1987) describes killer whale acoustic calls and how they can be identified to population and even to pod. While there is anecdotal information that Lolita shares acoustic characteristics with the members of the Southern Resident killer whale DPS found in the wild, this evidence is not as strong as the genetic data. In addition, morphological data, such as saddle patch pattern, are also consistent with, but not conclusive of, Lolita being a Southern Resident. This best available science supports Lolita's status as a member of the Southern Resident killer whale population.

Some commenters contend that Lolita not be included in the Southern Resident killer whale DPS, similar to other wild whales that are members of the North Pacific Resident subspecies (*i.e.,* Northern Resident and Alaska Resident killer whale populations). These commenters fail to recognize the previously discussed best available science defining the genetic characteristics that Lolita shares with the Southern Resident killer whale DPS and often highlighted individual test results rather than all of the available scientific information taken together. We find the multiple genetic characteristics constitute compelling lines of evidence that render Lolita and other members of the Southern Resident killer whale DPS discrete from and significant to the North Pacific Resident subspecies (NMFS, 2013; Ford, 2014). Additionally, while the ESA authorizes the listing, delisting, or reclassification of a species, subspecies, or DPS of a vertebrate species, it does not authorize the exclusion of the members of a subset or portion of a listed species, subspecies, or DPS from a listing decision. In 2001, the U.S. District Court in Eugene, Oregon (*Alsea Valley Alliance* v. *Evans,* 161 F. Supp.2d 1154 (D. Or. 2001)) (Alsea), ruled that once we had identified and listed a DPS (for Oregon Coast coho), the ESA did not allow listing only a subset (that which excluded 10 captive hatchery stocks) of that DPS. Accordingly, this case does not authorize the exclusion of Lolita from the Southern Resident Killer Whale DPS listing based on the best available science supporting her membership in the DPS.

Other comments note that there are other characteristics, such as behavior and habitat use, that Lolita does not share with the other wild members of the Southern Resident killer whales and suggest that NMFS could exercise its discretion to identify a separate captive only DPS. However, legislative history surrounding the 1978 amendments to the ESA that gave the Services the authority to identify DPSs indicates that Congress intended identification of DPSs to be used for the identification of wild populations, not separation of captive held specimens from wild members of the same taxonomic species (see Endangered Species Act Oversight: Hearing Before Senate Subcommittee on Resource Protection, Senate Committee on Environment and Public Works, 95th Cong. 50 (July 7, 1977)). Additionally, these arguments fail to adhere to Congress' directive to the Services that the authority to designate DPSs be exercised ''sparingly'' (Senate Report

151, 96th Congress, 1st Session). Finally, NMFS' decision making relevant to identifying a captive only DPS, in this context, is discretionary and not subject to judicial review (*Safari Club International* v. *Jewell,* 960 F. Supp. 2d 17 (DDC 2013)).

As described in the proposed rule (79 FR 4313; January 27, 2014), the ESA does not support the exclusion of captive members from a listing based solely on their captive status. On its face the ESA does not treat captives differently. Rather, specific language in section 9 and section 10 of the ESA presumes their inclusion in the listed entity, and captives are subject to certain exemptions to section 9. Section 9(a)(1)(A)–(G) of the ESA applies to endangered species regardless of their captive status. However, section 9(b) provides certain exemptions from the 9(a)(1)(A) and (a)(1)(G) prohibitions for listed animals held in captivity or in a controlled environment as of the date of the species' listing (or enactment of the ESA), provided the holding in captivity and any subsequent use is not in the course of commercial activity. Additionally, section 9(b)(2) refers to captive raptors and identifies that the prohibitions in 9(a)(1) shall not apply to raptors legally held in captivity. Section10(a)(1)(A) of the ESA allows issuance of permits to ''enhance the propagation or survival'' of the species. This demonstrates that Congress recognized the value of captive holding and propagation of listed species held in captivity but intended that such specimens would be protected under the ESA, with these activities generally regulated by permit.

We have specifically identified captive members as part of the listed unit during listing actions, such as for endangered smalltooth sawfish (68 FR 15674; April 1, 2003), and endangered Atlantic sturgeon (77 FR 5914; February 6, 2012), and in the final listing of five species of foreign sturgeon (79 FR 31222; June 2, 2014). Further, based upon the purposes of the ESA and its legislative history, courts have held and the USFWS has recently concluded that the ESA does not allow captive animals to be assigned different legal status from their wild counterparts on the basis of their captive status (*Safari Club International* v. *Jewell,* 960 F. Supp. 2d 17 (DDC 2013)). Subsequent to the submission of the petition regarding Lolita, USFWS published a proposed rule to amend the listing status of captive chimpanzees, so that all chimpanzees (wild and captive) would be listed as endangered (78 FR 35201; June 12, 2013). USFWS also published a 12-month finding that delisting the captive members of three listed antelope species was not warranted (78 FR 33790; June 5, 2013).

In a recent notice announcing a Final Policy of Interpretation of the Phrase ''Significant Portion of Its Range (SPR)'' in the Endangered Species Act's Definitions of ''Endangered Species'' and ''Threatened Species'' (79 FR 37578; July 1, 2014), the Services also confirmed the legal status of captive members of listed species. The notice explains, with regard to species found in captivity, the Services consider a captive population to have no ''range'' separate from that of the species to which it belongs (captive populations cannot be considered a SPR). The notice also states ''captive members have the same legal status as the species as a whole.''

Based on the preceding discussion, the information submitted during the public comment period, the peer reviews, and best available science and information, we find that captive members of the Southern Resident killer whale population should not be excluded from the listed Southern Resident killer whale DPS based on their captive status. Accordingly, this rule removes the exclusion for captive whales in the regulatory language describing the Southern Resident killer whale DPS. Our finding is consistent with the recent USFWS conclusions regarding the status of captive animals under the ESA and also with the Marine Mammal Commission recommendation to adopt a policy consistent with the USFWS in the proposed chimpanzee listing rule and treat all biological members of the Southern Resident killer whales as part of the DPS, regardless of whether those individuals are in the wild or in captivity (Marine Mammal Commission letter, August 13, 2013).

As part of the 2005 ESA listing of the Southern Resident killer whale DPS (70 FR 69903; November 18, 2005), we conducted an analysis of the five ESA section 4(a)(1) factors and concluded that the DPS was in danger of extinction and listed it as endangered. In March 2011, we completed a 5-year review of the ESA status of the Southern Resident killer whale DPS, concluding that no change was needed in its listing status and that the Southern Resident killer whale DPS would remain listed as endangered (NMFS, 2011). The petition and several public comments included an analysis of the five ESA section 4(a)(1) factors with respect to Lolita, although petitioners note that the analysis is not required to justify Lolita's inclusion in the DPS and that Lolita's genetic heritage is sufficient to support her inclusion in the listing. We agree that biological information regarding Lolita's origin and consideration of the applicability of the ESA to captive members of endangered species provide a sufficient basis for our determination and, therefore, do not include a review of the section 4(a)(1) factors for Lolita or the wild population.

While progress toward recovery has been achieved since the listing, as described in the 5-year review, the status of the DPS remains as endangered. Since the 5-year review was completed, additional actions have been taken to address threats, such as regulations to protect killer whales from vessel impacts (76 FR 20870; April 14, 2011), completion of a scientific review of the effects of salmon fisheries on Southern Resident killer whales (Hilborn, 2012), and ongoing technical working groups with the Environmental Protection Agency to assess contaminant exposure. However, the population growth outlined in the biological recovery criteria and some of the threats criteria have not been met. We have no new information that would change the recommendation in our 5-year review that the Southern Resident killer whale DPS remain classified as endangered (NMFS, 2011). This final rule amends the language describing the Southern Resident killer whale DPS by removing the exclusion of captive whales. With this change, Lolita, a female killer whale captured from the Southern Resident killer whale population in 1970, is not excluded from the Southern Resident killer whale DPS due to her captive status.

**Effects of Amendment to Listing**

Conservation measures provided for species listed as endangered or threatened under the ESA include concurrent designation of critical habitat if prudent and determinable (16 U.S.C. 1533(a)(3)(A)); recovery plans and actions (16 U.S.C. 1536(f)); Federal agency requirements to consult with NMFS and to ensure its actions do not jeopardize the species or result in adverse modification or destruction of critical habitat should it be designated (16 U.S.C. 1536); and prohibitions on taking (16 U.S.C. 1538). Following the listing, we designated critical habitat and completed a recovery plan for the Southern Resident killer whale DPS. We issued a final rule designating critical habitat for the Southern Resident killer whale DPS November 29, 2006 (71 FR 69055). The designation includes three specific areas: (1) The Summer Core Area in Haro Strait and waters around the San Juan Islands; (2) Puget Sound; and (3) the Strait of Juan de Fuca, which together comprise approximately 2,560

square miles (6,630 square km). The designation excludes areas with water less than 20 feet (6.1 m) deep relative to extreme high water. The designated critical habitat will not be affected by removing the exclusion of captive whales from the regulatory language describing the Southern Resident killer whale DPS. As the USFWS identified in its recent proposed chimpanzee rule, there is an ''anomaly of identifying the physical and biological features that would be essential to the conservation of a species consisting entirely of captive animals in an artificial environment'' (78 FR 35201; June 12, 2013). This observation also holds for a listed entity with only one captive member. In addition, the recent notice announcing a final policy interpreting Significant Portion of its Range under the ESA notes the Services consider a captive population to have no ''range'' separate from that of the species to which it belongs (79 FR 37578; July 1, 2014). We do not intend to modify the critical habitat designation to include consideration of Lolita and her captive environment.

After engaging stakeholders and providing multiple drafts for public comment, we announced the Final Recovery Plan for the Southern Resident killer whale DPS on January 24, 2008 (73 FR 4176). Lolita's capture and captivity is mentioned in the recovery plan; however, the recovery actions in the plan are focused on addressing the threats to and the recovery of the wild population. As the recovery plan is updated in the future, we will consider including an update that Lolita is included in the DPS.

Sections 7(a)(2) of the ESA requires Federal agencies to ensure that activities they authorize, fund, or carry out are not likely to jeopardize the continued existence of a listed species, or to adversely modify critical habitat. In the USFWS proposed rule for chimpanzees (78 FR 35201; June 12, 2013), USFWS identifies that ''the section 7 consultation process is not well suited to analysis of adverse impacts posed to a purely captive-held group of specimens given that such specimens are maintained under controlled, artificial conditions.'' This observation also holds for a listed entity with only one captive member. Previous guidance on examples of Federal actions that have the potential to impact Southern Resident killer whales was focused on activities that may affect wild whales. Additional considerations of actions that have the potential to affect Southern Resident killer whales, including Lolita, will be considered along with prohibitions on activities that affect the Southern Resident killer whale DPS. Some of these considerations are discussed below.

**Take Prohibitions and Identification of Those Activities That Might Constitute a Violation of Section 9 of the ESA**

On July 1, 1994, NMFS and USFWS published a policy (59 FR 34272) that requires us to identify, to the maximum extent practicable at the time a species is listed, those activities that would or would not constitute a violation of section 9 of the ESA. The ESA does not prohibit possession of animals lawfully taken into captivity, so a permit is required only if the person possessing the animal intends to engage in an otherwise prohibited act. Prohibited activities for ESA-listed endangered species include, but are not limited to: (1) ''take'' of such species, as defined in the ESA (including to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or attempt to engage in any such conduct); (2) delivering, receiving, carrying, transporting, or shipping in interstate or foreign commerce, in the course of a commercial activity, any such species; or (3) selling or offering for sale in interstate or foreign commerce any such species.

In the proposed rule, we said that, depending on the circumstances, we would not likely find continued possession, care, and maintenance of a captive animal to be a violation of section 9 (and that therefore, such activities would not require a section 10 permit). As noted above, we received numerous comments addressing the types of activities that might trigger section 9 concerns and/or warrant consideration for a section 10 permit. We believe these comments demonstrate the need for a more focused evaluation of these factors, which is more appropriately performed as part of a permit application process as opposed to this listing rule.

Likewise, we indicated in the proposed rule certain activities that we believe could result in violations of section 9 of the ESA, specifically including ''releasing a captive animal into the wild.'' 79 FR at 4318 (January 27, 2014). We based this on our proposed rule listing five species of sturgeon (since finalized at 79 FR 31222, June 2, 2014).

In this final rule, NMFS notes that issues surrounding any release of Lolita to the wild are numerous and complex and are not ripe for analysis in this listing rule. Such issues would be better evaluated in the context of a specific section 10 permit application. Any such process would include rigorous review by the scientific community, the Marine Mammal Commission, and the public, and be subject to an associated NEPA analysis, prior to action being taken.

**References Cited**

The complete citations for the references used in this document can be obtained by contacting NMFS (See **ADDRESSES** and **FOR FURTHER INFORMATION CONTACT**) or on our Web page at: *http://www.westcoast.fisheries.noaa.gov/protected_species/marine_mammals/killer_whale/lolita_petition.html.*

**Information Quality Act and Peer Review**

In December 2004, the Office of Management and Budget (OMB) issued a Final Information Quality Bulletin for Peer Review establishing minimum peer review standards, a transparent process for public disclosure of peer review planning, and opportunities for public participation. The OMB Bulletin, implemented under the Information Quality Act (Public Law 106–554), is intended to enhance the quality and credibility of the Federal government's scientific information, and applies to influential or highly influential scientific information disseminated on or after June 16, 2005. To satisfy our requirements under the OMB Bulletin, we obtained independent peer review of the information on Lolita in our status review update (NMFS, 2013). Four independent specialists were selected from the academic and scientific community, Federal and state agencies, and the private sector for this review (with two respondents). All peer reviewer comments were addressed in this final rule. The peer review process is detailed at: *http://www.cio.noaa.gov/services_programs/prplans/ID261.html.*

**Classification**

*National Environmental Policy Act (NEPA)*

The 1982 amendments to the ESA, in section 4(b)(1)(A), restrict the information that may be considered when assessing species for listing. Based on this limitation of criteria for a listing decision and the opinion in *Pacific Legal Foundation* v. *Andrus*, 657 F. 2d 829 (6th Cir. 1981), we have concluded that NEPA does not apply to ESA listing actions. (See NOAA Administrative Order 216–6.)

*Executive Order 12866, Regulatory Flexibility Act, and Paperwork Reduction Act*

As noted in the Conference Report on the 1982 amendments to the ESA, economic impacts cannot be considered

when assessing the status of a species. Therefore, the economic analysis requirements of the Regulatory Flexibility Act are not applicable to the listing process. In addition, this final rule is exempt from review under Executive Order 12866. This final rule does not contain a collection-of-information requirement for the purposes of the Paperwork Reduction Act.

*Executive Order 13122, Federalism*

In accordance with E.O. 13132, we determined that this final rule does not have significant federalism effects and that a federalism assessment is not required. In keeping with the intent of the Administration and Congress to provide continuing and meaningful dialogue on issues of mutual state and Federal interest, this final rule will be shared with the relevant state agencies in each state in which the species is believed to occur.

**List of Subjects in 50 CFR Part 224**

Administrative practice and procedure, Endangered and threatened species, Reporting and recordkeeping requirements.

Dated: February 4, 2015.

**Eileen Sobeck,**

*Assistant Administrator for Fisheries, National Marine Fisheries Service.*

For the reasons set out in the preamble, 50 CFR part 224 is amended as follows:

## PART 224—ENDANGERED MARINE AND ANADROMOUS SPECIES

■ 1. The authority citation for part 224 continues to read as follows:

**Authority:** 16 U.S.C. 1531–1543 and 16 U.S.C. 1361 *et seq.*

■ 2. In § 224.101, in the table in paragraph (h), revise the entry for ''Whale, killer (Southern Resident DPS)'' to read as follows:

**§ 224.101 Enumeration of endangered marine and anadromous species.**

* * * * *

(h) * * *

| Species[1] | | | Citation(s) for listing determination(s) | Critical habitat | ESA Rules |
|---|---|---|---|---|---|
| Common name | Scientific name | Description of listed entity | | | |
| **Marine Mammals** | | | | | |
| * | * | * | * | * | * |
| Whale, killer (Southern Resident DPS). | *Orcinus orca* ..... | Killer whales from the J, K, and L pods. | [*Insert citation] 2/10/2015* | 226.206 | 224.103 |
| * | * | * | * | * | * |

[1] Species includes taxonomic species, subspecies, distinct population segments (DPSs) (for a policy statement, see 61 FR 4722, February 7, 1996), and evolutionarily significant units (ESUs) (for a policy statement, see 56 FR 58612, November 20, 1991).

* * * * *

[FR Doc. 2015–02604 Filed 2–9–15; 8:45 am]

**BILLING CODE 3510–22–P**

## DEPARTMENT OF COMMERCE

### National Oceanic and Atmospheric Administration

### 50 CFR Part 660

**[Docket No. 131119977–4381–02]**

**RIN 0648–XD640**

## Magnuson-Stevens Act Provisions; Fisheries Off West Coast States; Pacific Coast Groundfish Fishery; Pacific Whiting Allocations and Fishery Closure; Pacific Whiting Seasons

**AGENCY:** National Marine Fisheries Service (NMFS), National Oceanic and Atmospheric Administration (NOAA), Commerce.

**ACTION:** Reapportionment of tribal Pacific whiting allocation, and implementation of an Ocean Salmon Conservation Zone to protect Chinook salmon.

**SUMMARY:** This document announces the reapportionment of 45,000 metric tons (mt) of Pacific whiting from the tribal allocation to the non-tribal commercial fishery sectors via two actions, in order to allow full utilization of the Pacific whiting resource. It also announces the implementation of an Ocean Salmon Conservation Zone that prohibited the targeting of Pacific whiting with midwater trawl gear shoreward of approximately 100 fathoms (fm) (183 m) to reduce Chinook salmon bycatch in the Pacific whiting fishery.

**DATES:** The rules set out in this document were made through automatic action, and are published in the **Federal Register** as soon as practicable after they are issued. The Ocean Salmon Conservation Zone was effective 0800 local time October 20, 2014 until December 31, 2014. The reapportionments of Pacific whiting were effective from 1200 local time, September 12, 2014 (25,000 mt) and 2000 local time October 23, 2014 (additional 20,000 mt), until December 31, 2014. Comments will be accepted through February 25, 2015.

**ADDRESSES:** You may submit comments, identified by NOAA–NMFS–2014–0020 by any of the following methods:

- *Electronic Submissions:* Submit all electronic public comments via the Federal eRulemaking Portal at *www.regulations.gov/#!docketDetail;D=NOAA-NMFS-2014-0020,* click the ''Comment Now!'' icon, complete the required fields, and enter or attach your comments.
- *Mail:* William W. Stelle, Jr., Regional Administrator, West Coast Region, NMFS, 7600 Sand Point Way NE., Seattle, WA 98115–0070, Attn: Miako Ushio.

*Instructions:* Comments sent by any other method, to any other address or individual, or received after the end of the comment period, may not be considered by NMFS. All comments received are a part of the public record. All personal identifying information (*e.g.,* name, address, etc.), confidential business information, or otherwise sensitive information submitted voluntarily by the sender will be publicly accessible. NMFS will accept anonymous comments (enter ''N/A'' in the required fields if you wish to remain anonymous).

**FOR FURTHER INFORMATION CONTACT:** Miako Ushio (West Coast Region, NMFS), phone: 206–526–4644 or email: *miako.ushio@noaa.gov.*

**SUPPLEMENTARY INFORMATION:**

**In re: STEARNS ZOOLOGICAL RESCUE & REHAB CENTER, INC., a Florida corporation d/b/a DADE CITY WILD THINGS.**
**Docket No. 15-0146.**
**Decision and Order.**
**Filed February 15, 2017.**

**AWA.**

Samuel D. Jockel, Esq., for Complainant.
William J. Cook, Esq., for Respondent.
Initial Decision and Order entered by Bobbie J. McCartney, Chief Administrative Law Judge.

## DECISION AND ORDER

The Animal Welfare Act (7 U.S.C. §§ 2131 *et seq.*) (AWA or Act) regulates the commercial exhibition, transportation, purchase, sale, housing, care, handling, and treatment of "animals," as that term is defined in the Act and in the regulations issued under the Act (9 C.F.R. Part 1, *et seq.*) (Regulations). Congress delegated to the Secretary of Agriculture (USDA) authority to enforce the Act.

On July 17, 2015, Complainant filed a complaint alleging that respondent Stearns Zoological Rescue & Rehab Center, Inc., violated the AWA and the Regulations on multiple occasions between July 27, 2011 and November 21, 2013. On August 5, 2015, Stearns Zoo filed an answer admitting the jurisdictional allegations and denying the material allegations of the complaint. An oral hearing was held before me, Chief Administrative Law Judge Bobbie J. McCartney, on June 27, 28, 29, and 30, 2016 in Tampa, Florida.

### I. Identification of Animals

The Regulations provide:

> A class "C" exhibitor shall identify all live dogs and cats under his or her control or on his or her premises, whether held, purchased, or otherwise acquired:

(1) As set forth in paragraph (b)(1) or (b)(3) of this section, or

(2) By identifying each dog or cat with:

(i) An official USDA sequentially numbered tag that is kept on the door of the animal's cage or run;

(ii) A record book containing each animal's tag number, a written description of each animal, the data required by § 2.75(a), and a clear photograph of each animal; and

(iii) A duplicate tag that accompanies each dog or cat whenever it leaves the compound or premises.

9 C.F.R. § 2.50(c).

The Complaint alleges that on November 21, 2013, Stearns Zoo willfully violated the Regulations by failing to identify a dog used for exhibition. (Compl. at 3 ¶ 6). In his inspection report, Dr. Navarro wrote, "[t]he dog used during interaction sessions had no official USDA identification." (CX-19 at 1). Dr. Navarro testified that during the inspection Ms. Stearns represented to him that the dog was being used for interaction sessions:

Q How do you know that the dog was being used for interactive sessions?

A Because Mrs. Stearns told us when we asked her.

Transcript (Vol. 2), 133:19-134:2.

However, Ms. Stearns testified that the dog was *not* used for exhibition, but rather that this was a family pet. (Tr. 4, 21). On balance, the testimony provided at hearing by the responsible party is more probative. Accordingly, an essential element of the subject alleged violation has not been established and is, therefore, not sustained.

**II. <u>Access for Inspection</u>**

The Act provides:

> (a) … the Secretary shall, at all reasonable times, have access to the places of business and the facilities, animals, and those records required to be kept pursuant to section 2140 of this title of any such dealer, exhibitor, intermediate handler, carrier, research facility, or operator of an auction sale…[1]

The Regulations provide:

> (a) Each dealer, exhibitor, intermediate handler, or carrier, shall, during business hours, allow APHIS officials:
>
> (1) To enter its place of business;
> (2) To examine records required to be kept by the Act and the regulations in this part;
> (3) To make copies of the records;
> (4) To inspect and photograph the facilities, property and animals, as the APHIS officials consider necessary to enforce the provisions of the Act, the regulations and the standards in this subchapter; and
> (5) To document, by the taking of photographs and other means, conditions and areas of noncompliance.[2]

The Complaint alleges that on two occasions (January 26, 2012 and September 9, 2013) Stearns Zoo willfully violated the Act and the Regulations by failing to have a responsible person available to provide access to APHIS officials to inspect their facilities, animals, and records during normal business hours. (Compl. at 3 ¶ 7). These allegations are supported by the evidence of record and are therefore sustained.

[1] 7 U.S.C. § 2146(a).
[2] 9 C.F.R. § 2.126(a).

Ms. Stearns admitted that she was not available for the inspection on January 26, 2012. She was at a doctor's appointment. (Tr. 4, 184). She argues that because the inspector never reached her, Complainant cannot say that she denied them access. This position is not supportable. It is well settled that the failure of an exhibitor either to be available to provide access for inspection or to designate a responsible person to do so constitutes a willful violation of 7 U.S.C. § 2146(a) and 9 C.F.R. § 2.126(a). Accordingly, this violation is sustained.[3]

On September 9, 2013, Dr. Brandes was unable to conduct an inspection at Stearns Zoo's facility because no one was available to accompany him. In his inspection report, Dr. Brandes wrote: "A responsible adult was not available to accompany APHIS Officials during the inspection process at 1:00 P.M. on 09/09/2013." (CX 18). At the hearing, Dr. Brandes testified that he rang the bell at the facility and called Ms. Stearns, who told him that the facility was closed on Monday and she was busy. In support of Respondent's position that the attempted inspection was not made during normal "business hours" as required to establish the alleged violation, Ms. Stearn's testified that the Zoo is a public facility that is closed on Mondays. (*See* Tr. (Vol. 4), 215:2-14). However, the Regulations provide: "*Business hours* means a reasonable number of hours between 7 a.m. and 7 p.m., Monday through Friday, except for legal Federal holiday, each week of the year, during which inspections by APHIS may be made." 9 C.F.R. § 1.1.

Further, the Judicial Officer has previously rejected a similar argument:

> I reject Mr. Perry and PWR's contention that Dr. Bellin and Mr. Watson did not attempt to conduct an inspection during "business hours," as that term is used in 9 C.F.R. § 2.126, merely because Mr. Perry and PWR's business was not open to the public at the time Dr. Bellin and Mr. Watson attempted to conduct the inspection. The time of the attempted inspection was 10:00 a.m., Thursday, January 20, 2005, which was not a holiday, and Mr. Perry was present loading animals to be moved to La Crosse, Wisconsin, for exhibition…. I find, under these

[3] Tr. (Vol. 2), 164:12-20.

> circumstances, Dr. Bellin and Mr. Watson attempted to conduct an inspection of Mr. Perry and PWR's business during business hours, even though the business was not open to the public at that time. Therefore, I conclude Mr. Perry and PWR willfully violated 7 U.S.C. § 2146(a) and 9 C.F.R. § 2.126(a), on January 20, 2005.

*Perry*, 71 Agric. Dec. 876, 880 (U.S.D.A. 2012).

Accordingly, Respondent's position is not supportable, and this violation must be sustained.

## III. Handling

Congress intended for the exhibition of animals to be accomplished in a manner that is safe for both animals and humans. The Regulations provide:

> "Handling of all animals shall be done as expeditiously and carefully as possible in a manner that does not cause trauma, overheating, excessive cooling, behavioral stress, physical harm, or unnecessary discomfort." 9 C.F.R. § 2.131(b)(1).
>
> "Physical abuse shall not be used to train, work, or otherwise handle animals." 9 C.F.R. § 2.131(b)(2)(i).
>
> "During public exhibition, any animal must be handled so there is minimal risk of harm to the animal and to the public, with sufficient distance and/or barriers between the animal and the general viewing public so as to assure the safety of animals and the public." 9 C.F.R. § 2.131(c)(1).
>
> "Young or immature animals shall not be exposed to rough or excessive public handling or exhibited for periods of time which would be detrimental to their health or well-being." 9 C.F.R. § 2.131(c)(3).
>
> "Animals shall be exhibited only for periods of time and under conditions consistent with their good health and well-being." 9 C.F.R. § 2.131(d)(1).

The Regulations define "handling" as: "petting, feeding, watering, cleaning, manipulating, loading, crating, shifting, transferring, immobilizing, restraining, treating, training, working, and moving, or any similar activity with respect to any animal." 9 C.F.R. § 1.1.

### A. Respondent's Baby Tiger Swim Program

Despite credible testimony from Respondent that Respondent attempted to develop its baby tiger swim program with care and attention to the well-being of its animals, and despite my finding that Respondent did not use physical abuse to train, work, or otherwise handle its animals; for the reasons discussed more fully herein below, it is my determination that Stearns Zoo's baby tiger swim sessions failed to provide sufficient distance and/or barriers between the animals and the public as required by the applicable regulations at 9 C.F.R. §§ 2.131(b)(1)), 2.131(b)(2)(i), 2.131(c)(1),[4] and, further, that the baby tiger swim program is not consistent with the requirements of 9 C.F.R. § 2.131(c)(3) that "(y)oung or immature animals shall not be exposed to rough or excessive public handling or exhibited for periods of time which would be detrimental to their health or well-being."[5] Therefore, this practice must cease and desist.

1. Respondent attempted to develop its baby tiger swim program with care and attention to the well-being of its animals.

Respondent provided credible testimony during the hearing that it attempted to develop its baby tiger swim program with care and attention to the well-being of its animals. Respondent developed the baby tiger swim program over several years as part of its tiger training program as a means to acclimate captive bred tigers to the presence of humans and to build a greater bond with the public in the animal world. (Tr. 3, 19). Kathy Stearns developed her tiger protocols with the assistance of qualified veterinarians. (Tr. 4, 19; RX 14-16). She also limits the tigers' swims to three booking slots a day, the tigers do not swim for more than a couple minutes total, she prohibits visitors from taking pictures that might distract

[4] Compl. ¶¶ 8b, 9a, 10c.
[5] C.F.R. § 2.131(c)(3).

the tigers, and visitors may not restrain the tigers. (Tr. 4, 24-27, 37). Respondent also takes several steps to account for the tiger's needs. (Tr. 4, 39). First, the tigers are checked in the morning to see how they are feeling. They are checked again before the swim. If the tiger is sleeping, Respondent does not wake it up. (Tr. 4, 39-40). Respondent never forces a tiger to swim. (Tr. 4, 49). The trainers have full authority to cancel or change a swim based on the tiger's condition and this sometimes happens. (Tr. 4, 51-52). Although three slots are available, Respondent averages one swim per day. (Tr. 4, 43-44).

Further, Respondent's veterinarian, Dr. Don Woodman, had no concerns about undue stress so long as the protocol was followed. (RX 13).[6] Signs of undue stress would include abnormal stools, abnormal feeding patterns, growling, listlessness, changes in sleep/wake cycles, changes in gross physical appearance such as a dull sheen to the hair coat or dull look to the eyes or other marked changes in physical condition or mentation. (RX 13; Tr. 3, 48-54). It is undisputed that Respondent's tigers are quite healthy and active and have shown no signs of undue stress, abuse or neglect. (Tr. 3, 42-43). Similarly, Vernon Yates, a humane officer who investigates animal abuse and who owns and trains tigers, testified that he has seen how Respondent's tigers are trained and he has not found any instances of animal cruelty. (Tr. 3, 157).

After reviewing a segment of ABC's "Good Morning America" video footage at the hearing, Dr. Gage testified that "[i]t appeared to me to be an animal in the water that does not want to be in the water and was trying to find the easiest place to get out of the water, and that seemed to be the reporter."[7] However, unlike Dr. Gage, who only saw the broadcast video, both Kathy and Randy Stearns were present during the entire interaction. (Tr. 4, 130-135). Contrary to Gage's view that the tiger was in distress and did not want to swim, Kathy Stearns testified that the tiger was not under any distress and just wanted to play. (Tr. 4, 134-135). Randy Stearns also testified that the tiger was not under distress and simply wanted to play and swim. (Tr. 3, 213, 216-217).

[6] In addition to his veterinary qualifications, Dr. Woodman has treated and raised tigers. In raising tigers, he trained them to get used to humans, including by taking them in his pool. (Tr. 3, 40-41).
[7] Tr. (Vol. 2), 206:16-20.

Dr. Gage also noted that there were several occasions in the segment where the trainer pulled or held the smaller cub by the tail while it was in the water.[8] It is undisputed that Respondent's employees are trained to hold the base of the tiger's tail to provide balance and support while the tiger learns to swim. (RX 22; Tr. 4, 151). Although Dr. Gage admitted that she had never trained a tiger to swim, she testified, "If you're supporting it under the base of the tail, it's truly support, and that may be acceptable, but I feel that pulling on the tail is just a rotten thing to do." (Tr. 2, 274, 277). She added, "just support, I don't really see that as being a big issue, but I watched quite a number of these videos and pictures where it looked like the trainer was pulling the animal by the tail." (Tr. 2, 278). She did not specify which videos or pictures depicted pulling the animal by the tail, and she actually only saw two videos prior to her testimony, neither of which showed a tiger being pulled by the tail.

Randy Stearns adamantly denied pulling or yanking a tiger's tail. He testified that he would never do that because he works with these cats throughout their lives, "So I don't want bad blood between a tiger that's going to be five, 600 pounds later. So it's kind of a mutual respect. So we do have a good bond. So I wouldn't want to do anything – you know, especially anything to harm an animal, let alone make it upset." (Tr. 3, 28). Consistent with this testimony, one picture from Seiler's encounter shows Randy Stearns directing a customer not to grab the tiger's tail. (Tr. 3, 199). Randy Stearns explained that in the pictures Ms. Seiler presented, he was not actually pulling the tiger's tail. In the pictures taken on land, he was simply supporting the tiger by its belly with his hand on the tiger's tail to ensure that the animal did not flip over and fall on his head. The cat was not vocalizing when he had his hand on the tail. (Tr. 3, 29). In one of the water photographs, Stearns's hand was on the very tip of the tail. He was moving it away after letting the tiger go to swim on its own. In another picture, Stearns had his hand on the tail as the tiger was getting out of the water to keep the tiger from falling back into the water and going under. At the same time, he was moving his right hand under the tiger to support him. (Tr. 3, 33-34). As for holding a tiger by the neck, this allegation apparently was taken from Seiler's affidavit, which she corrected during the hearing to reflect that the tiger was being held by the scruff of the neck and not strangled. (Tr.1, 85). Dr. Gage testified that scruffing is a common

[8] CX 6 at 2.

practice, and tigers will relax when held by the scruff, as the mother would do. (Tr. 2, 218, 267).

It is my determination that, taken as a whole, the evidence of record does not support a finding that Stearns Zoo violated section 2.131(b)(2)(i) by using physical abuse to work the tigers.

2. Stearns Zoo's baby tiger swim sessions failed to provide sufficient distance and/or barriers between the animals and the public as required by the applicable regulations.

Despite credible testimony from Respondent that Respondent attempted to develop its baby tiger swim program with care and attention to the well-being of its animals, and despite my finding that Respondent did not use physical abuse to train, work, or otherwise handle its animals; for the reasons discussed more fully herein below, it is my determination that Stearns Zoo's baby tiger swim sessions failed to provide sufficient distance and/or barriers between the animals and the public as required by the applicable regulations at 9 C.F.R. §§ 2.131(b)(1)), 2.131(b)(2)(i), 2.131(c)(1),[9] and, further, that the baby tiger swim program is not consistent with the requirements of 9 C.F.R. § 2.131(c)(3) that "(y)oung or immature animals shall not be exposed to rough or excessive public handling or exhibited for periods of time which would be detrimental to their health or well-being."[10]

a. *September 30, 2011 (Baby Tiger Swim Session)*

The evidence shows that on September 30, 2011, Barbara Keefe paid for a "tiger swim session" at Stearns Zoo's facility.[11] In a letter to APHIS and an affidavit, Ms. Keefe described in detail what she observed at the facility.[12] She recalled that at least three separate groups participated in three tiger swim sessions that day.[13]

[9] Compl. ¶¶ 8b, 9a, 10c.
[10] 9 C.F.R. § 2.131(c)(3).
[11] CX- 9.
[12] CX-9 at 1.
[13] CX-9 at 2; Tr. (Vol. 2), 17:2-6, 75:3-8, 78:1-14.

While there was quite a bit of testimony from various witnesses opining as to whether the baby tigers were in distress or enjoyed the swim sessions, the dispositive point to be made here is that exhibitions where dangerous animals are potentially or actually in direct contact with the public violate both section 2.131(c)(1) and 2.131(b)(1):

> The evidence demonstrates the public was extremely close to animals that were controlled solely by two volunteers who are familiar with the animals but have no special training in containing them, preventing their escape, or controlling them in the event of an attack. Given the limited handling training for the volunteers, the number of people in attendance, the close proximity of dangerous animals, the lack of a formal plan to control animals in the event of escape, combined with the potential for people to physically come into contact with the animals, I find, during the behind-the-scenes exhibitions, such as were observed on June 2, 2008, Tri-State and Mr. Candy violated 9 C.F.R. § 2.131(c)(1) by failing to handle animals so there was minimal risk of harm to the animals and to the public.

*Tri-State Zoological Park of Western Maryland, Inc.*, 72 Agric. Dec 128, 138 (U.S.D.A. 2013). *See also Williams*, 64 Agric. Dec. 1347, 1361 (U.S.D.A. 2005).

b. *October 10, 2012 (Good Morning America Swim Session)*

On October 10, 2012, Stearns Zoo exhibited two tigers at Stearns Zoo's facility on a segment of ABC's "Good Morning America." Video footage of the event shows an ABC reporter directly handling two tigers in the pool.[14] Dr. Laurie Gage testified regarding the younger tiger (Tony) that

> . . . the size of the animal, the age of the animal . . . it's an animal which . . . should be in the nursery . . . They should be fully vaccinated, because people can carry a virus that's very tough in the environment, hard to kill, and

[14] CX-4 at 00:18

> lives for a long time and can be carried on people's clothing and their hands and brought into a situation like this . . . you're putting this animal in an unusual situation for its age."[15] Dr. Gage noted that adding members to the public that are not trained to handle the animal causes an issue as, "[t]hey don't necessarily understand how to respond if it misbehaves, or they're not trained to handle baby tigers.[16]

In her declaration (and in her testimony), Dr. Gage noted that APHIS Animal Care considers news reporters, such as the one in the video, to be members of the public.[17]

Later in the footage, an additional tiger-a large juvenile (Tarzan) was brought into the pool, where the reporter was in direct contact with the juvenile.[18] Dr. Gage testified that ". . . this is a large tiger that should not be anywhere close to a member of the public. This is an animal that's too big and too strong, too fast. It could cause damage not only to his handler, but to a member of the public."[19] She noted that the animal was sixty pounds, if not more.[20] Even Stearns Zoo's attending veterinarian would agree, "[o]ver 40 pounds, at that point, I think that they could start becoming dangerous to the public. They can start causing bites that would be significant or scratches that would be significant."[21]

"Respondents' lions and tigers are simply too large, too strong, too quick, and too unpredictable for a person (or persons) to restrain the animal or for a member of the public in contact with one of the lions or tigers to have the time to move to safety." *International Siberian Tiger Foundation*, 61 Agric. Dec. 53, 78 (U.S.D.A. 2002).

---

[15] Tr. (6/28/16), 197:7-198:7.
[16] Tr. (6/28/16), 198:19-199:9.
[17] CX-6 at 1.
[18] CX-4 at 02:50.
[19] Tr. (6/28/16), 204:13-18.
[20] Tr. (6/28/16), 211:12.
[21] Tr. (6/28/16), 211:12.

It is well settled that exhibitions where dangerous animals are potentially or actually in direct contact with the public violate both sections 2.131(c)(1) and 2.131(b)(1):

> The evidence demonstrates the public was extremely close to animals that were controlled solely by two volunteers who are familiar with the animals but have no special training in containing them, preventing their escape, or controlling them in the event of an attack. Given the limited handling training for the volunteers, the number of people in attendance, the close proximity of dangerous animals, the lack of a formal plan to control animals in the event of escape, combined with the potential for people to physically come into contact with the animals, I find, during the behind-the-scenes exhibitions, such as were observed on June 2, 2008, Tri-State and Mr. Candy violated 9 C.F.R. § 2.131(c)(1) by failing to handle animals so there was minimal risk of harm to the animals and to the public.

*Tri-State Zoological Park of Western Maryland, Inc.*,72 Agric. Dec 128, 138 (U.S.D.A. 2013). *See also Williams*, 64 Agric. Dec. 1347, 1361 (U.S.D.A. 2005).

c. *October 13, 2012 (Baby Tiger Swim Session)*

The evidence reflects that on October 13, 2012, Ms. Jayanti Seiler participated in a "tiger swim" at Stearns Zoo. Ms. Seiler, along with five to seven other people,[22] were shuttled to the area where the animals were kept. Randy Stearns was the trainer during her session, and the juvenile tiger, Tony was brought out to interact with the customers.[23] While there was quite a bit of testimony from various witnesses opining as to whether the baby tigers were in distress or enjoyed the swim sessions, the dispositive point to be made here is that exhibitions where dangerous animals are potentially or actually in direct contact with the public violate both sections 2.131(c)(1) and 2.131(b)(1):

[22] Tr. (Vol. 1), 35:18-20.
[23] CX-8 at 1.

> The evidence demonstrates the public was extremely close to animals that were controlled solely by two volunteers who are familiar with the animals but have no special training in containing them, preventing their escape, or controlling them in the event of an attack. Given the limited handling training for the volunteers, the number of people in attendance, the close proximity of dangerous animals, the lack of a formal plan to control animals in the event of escape, combined with the potential for people to physically come into contact with the animals, I find, during the behind-the-scenes exhibitions, such as were observed on June 2, 2008, Tri-State and Mr. Candy violated 9 C.F.R. § 2.131(c)(1) by failing to handle animals so there was minimal risk of harm to the animals and to the public.

*Tri-State Zoological Park of Western Maryland, Inc.*, 72 Agric. Dec 128, 138 (U.S.D.A. 2013). *See also Williams*, 64 Agric. Dec. 1347, 1361 (U.S.D.A. 2005).

d. *October 18, 2012 (Fox and Friends Swim Session)*

On October 18, 2012, Stearns Zoo exhibited a young tiger, Tony, in a simulated swim encounter staged in New York, which was presented on "Fox and Friends."[24] The video footage shows Randy Stearns handling "Tony" in front of a public crowd pressed in tightly to the makeshift pool in an effort to see the baby tiger.[25] Contrary to Respondent's request, a kiddie pool had been provided, and Tony was unable to swim properly. (Tr. 4, 139). Randy Stearns testified that the tiger made noises indicating that he was excited by the cameras, and that the flimsiness of the pool was a problem for him. (Tr. 4, 140) (Tr. 3, 227). According to Mr. Stearns, the camera was too close to the tiger, and the tiger wanted to play with it. (Tr. 3, 226). He was following the camera until he became distracted by a toy moose. (Tr. 3, 227). The tiger was not under distress or even scared of the

[24] This was the same tiger depicted in the ABC show a week earlier. Tony was ten weeks old and weighed about twenty-two pounds. (Tr. 4, 140).
[25] CX-5.

camera. He wasn't doing anything abnormal. (Tr. 3, 228). After this swim, Mr. Stearns testified that "Tony" was perfectly healthy. (Tr. 4, 141-142).

Based on her observation of the video evidence, Dr. Gage concluded that the baby tiger did not want to swim under those circumstances. (CX 6; Tr. 2, 263). While she admitted that it was possible that the tiger wanted to leave the pool because he was curious about something on the outside, Dr. Gage stated that "the animal did not appear to enjoy being in the water . . . it made numerous and consistent attempts to exit the water but was held in the pool by its handler holding the leash."[26]

Again, the dispositive point to be made here is that exhibitions where dangerous animals are potentially or actually in direct contact with the public violate both section 2.131(c)(1) and 2.131(b)(1):

> The evidence demonstrates the public was extremely close to animals that were controlled solely by two volunteers who are familiar with the animals but have no special training in containing them, preventing their escape, or controlling them in the event of an attack. Given the limited handling training for the volunteers, the number of people in attendance, the close proximity of dangerous animals, the lack of a formal plan to control animals in the event of escape, combined with the potential for people to physically come into contact with the animals, I find, during the behind-the-scenes exhibitions, such as were observed on June 2, 2008, Tri-State and Mr. Candy violated 9 C.F.R. § 2.131(c)(1) by failing to handle animals so there was minimal risk of harm to the animals and to the public.

*Tri-State Zoological Park of Western Maryland, Inc.*, 72 Agric. Dec 128, 138 (U.S.D.A. 2013). *See also Williams*, 64 Agric. Dec. 1347, 1361 (U.S.D.A. 2005).

[26] Tr. 2, 264; CX-6 at 2.

3. The baby tiger swim program is not consistent with the requirements of 9 C.F.R. § 2.131(c)(3) that "(y)oung or immature animals shall not be exposed to rough or excessive public handling or exhibited for periods of time which would be detrimental to their health or well-being.

Further, and perhaps more importantly, Stearns Zoo's baby tiger swim program is not consistent with the requirements of 9 C.F.R. § 2.131(c)(3) that "(y)oung or immature animals shall not be exposed to rough or excessive public handling or exhibited for periods of time which would be detrimental to their health or well-being."[27]

As referenced *supra*, Dr. Laurie Gage testified regarding the younger tiger (Tony):

> . . . the size of the animal, the age of the animal…it's an animal which…should be in the nursery . . . They should be fully vaccinated, because people can carry a virus that's very tough in the environment, hard to kill, and lives for a long time and can be carried on people's clothing and their hands and brought into a situation like this...you're putting this animal in an unusual situation for its age.[28]

This testimony is equally applicable to all of the baby tiger swim sessions.

**B. Macaque Monkey**

The Complaint alleges that on or about July 27, 2011, Stearns Zoo willfully violated the Regulations (9 C.F.R. § 2.13(c)(1)) by exhibiting a macaque without sufficient distance and/or barriers between the macaque and the public so as to minimize the risk of harm to the animals and the public.[29] Dr. Navarro testified that he received an incident report dated July 21, 2011 from a representative from State Department of Health with respect to an individual who sought treatment for injuries from a monkey

[27] 9 C.F.R. § 2.131(c)(3).
[28] Tr. (6/28/16), 197:7-198:7.
[29] Compl. ¶ 10a.

bite at Stearns Zoo.[30] According to the report, during an encounter with a monkey, the monkey slapped the victim's face and repeatedly bit the victim's arm, breaking the skin.[31] Dr. Navarro included this information in an inspection report dated July 27, 2011.[32]

The Judicial Officer has observed, "the probative value of a report depends on the extent to which the inspector documents the facts supporting [the inspector's] findings." *Hansen*, 57 Agric. Dec. 1072 (U.S.D.A. 1998). Inspector Navarro did not investigate or verify the facts in the subject report and instead relied on the statement of an unidentified health official who simply reported the bite complaint of an unidentified customer. (CX-14, CX-21). He did not speak to the person claiming to have been bitten or the health official, nor did he show Kathy Stearns the complaint. (Tr. 2, 147-148).

Ms. Stearns testified that she personally handled the monkey and interacted with the customer. She testified that the monkey was on a leash and did not bite the customer. (Tr. 4, 174-175). The FWC also investigated the complaint, and Ms. Stearns provided the agency with photos of the session; however, nothing came of it. She similarly told the USDA inspector that the incident did not happen and offered to show pictures. (Tr. 4, 176-177, 181). Ms. Stearns believed that she appealed the inspection report but she did not keep the paperwork. She felt that the issue had been put to bed since the FWC had found no violation. The first she heard of it again was in this case.[33] (Tr. 4, 183).

The most probative evidence regarding this disputed violation came from Ms. Stearns, who had personal knowledge of the encounter, and who testified that she was personally handled the monkey during the encounter, that the monkey was on a leash, and that the monkey did not bite the customer. (Tr. 4, 174-175). Accordingly, Complainant has failed to meet its burden of proof regarding this alleged violation and this alleged violation is not sustained.

## IV. Standards

[30] Tr. (Vol. 2), 119:15-120:1; 120:14-21.
[31] CX-21.
[32] CX-14.
[33] The incident was not included in Respondent's May 31, 2012 official warning. (CX-3).

Section 2.100(a) of the Regulations provides: "Each exhibitor . . . shall comply in all respects with the regulations set forth in part 2 of this subchapter and the standards set forth in part 3 of this subchapter for the humane handling, care, treatment, and transportation of animals. . ."[34]

The Complaint alleges that in five separate instances, Stearns Zoo failed to meet the minimum standards with respect to drainage, structural strength, and shelter from inclement weather.

**A. May 1, 2013 (Drainage)**

Section 3.127(c) of the Standards provides: "Drainage. A suitable method shall be provided to rapidly eliminate excess water. The method of drainage shall comply with applicable Federal, State, and local laws and regulations relating to pollution control or the protection of the environment."[35]

The evidence shows that on May 1, 2013, Stearns Zoo's tiger enclosures had an accumulation of mud and water.[36] In his inspection report, Dr. Navarro wrote:

> A few of the Tiger enclosure[s] had water and mud accumulation due to rainy weather during the night. The owner recognized the problem and started working on it by putting new substrate on the ground inside the enclosure. According to the owner cement is going to be pour[ed] within the next month.[37]

Dr. Navarro testified that more than one enclosure had "a lot of mud, and the tigers were muddy, and there was a drainage issue. . ."[38] His photographs show two separate enclosures: (1) a tiger laying on the ground

[34] 9 C.F.R. § 2.100(a). This Regulation applies to all of the alleged noncompliance with the standards promulgated under the Act (Standards).
[35] 9 C.F.R. § 3.127(c).
[36] Compl. ¶ 12a.
[37] CX-17 at 1.
[38] Tr. (Vol. 2), 129:18-22.

with mud in one enclosure;[39] and (2) another enclosure with muddy ground and drainage issues.[40] The accumulation of water and mud caused mud to get on the tigers because, ". . . I don't see anywhere where they can lay down without being muddy."[41] Dr. Navarro testified that the mud contains bacteria that could create an infection of the skin and intestinal problems if it were consumed.[42]

Stearns Zoo's asserts that, "it was really wet from the bad storms.[43] Inspections of outdoor facilities conducted on rainy days will often reveal pools of water; however, the Standard requires a suitable method to rapidly eliminate excess water.[44] Stearns Zoo had no method to rapidly eliminate excess water on May 1, 2013. Although Stearns Zoo asserts that it corrected the problem after the inspection,[45] again, subsequent correction does not obviate violations.[46] Accordingly, the violation is sustained.

**B. September 6, 2012 (lion enclosure)**

Section 3.125(a) of the Standards provides: "Structural strength. The facility must be constructed of such material and of such strength as appropriate for the animals involved. The indoor and outdoor housing facilities shall be structurally sound and shall be maintained in good repair to protect the animals from injury and to contain the animals."[47]

As alleged in the Complaint, the evidence shows that on September 6, 2012, Stearns Zoo failed to maintain the lion enclosure in good repair as there was a loose electric wire hanging inside the enclosure.[48] In his

[39] CX-17 at 2, 3; Tr. (Vol. 2), 130:6-10.
[40] CX-17 at 4, 5; Tr. (Vol. 2), 130:15-18.
[41] Tr. (Vol. 2), 131:9-12.
[42] Tr. (Vol. 2), 131:15-19.
[43] Tr. (Vol. 4), 204:20.
[44] White, Docket No. 12-0277, 2014 WL 4311058, at *10 (U.S.D.A. May 13, 2014).
[45] Tr. (Vol. 4), 208:13-209:2.
[46] Pearson, 68 Agric. Dec. 685, 727-28 (U.S.D.A. 2009), *aff'd*, 411 F. App'x 866 (6th Cir. 2011); Bond, 65 Agric. Dec. 92, 109 (U.S.D.A. 2006), *aff'd per curiam*, 275 F. App'x 547 (8th Cir. 2008); Drogosch, 63 Agric. Dec. 623, 643 (U.S.D.A. 2004); Parr, 59 Agric. Dec. 601, 644 (U.S,D,A, 2000), *aff'd per curiam*, 273 F.3d 1095 (5th Cir. 2001) (Table); DeFrancesco, 59 Agric. Dec. 97, 112 n.12 (U.S.D.A. 2000); Huchital, 58 Agric. Dec. 763, 805 n.6 (U.S.D.A. 1999); Stephens, 58 Agric. Dec. 149, 184-85 (U.S.D.A. 1999).
[47] 9 C.F.R. § 3.125(a).
[48] Compl. ¶ 12b.

inspection report, Dr. Navarro wrote: "The electric wire inside the lion enclosure was hanging lose due to a tree limb that fell and hit the horizontal holding wire clamp."[49]

At the hearing, Dr. Navarro testified that the purpose of the electric wire, which goes around the lion enclosure, was to have a continuous ". . . electrical circuit that it prevents the animals from going over it because they receive like an electrical shock. It has impulses, and that prevents the animals from climbing out of the enclosure."[50] Dr. Navarro's photographs show the clamp facing down, allowing the electric wire to touch the fence.[51] The electric wire was not operating as it was designed to operate because "it was too close to the chain link . . . if an animal decided to climb over it, it could walk over it because it didn't have enough separation from the chain-link fence."[52] Accordingly, the violation is sustained.

**C. May 1, 2013 (baboon enclosure)**

The evidence shows that on May 1, 2013, Stearns Zoo failed to maintain an enclosure for two baboons in good repair.[53] Section 3.75(a) of the Standards provides:

> Structure: construction. Housing facilities for nonhuman primates must be designed and constructed so that they are structurally sound for the species of nonhuman primates housed in them. They must be kept in good repair, and they must protect the animals from injury, contain the animals securely, and restrict other animals from entering.

9 C.F.R. § 3.75(a).

In his inspection report, Dr. Navarro wrote:

[49] CX-16 at 1.
[50] Tr. (Vol. 2), 125:13-16.
[51] CX-16 at 3, 4; Tr. (Vol. 2), 126:18-126:1.
[52] Tr. (Vol. 2), 125:14-18.
[53] Compl. ¶ 12c.

> The enclosure housing the 2 male baboon[s] had a detached welded pole on the side and front panel area of the enclosure in which the primates are exhibited. The constant pushing and pulling on the chain link by the primates on the side and front area of the enclosure may result in a debilitated structure and makes the enclosure vulnerable to escape of the animals.

CX-17 at 1.

Photographs taken during the inspection show detached poles on the side panels of the enclosure, caused by the primates banging on the chain-link fence.[54] Given the strength of the nonhuman primates, Dr. Navarro testified that the issue with the detached poles lay in the danger for escape if the chain-link fence became unattached by the nonhuman primates.[55] The purpose of the enclosure is to protect the animals from injury and to contain them securely.[56] The photographic evidence demonstrates the effect of the baboons' strength,[57] and that the enclosure was structurally compromised due to the detached pole. Accordingly, the violation is sustained.

**D. November 21, 2013 (pig enclosure)**

The evidence shows that on November 21, 2013, Stearns Zoo failed to maintain an enclosure for a pig so as to protect the animal from injury.[58] Section 3.125(a) of the Standards provides:

> Structural strength. The facility must be constructed of such material and of such strength as appropriate for the animals involved. The indoor and outdoor housing facilities shall be structurally sound and shall be maintained in good repair to protect the animals from injury and to contain the animals.[59]

[54] CX-17 at 6, 7; Tr. (Vol. 2) 128:20-129:3.
[55] Tr. (Vol. 2), 128:6-9.
[56] *See* 9 C.F.R. § 3.75(a).
[57] Tr. (Vol. 2), 128:20-129:3.
[58] Compl. ¶ 12d.
[59] 9 C.F.R. § 3.125(a).

In his inspection report, Dr. Navarro wrote: "The enclosure housing the pig had a rusted pipe with jagged edges."[60] His photographs depict a rusty vertical pipe that was used to close the door of the pig enclosure.[61] The rust's location-at the bottom edges-posed a risk of harm to the pig as, ". . . the jagged edges, along with the rust . . . if he uses his snout, like some of the pigs do, he could cut his snout on the jagged edges."[62] Accordingly, the violation is sustained.

**E. November 21, 2013 (shelter for tigers)**

The evidence shows that on November 21, 2013, Stearns Zoo failed to provide tigers with adequate shelter from inclement weather.[63] Section 3.127(b) of the Standards provides: "Natural or artificial shelter appropriate to the local climatic conditions for the species concerned shall be provided for all animals kept outdoors to afford them protection and to prevent discomfort to such animals. . . ."[64] Exhibitors are required to provide *each* animal housed outdoors with adequate shelter from the elements.

> On a July 28, 1992, inspection of Big Bear Farm, Inc., two APHIS inspectors found that "the petting zoo enclosure housed 1 potbellied pig, 5 sheep and 7 goats was equipped with 2 wood shelter boxes and 1 plastic barrel. There was not enough total shelter space to accomodate [*sic*] all animals housed in this enclosure at the same time.

*Big Bear Farm, Inc.*, 55 Agric. Dec. 107, 122-23 (U.S.D.A. 1996).[65]

---

[60] CX-19 at 1.
[61] Tr. (Vol. 2), 134:13-16.
[62] Tr. (Vol. 2), 134:9-12.
[63] Compl. ¶ 12e.
[64] 9 C.F.R. § 3.127(b).
[65] Pearson, 68 Agric. Dec. 685, 709 (U.S.D.A. 2009) ("On or about September 9, 1999, Mr. Pearson housed a bobcat in an enclosure with a damaged roof that did not provide the animal with shelter from inclement weather, in willful violation of section 3.127(b) of the Regulations…."); Parr, 59 Agric. Dec. 601, 613 (U.S.D.A. 2000) ("Mr. Currer testified that he observed a tiger in an enclosure that had a roof but had no protection on its sides from wind or blowing rain….Respondent states that he completed the repairs necessary to comply with 9 C.F.R. § 3.127(b) by April 20, 1997…. I conclude that on April 9, 1997,

In his inspection report, Dr. Navarro wrote: "One tiger enclosure had a shelter that was not tall enough for the tigers to go into it and make normal postural movements."[66] Dr. Navarro's photographs show a shelter that, "was not high or tall enough for the animals to get in there in case there was rain and they wanted to get shelter from the elements."[67] He testified that the opening in the enclosure was two feet by two feet, not sufficient for both of the tigers.[68] Accordingly, the violation is sustained.

## Findings of Fact

1. The Secretary of Agriculture has jurisdiction in this AWA administrative enforcement matter. 7 U.S.C. §§ 2149(a), (b).

2. Stearns Zoological Rescue & Rehab Center, Inc. (Stearns Zoo), is a Florida corporation (N07000007224) that does business as Dade City Wild Things, and whose registered agent for service of process is Kathryn P. Stearns, 36909 Blanton Road, Dade City, Florida 33523. (Compl. ¶ 1; Answer at ¶ 1; CX-1; CX-2). Stearns Zoo exhibits domestic, wild, and exotic animals at its Blanton Road facility and off-site. (CX-1, CX-2, CX-5; Stipulations as to Facts, Witnesses and Exhibits (Stipulations) ¶ 1.E).

3. Randall (Randy) Stearns is a director and the President of Stearns Zoo, and Kathryn Stearns is a director and the Secretary of Stearns Zoo. (CX-2).

4. At all times mentioned in the Complaint, Stearns Zoo was an exhibitor, as that term is defined in the Act and the Regulations, and held AWA license number 58-C-0883. (Compl. ¶ 1; Answer ¶ 1; CX-1, CX-2).

5. In 2011, Stearns Zoo represented to APHIS that it held sixty-one animals; in 2012, Stearns Zoo represented that it held ninety-seven

---

Respondent willfully violated section 3.127(b) of the Standards…by failing to provide an animal shelter from inclement weather.").
[66] CX-19 at 2.
[67] CX-19 at 6, 7; Tr. (Vol. 2), 135:22-136:4.
[68] Tr. (Vol. 2), 136:13-21.

animals; in 2013, Stearns Zoo represented that it held 126 animals; in 2014, Stearns Zoo represented that it held ninety-eight animals; and in 2015, Stearns Zoo represented that it held 139 animals. (Compl. ¶ 2; CX-1).

6. On May 31, 2012, APHIS issued an Official Warning to Stearns Zoo with respect to noncompliance documented during five inspections: May 4, 2010 (perimeter fence); September 21, 2010 (veterinary care, facilities, drainage); May 17, 2011 (non-human primate enclosure); September 14, 2011 (handling of a tiger); and February 23, 2012 (serval enclosure). (Answer ¶ 4; CX-3; Tr. (Vol. 2), 101:12-116:15 (Navarro); 157:18-163:17 (Brandes); 173:6-179:18 (Gaj)).

7. On November 21, 2013, Veterinary Medical Officer (VMO) Dr. Luis Navarro conducted a compliance inspection of Stearns Zoo's facilities, equipment, and animals, and asserted that Stearns Zoo had failed to identify a dog as required; however, the evidence of record reflects that the dog was *not* used for exhibition, but rather that this was a family pet. (Tr. 4, 21).

8. On January 26, 2012, Dr. Navarro attempted to conduct a compliance inspection at Stearns Zoo's facility, but no one was available to provide access or to accompany him. VMO Navarro prepared a contemporaneous inspection report. (CX-15; Stipulations ¶ I.A; Tr. (Vol. 2), 122:14-124:12).

9. On September 9, 2013, VMO Dr. Robert Brandes attempted to conduct an inspection at Stearns Zoo's facility. No one from Stearns Zoo was available to provide access or to accompany him. He prepared a contemporaneous inspection report. (CX-18; Stipulations ¶ I.B; Tr. (Vol. 2), 163:18-167:6).

10. On July 27, 2011, it was alleged that Stearns Zoo, during exhibition, allowed members of the public to have direct contact with a macaque without any distance and/or barriers between the macaque and the public; however, this alleged violation was based solely on unsubstantiated third-party information that was directly rebutted by

the sworn testimony of Ms. Stearns at hearing based on her personal knowledge. (CX-14, 21; Tr. 2, 147-148; Tr. 4, 174-175).

11. On September 30, 2011 and on October 13, 2012, Stearns Zoo exhibited a young tiger to the public, including Barbara Keefe and Jayanti Seiler, respectively, in a pool, without any distance and/or barriers between the tiger and the public. (CX-9, CX-10, CX-11, CX-12; Tr. (Vol. 2), 25:22-32:2 (Keefe). Tr. (Vol. 1), 38:10-20; 141:1-12 (Seiler)).

12. On October 10, 2012, Stearns Zoo exhibited a young tiger (Tony) in a pool with a member of the public (a television reporter) who was permitted to handle the tiger directly. (CX-4, CX-6; Tr. (Vol. 2), 192:12-194:14, 202:9-203:2, 205:21-208:1 (Gage); Stipulations ¶ D).

13. On October 10, 2012, Stearns Zoo also exhibited a large juvenile tiger (Tarzan) in a pool with a member of the public (a reporter) without any distance and/or barrier between the tiger and the reporter. (CX-4, CX-6; Tr. (Vol. 2), 192:12-206:5, 211:2-18 (Gage); Stipulations ¶ D).

14. On October 18, 2012, Stearns Zoo exhibited a juvenile tiger (Tony) in a pool outdoors in New York City, as part of a television show, without any barrier and scant distance between the tiger and a television reporter. (CX-5, CX-6; Tr. (Vol. 2), 213:18-22, 217:13-219:5 (Gage); Stipulations ¶ E).

15. On May 1, 2013, VMO Navarro conducted a compliance inspection at Stearns Zoo. (CX-17). He observed and documented in an inspection report that there was not a method to rapidly eliminate excess water from tiger enclosures, which had an accumulation of mud and water, and that the enclosure for two baboons had a support pole that had detached from the side and front of the enclosure. (CX-17; Tr. (Vol. 2), 129:130:10 (Navarro); Stipulations at 1 ¶ G).

16. On September 6, 2012, Dr. Navarro conducted a compliance inspection at Stearns Zoo. (CX-16). He observed and documented in an inspection report that there was a loose electrical wire hanging inside the lion enclosure and accessible to the lion. (CX-16; Tr. (Vol. 2), 124:13-127:19 (Navarro); Stipulations at 1-2 ¶ H).

17. On November 21, 2013, Dr. Navarro conducted a compliance inspection at Stearns Zoo. (CX-19). He observed and documented in an inspection report that Stearns Zoo's enclosure for a pig contained a rusted jagged pipe, and that there was inadequate shelter from inclement weather for tigers. (CX-19; Tr. (Vol. 2), 132:16-137:19 (Navarro); Stipulations at 1 ¶ C).

18. On September 30, 2011, October 10, 2012, October 13, 2012, and October 18, 2012, Stearns Zoo's baby tiger swim program was not consistent with the requirements of 9 C.F.R. § 2.131(c)(3) in that young or immature baby tigers were exposed to rough or excessive public handling or exhibited for periods of time which would be detrimental to their health or well-being. For example, Dr. Laurie Gage testified regarding the younger tiger (Tony), ". . . the size of the animal, the age of the animal . . . it's an animal which . . . should be in the nursery…They should be fully vaccinated, because people can carry a virus that's very tough in the environment, hard to kill, and lives for a long time and can be carried on people's clothing and their hands and brought into a situation like this . . . you're putting this animal in an unusual situation for its age." (Tr. (6/28/16), 197:7-198:7).

## Conclusions of Law

1. On November 21, 2013, Stearns Zoo did not violate the Regulations by failing to identify a dog because the dog was ***not*** used for exhibition but rather was a family pet. (Tr. 4, 21). 9 C.F.R. § 2.50(c).

2. On or about January 26, 2012 and September 9, 2013, Stearns Zoo willfully violated the Act and the Regulations by failing to have a responsible person available to provide access to APHIS officials to inspect its facilities, animals, and records during normal business hours. 7 U.S.C. § 2146(a); 9 C.F.R. § 2.126(a).

3. On July 27, 2011, Stearns Zoo did not violate the Regulations, 9 C.F.R. § 2.131(c)(1), by failing to handle a macaque properly during public exhibition.

4. On September 30, 2011, October 10, 2012, October 13, 2012, and October 18, 2012, Stearns Zoo willfully violated the Regulations, 9 C.F.R. § 2.131(c)(1), by failing to handle tigers during public exhibition with minimal risk of harm to the animals and the public, and with sufficient distance and/or barriers between the animals and the public.

5. On September 30, 2011, October 10, 2012, October 13, 2012, and October 18, 2012, Stearns Zoo willfully violated the Regulations, 9 C.F.R. §§ 2.131(c)(3) and 2.131(d)(1), by exposing young or immature tigers to rough or excessive handling and/or by exhibiting them for periods of time and/or under conditions that were inconsistent with their good health and well-being.

6. In five instances on the following dates, Stearns Zoo willfully violated the Regulations, 9 C.F.R. § 2.100(a), by failing to meet the minimum Standards promulgated under the AWA (9 C.F.R. Part 3) (Standards), as follows:

    i. September 6, 2012. Loose electric wire inside lion enclosure. 9 C.F.R. § 3.125(a).

    ii. May 1, 2013. No method to rapidly eliminate excess water from tiger enclosures. 9 C.F.R. § 3.127(c).

    iii. May 1, 2013. Detached support pole for enclosure housing two baboons. 9 C.F.R. § 3.75(a).

    iv. November 21, 2013. Rusted pipe with jagged edges in pig enclosure. 9 C.F.R. § 3.125(a).

    v. November 21, 2013. Inadequate shelter from inclement weather for tigers. 9 C.F.R. § 3.127(b).

## V. Sanctions

The evidence establishes that, *inter alia*, Stearns Zoo repeatedly handled animals in a manner that placed the animals (and people) at risk

of harm, and repeatedly failed to provide access for inspection, in willful violation of the Regulations. For these reasons alone, Complainant requests that license 58-C-0883 be revoked. The Complainant also requests that Stearns Zoo be ordered to cease and desist from future violations, and that a civil penalty be assessed. APHIS believes that the evidence supports a finding that Stearns Zoo committed twenty-three violations and seeks the assessment of a civil penalty of $23,000.[69]

The Secretary may revoke an AWA license following a single, willful violation. U.S.C. § 2149(a); *Pearson v. USDA*, 411 F. App'x 866, 872 (6th Cir. 2011) ("An AWA license may be revoked following a single, willful violation of the Animal Welfare Act.") (citing *Cox v. USDA*, 925 F.2d 1102, 1 105 (8th Cir. 1991)). A willful act is an act in which the violator intentionally does an act which is prohibited, irrespective of evil motive or reliance on erroneous advice, or acts with careless disregard of statutory requirements. *Ash*, 71 Agric. Dec. 900, 913 (U.S.D.A. 2012); *Bauck*, 68 Agric. Dec. 853, 860-61 (U.S.D.A. 2009), *appeal dismissed*, No. 10-1138 (8th Cir. Feb. 14, 2010); *D&H Pet Farms Inc.*, 68 Agric. Dec. 798, 812-13 (U.S.D.A. 2009): *Bond*, 65 Agric. Dec 92, 107 (U.S.D.A. 2006), *aff'd per curium*, 275 F. App'x 547 (8th Cir. 2008); *Stephens*, 58 Agric. Dec. 149, 180 (U.S.D.A. 1999); *Arab Stock Yard, Inc.*, 37 Agric. Dec. 293, 306 (U.S.D.A. 1978), *aff'd mem.*, 582 F.2d 39 (5th Cir. 1978). However, as reflected in *Esposito*, 38 Agric. Dec. 613, 633 (U.S.D.A. 1979), different degrees of seriousness of violations are recognized by the Judicial Officer and, of course, mitigating circumstances are always considered in determining the sanction to be issued and may be grounds for imposing a lesser sanction.

The Act authorizes the Secretary to assess a civil penalty of up to $10,000 for each violation of the Act or the Regulations. When determining the amount of the civil penalty to be assessed for violations of the Animal Welfare Act and the Regulations, the Secretary of Agriculture is required to give due consideration to four factors: (1) the size of the business of the person involved; (2) the gravity of the violations; (3) the person's good faith; and (4) the history of previous violations. 7 U.S.C. § 2149(b).

[69] The maximum civil penalty that could be assessed under the Act is $230,000.

**A. Size of the business**

Respondent operates a zoo on twenty-two acres with approximately 300 animals. Respondent has been in business for sixteen years and has grown from nothing to being open six days a week. (Tr. 4, 6-9, 13). Therefore, Stearns Zoo operates a large business exhibiting animals. *Huchital*, 58 Agric. Dec. 763, 816-17 (U.S.D.A. 1999) (finding the respondent, who held approximately eighty rabbits, operated a large business); *Browning*, 52 Agric. Dec. 129, 151 (U.S.D.A. 1993) (finding the respondent, who held seventy-five to eighty animals, operated a moderately large business), *aff'd per curiam*, 15 F.3d 1097 (11th Cir. 1994).

**B. Gravity of the violations**

The gravity here is great because several of the violations put both people and animals at risk of injury.

**C. Respondent's Good Faith**

The evidence of record reflects that Kathy Stearns has been working with exotic animals most of her life and that she is devoted to the care and well-being of her animals. She is involved with conferences and compliance training, including first responder training, and she was a member of the Florida Fish and Wildlife Conservation Commission ("FWC") Technical Advisory Group involved with revisions to Florida's captive wildlife regulations. (Tr. 4, 11-12). She is also involved with tiger genome research, and has created an endangered species conservation fund. She has given money to the University of Arizona to buy cameras for identifying cats in South America and has funded other projects. (Tr. 4, 72-73).

Complainant contends that Stearns Zoo has not shown good faith because despite having been issued an Official Warning on May 31, 2012, Stearns Zoo has continued to violate the same Regulations. However, the May 31, 2012 Official Warning is simply a composite of inspection reports, and the Judicial Officer has made clear that inspectors do not determine whether a violation exists:

> It bears repeating that an inspector is only an evidence gatherer. The inspector has no authority to find that anyone violated the Animal Welfare Act or the Regulations and Standards, but merely presents evidence, first to the agency and the agency's counsel, and then before an administrative law judge.

*Hansen*, 57 Agric. Dec. 1072, 1123 (U.S.D.A. 1998).

Further, a closer look at the May 31, 2012 Official Warning does not support a finding of bad faith. There are seven alleged violations listed on the official warning. (CX 3). Complainant presented evidence on five of them.[70]

- September 21, 2010 – splintered resting surface – This allegation is unrelated and different from other alleged violations, and there is no suggestion that the resting surface was not repaired. (Tr. 4, 160-161).

- September 21, 2010 - drainage – Stearns testified that only two enclosures had drainage issues and Respondent installed concrete floors. (Tr. 4, 208).

- May 17, 2011 - non-human primate enclosure – The inspector found a welded pole that had become detached from the roof of a macaque enclosure. Again, there is no suggestion that this alleged violation continued and was not repaired.

- February 23, 2012 – rusted pipe in serval enclosure – The inspector testified that Respondent repaired the pipe. (Tr. 2, 116).

- September 14, 2011 – tiger swim - The inspection report and subsequent warning stated:

> During the tiger swim session the cub #2 (blue collar, black leash) was reluctant to move to the edge of the pool

[70] Complainant's counsel stated on the record that it was not contending that an allegation of failure to provide adequate veterinary care to Cleo the black leopard was evidence of bad faith. (Tr. 3, 103-104). Complainant also abandoned the alleged prior violation of May 4, 2010 (perimeter fence).

> and the handler pulled him by the leash. The cub was later passed from the side of the pool to the handler inside the pool and the cub was apparently under distress by vocalizing and moving around when handled inside the pool in apparent discomfort. After swimming for a short distance the cub swam towards the handler located at the pool wall and extended his paws towards the edge of the pool apparently wanting to get out of the pool. Instead of pulling the cat out of the water and stopping the encounter the handler decided to continue the swimming.

CX-3 at 53.

Respondent videotaped the inspection and strongly contends that the video tells a different story from the subjective allegations contained in the inspection report regarding the issue of whether the baby tiger was in discomfort. (RX-7; Tr. 4, 94-116). Consequently, Respondent appealed the report and sent APHIS the portion of the video showing the second cub referenced in the report. (RX-8; Tr. 4, 120). The agency then sent Stearns a letter advising that it had not received the video. (RX-9). Apparently it had become separated from the appeal and sent to Dr. Gaj. (Tr. 4, 122). The agency then denied the appeal without viewing the video. (RX-11). The agency's letter, written by Dr. Robert Willems, dated February 12, 2012, stated that the cub referenced in the inspection report (the second cub) was showing signs of distress. In contrast, "the other cub in the pool which did not exhibit these same signs of distress but seemed content with being in the water." (RX-11).

Dr. Willems wrote to Respondent again on February 24, 2012, stating that after review of the video, "it appears that the cub pictured is not the same one for which the citation was written. The cub in the video you submitted appears to be the other cub that was swimming in the pool at the time of the inspections. This was the cub we acknowledged was not distressed." (RX-27). Stearns was positive that she sent the agency video of cub two. (Tr. 4, 128). The video that Dr. Willems reviewed shows a cub that he admitted was not in distress. (Tr. 4, 129). After receiving the letter, Stearns called Dr. Willems and sent him the full version of the video with both cubs. She has yet to hear back. (Tr. 4, 126-127). Thus, Respondent

was not advised of any violation on September 14, 2011 regarding its tiger swim encounter.

Even more importantly, for purposes of considering Complainant's request to *revoke* Respondent's license, is that fact that the full nature and scope of the dangers posed by the Respondents swim program to the baby tigers were not clearly communicated to the Respondent even at the time of the inspections giving rise to the subject violations. The record reflects that the USDA investigators were not particularly concerned with the fact that the baby tigers weighed only about twenty pounds and were only about eight weeks old and should not have been in the unnatural and unprotected environment of a chlorinated swimming pool at all or that there were members of the public swimming in the pool with these wild animals. Luis Navarro, a veterinarian medical officer for the United States Department of Agricultural, APHIS Animal Care, and Mr. Gregory S. Gaj testified as follows:

**Testimony of Dr. Navarro:**
**6/28/16 In Re: Stearns Zoological Rescue & Rehab Center**

**Page: 106**
BY MR. JOCKEL:
Q. Let's look at Complainant's Exhibit 3, page 53. Are you there?
A Yes.
Q. And can you identify this document?
A Yes. This is an inspection report conducted September 14, 2011.
Q. Where did this inspection occur?
A At the facility on Blanton Road. That's the site 1 facility.
Q. And where in that facility particularly did that occur?
A. Let me read it here. The swim with the tiger session happens usually at the pool that's on the facility. At the time, there was one pool,

**Page: 107**
I think, and now they have two pools; but I don't

think they use this other pool anymore.
Q. Was there a facility representative present?
A. Yes. Mrs. Stearns was present.
Q. And was anyone else from APHIS present?
A. Yes. Dr. Gaj was with me during that inspection. He's my supervisor.
Q. Okay. What can you recall was the problem that you observed with the tiger-swim session?
A. There were two tigers -- young tigers. The first tiger that did the swim session, we didn't notice too much issues with the tiger going into the water or during the swim session. At the end, he was getting tired, and I believe he was trying to reach for the border of the pool to get out.
The second tiger is the one that -- was the one we had an issue with, and it was because he was kind of reluctant to go into the water, and the handler had to pick him up, take him to the

**Page: 108**

corner. He would come back from the pool and he would -- he didn't want to get into the water. And once he got into the water, he tried to swim out of the water, and that's where we find the issue with the tiger. He was kind of reluctant, and he had to be pulled by the leash to bring him towards the corner of the pool -- to the corner of the pool.

**6/28/16 In Re: Stearns Zoological Rescue & Rehab Center**

**Page: 108**

Q. Let's start from the beginning. Were there members of the public present?
A. Yes.
Q. How many?

A. There were approximately two to four. I can't recall the exact number.
Q. And were they located in the pool with the tiger?
A. Yes. They would go into the pool with the tiger.
Q. And you just testified that there were two different tigers. What was the size of those tigers?
A. These tigers were approximately -- I

**Page: 109**

would have to say approximately because I didn't weigh them, but they were approximately 20, 22 pounds of weight, and I asked the owner, and she told me it was around eight weeks of age approximately.

**Page: 110**

BY MR. JOCKEL:
Q. How large was the pool?
A. Approximately like 20 feet by 15, I would say, and they would use just half the pool for exhibition. I guess they would use the lower end where it was shallower.
Q. And how close did the patrons get to the tigers?
A. They got close enough to take pictures with them, and they could pet the tigers.

**Testimony of Gregory S. Gaj**
**6/28/16 In Re: Stearns Zoological Rescue & Rehab Center**

**Page:113**

Q. Have you conducted inspections along with VMO Dr. Navarro at this particular facility?
A. Yes, I have.
Q. And did you conduct an inspection with Dr. Navarro in September of 2011 that involved a

tiger swim?
A. Yes, I did.
Q. What happened during that inspection?
A. When we were doing the inspection for the tiger swim, we went to the pool, which was at Mrs. Stearns' home and that's where they were doing the tiger swim. We watched them take the first tiger, approximately eight weeks, and take it and put it into the pool to swim with the public.
JUDGE McCARTHY [*sic*]: Can I ask you a few questions about the pool. Is that a chlorinated?

**6/28/16 In Re: Stearns Zoological Rescue & Rehab Center**

**Page: 174**

pool?
THE WITNESS: Yes, I believe it is.
JUDGE McCARTHY [*sic*]: Is that a standard-size pool for residential purposes, or was it a pool constructed specifically for the utilization of display with these animals?
THE WITNESS: It appeared to be just a standard pool for, you know, the owner. I don't think it was specifically designed in any way for exhibition.
JUDGE McCARTHY [*sic*]: All right, thank you.
THE WITNESS: So, we watched the first juvenile tiger do the swim with the tiger program, and what they did was they led him to the pool, picked up the tiger, handed it to a trainer, put it into the pool, and with the first juvenile tiger, they did have a momentary, you know, uncomfortableness in my opinion with him being put in the water, but the animal appeared to calm down fairly quickly. And then they proceeded to do the swim program, which allowed a member of the public to swim next to the tiger as it was swimming from

**6/28/16 In Re: Stearns Zoological Rescue & Rehab Center**

**Page: 175**

one handler across the pool to the other. When they did the first swim with the tiger, I did not feel that there was enough of a problem to -- to say that it was dangerous for the public at that point. The animal seemed to calm down and be acclimated enough to the water to do the program.
JUDGE McCARTHY [*sic*]: When you say it swam from one handler to the other, was the animal restrained by a leash at all times?
THE WITNESS: I think there was a leash dangling behind the tiger, but it wasn't one that it was actually -- the tiger was actually swimming on its own. There may have been a leash behind it dragging in the water, but I don't think so.

The record reflects that it was not until the hearing that compelling testimony provided by USDA expert witness Dr. Laurie Gage fully demonstrated that Respondent's baby tiger swim program is simply not consistent with the requirements of 9 C.F.R. § 2.131(c)(3) that "(y)oung or immature animals shall not be exposed to rough or excessive public handling or exhibited for periods of time which would be detrimental to their health or well-being."[71] Dr. Gage provided detailed testimony in support of her position on this issue including, but not limited to, testimony that

> . . . the size of the animal, the age of the animal . . . it's an animal which . . . should be in the nursery. . . They should be fully vaccinated, because people can carry a virus that's very tough in the environment, hard to kill, and lives for a long time and can be carried on people's clothing and their hands and brought into a situation like

[71] 9 C.F.R. § 2.131(c)(3).

> this...you're putting this animal in an unusual situation for its age.[72]

In light of the lack of clear communication to the Respondent regarding the full nature and scope of the problems with its baby tiger swim program, I cannot find bad faith based on prior warnings.

**D. History of previous violations**

Prior inspection reports show that Respondent has been inspected repeatedly without being written up. (RX-1; Tr. 4, 190-196).

The evidence establishes that, *inter alia*, Stearns Zoo repeatedly handled animals in a manner that placed the animals (and people) at risk of harm, and repeatedly failed to provide access for inspection, in willful violation of the Regulations. Complainant requests that Stearns Zoo be ordered to cease and desist from future violations, and that a civil penalty of $23,000.00 be assessed because APHIS believes that the evidence supports a finding that Stearns Zoo committed twenty-three violations. (The maximum civil penalty that could be assessed under the Act is $230,000.00). Because two of the alleged violations were not sustained, the civil money penalty is hereby adjusted to $21,000.00.

Complainant also requests that license 58-C-0883 be revoked. The Secretary may revoke an AWA license following a single, willful violation. U.S.C. § 2149(a); *Pearson v. USDA*, 411 F. App'x 866, 872 (6th Cir. 2011) ("An AWA license may be revoked following a single, willful violation of the Animal Welfare Act . . ") (citing *Cox v. USDA*, 925 F.2d 1102, 1 105 (8th Cir. 1991)). A willful act is an act in which the violator intentionally does an act which is prohibited, irrespective of evil motive or reliance on erroneous advice, or acts with careless disregard of statutory requirements. *Ash*, 71 Agric. Dec. 900, 913 (U.S.D.A. 2012); *Bauck*, 68 Agric. Dec. 853, 860-61 (U.S.D.A. 2009), *appeal dismissed*, No. 10-1138 (8th Cir. Feb. 14, 2010); *D&H Pet Farms Inc.*, 68 Agric. Dec. 798, 812-13 (U.S.D.A. 2009): *Bond*, 65 Agric. Dec 92, 107 (U.S.D.A. 2006), *aff'd per curium*, 275 F. App'x 547 (8th Cir. 2008); *Stephens*, 58 Agric. Dec. 149, 180 (U.S.D.A. 1999); *Arab Stock*

[72] Tr. (6/28/16), 197:7-198:7.

*Yard, Inc.*, 37 Agric. Dec. 293, 306 (U.S.D.A. 1978), *aff'd mem.*, 582 F.2d 39 (5th Cir. 1978). However, as reflected in *Esposito*, 38 Agric. Dec. 613, 633 (U.S.D.A. 1979), different degrees of seriousness of violations are recognized by the Judicial Officer and, of course, mitigating circumstances are always considered in determining the sanction to be issued and may be grounds for imposing a lesser sanction.

It is my determination that the lack of clear communication to the Respondent regarding the full nature and scope of the problems with its baby tiger swim program, the most serious of the subject violations, demonstrates mitigating circumstances which are appropriate for consideration of the imposition of a lesser sanction than revocation. The Judicial Officer has held that "[i]f the remedial purpose of the Animal Welfare Act is to be achieved, the sanction imposed must be adequate to deter Respondent and others from violating the Animal Welfare Act, the Regulations, and the Standards." *Volpe Vito*, 56 Agric. Dec. 269, 273 (U.S.D.A. 1997). The assessment of a $21,000.00 civil money penalty and a sixty-day suspension is supported by the record and will ensure address the Secretary's legitimate enforcement concerns without putting Respondent out of business.[73]

**ORDER**

1. Stearns Zoo, it agents and employees, successors and assigns, directly or through any corporate or other device, shall cease and desist from violating the Act and the Regulations.

2. AWA license number 58-C-0883 is hereby suspended for a period of sixty (60) days from the date this Decision and Order becomes final.

3. Stearns Zoo is assessed a civil penalty of $21,000.00, to be paid by check made payable to the Treasurer of the United States and remitted

[73] The agency's regulations provide that no license may be issued to any applicant whose license has been revoked, and any person whose license has been revoked shall not be licensed. *See* 9 C.F.R. § 2.11(a)(3); 9 C.F.R. § 2.10(b); *see also* Ash, 72 Agric. Dec. 340, 343 (U.S.D.A. 2013) (Remand Order) ("[R]evocation of a person's Animal Welfare Act license bars that person from obtaining an Animal Welfare Act license at any time in the future.").

either by U.S. Mail addressed to USDA, APHIS, Miscellaneous, P.O. Box 979043, St. Louis, MO 63197-9000, or by overnight delivery addressed to:

US Bank, Attn: Govt
Lockbox 979043
1005 Convention Plaza
St. Louis, MO 63101

This Decision and Order shall be final and effective without further proceedings thirty-five (35) days after service unless an appeal to the Judicial Officer is filed with the Hearing Clerk within thirty (30) days after service, pursuant to section 1.145 of the Rules of Practice (7 C.F.R. § 1.145).

Copies of this Decision and Order shall be served by the Hearing Clerk upon each of the parties.

___

**In re: GRETCHEN MOGENSEN.**
**Docket No. 16-0042.**
**Decision and Order.**
**Filed March 22, 2017.**

**AWA.**

Gretchen Mogensen, Petitioner, pro se.
Colleen A. Carroll, Esq., for Respondent.
Initial Decision and Order by Bobbie J. McCartney, Chief Administrative Law Judge.

## DECISION AND ORDER GRANTING RESPONDENT'S MOTION FOR SUMMARY JUDGMENT

### Introduction

The Rules of Practice Governing Formal Adjudicatory Proceedings Instituted by the Secretary Under Various Statutes [Rules of Practice], set forth at 7 C.F.R. § 1.130 *et seq.*, apply to adjudication of the instant matter. This case involves a letter filed by pro-se petitioner Gretchen Mogensen

# EXPERT REPORT OF DR. JENNIFER CONRAD, DVM

*Amended as of January 22, 2020*

My testimony is focused on the veterinary care, health, and wellbeing of the lions, tigers, and hybrids of those species (collectively, big cats) housed at Wildlife in Need and Wildlife in Deed, Inc. (WIN) in Charlestown, Indiana.

## Background and Qualifications

I am a doctor of veterinary medicine currently practicing in Los Angeles. I hold a Doctorate of Veterinary Medicine (1994) from the University of California, Davis, School of Veterinary Medicine, where I took the Wildlife Medicine Track, and a Bachelor of Arts in Biology (1989) from the University of California, Berkeley. I am a member of the American Veterinary Medicine Association (AVMA), the American Association of Zoo Veterinarians (AAZV), and the European Association of Zoo and Wildlife Veterinarians (EAZWV), and the International Veterinary Academy of Pain Management (IVAPM), the American Association of Feline Practitioners (AAFP), and the International Society of Feline Medicine (ISFM).

I work with and provide humane care to captive wildlife. At present, I care for approximately 30 lions and tigers, having cared for some 200 over the course of my 25 years as a veterinarian. I have been the attending veterinarian, within the meaning of the Animal Welfare Act, for six USDA-licensed facilities housing big cats during those 25 years. I am experienced in all aspects of veterinary care for big cats, including performing reparative surgery to their declawed paws, and providing adequate comprehensive veterinary care, including in the areas of neonatal care, nutrition, enrichment, and housing. Since graduating from veterinary school, I have also participated in many programs to protect and improve the lives of wild animals, including conservation efforts in Namibia, Nepal, and the Galapagos Islands, among other locations. In the field of veterinary medicine, to my knowledge, there is no one with more experience than I have

in the care, treatment, and morbidity of declawed big cats, such as the big cats involved in this case.

In addition to my veterinary work, I started the Paw Project in 1999, a nonprofit that rehabilitates big cats such as lions, tigers, cougars, jaguars, and even domestic cats maimed by declawing. I have participated in programs and activities to educate the public about the physiological and behavioral effects of feline declawing. I have been called to write letters in animal abuse cases both in the United States and abroad that require expert testimony on declawing. In 2006, the United States Department of Agriculture (USDA), the federal agency that oversees animals bred, exhibited, or sold in commerce, issued guidance under the Animal Welfare Act (AWA) that declawing or defanging large carnivores, including big cats, constituted a failure to provide adequate veterinary care. This change was based in part on information and guidance provided by the Paw Project. In 2018, the Big Cat Sanctuary Alliance, an organization of sanctuaries that house and care for big cats, had me speak at their national conference regarding the deleterious effects of declawing big cats and subsequent need to provide chronic pain management for big cats they rescue already declawed because they recognize my expertise in these areas.

During the hearing in this matter on the Plaintiff's Motion for Preliminary Injunction, the Court found that I am qualified to testify as an expert on "declawing and the treatment of big cats, specifically in the repairing of declawed big cats." PI Hr'g Tr. 23:10-13. This report incorporates by reference my testimony provided during that hearing. *Id.* at 16:7-47:9.

A copy of my curriculum vitae is attached as Exhibit 1 to this Report. I have testified as an expert regarding the proper care of captive tigers in the matter *Kuehl v. Sellner*, 161 F. Supp. 3d 678 (N.D. Iowa 2016).

I am being compensated at a rate of $100.00 per hour, up to a maximum of $800.00 per day, for my time spent in connection with this matter.

**Resources**

In preparing this testimony, I have considered:[1]

- USDA inspection reports of Defendants' facility, dated June 25, 2013 (PETA-WIN_002524 – 2530); January 17, 2014 (PETA-WIN_002361 – 2362); May 6, 2014 (PETA-WIN_002370 – 2372); August 20, 2014 (PETA-WIN_002363 – 2366); September 13, 2015 (PETA-WIN_002367 – 2369); January 20, 2016 (PETA-WIN_002547 – 2551); March 17, 2017 (PETA-WIN_002581 – 2588); March 17, 2017 (PETA-WIN_003617 – 3620); March 18, 2017 (PETA-WIN_003629); and March 29, 2017 (PETA-WIN_002589 – 2591);
- the USDA's Complaint against Timothy L. Stark and Wildlife in Need and Wildlife in Deed, Inc. (Complaint, In re: Timothy L. Stark, et al., AWA Docket Nos. 16-0124 and 16-0125 (July 8, 2016);
- the testimony of USDA officials and the government's exhibits against Timothy L. Stark and Wildlife in Need and Wildlife in Deed, Inc., given during the hearing on the merits before the Administrative Law Judge in AWA Docket Nos. 16-0124 and 16-0125;
- the Complaint and Motions for Temporary Restraining Order and for Preliminary Injunction, with supporting exhibits, in this matter;
- the transcripts of witnesses deposed in this matter;
- the expert declarations previously filed in this matter;
- the videos and photographs taken, and the report written, by PETA's confidential informant;
- the transcripts of hearings in this matter;
- video footage, photographs, and other documentary evidence of the Defendants' "Tiger Baby Playtime" events;
- video and photographs of the site inspection of Defendants' premises and big cats, which occurred March 22, 2019
- video and photos of a Big Cat, declawed by the Defendants, now residing at The Wild Animal Sanctuary;
- text messages of Defendant Tim Stark;
- the Defendants' veterinary records, transfer records, and medical logs regarding the big cats in their possession; and
- photographs and videos, provided by the Defendants in discovery in this matter, of the big cats who were the subject of the USDA inspection dated March 17, 2017.

[1] After completing my expert report, I reviewed a number of documents subsequently produced by PETA. These documents—PETA-WIN_009978, PETA-WIN_009982, PETA-WIN_009985, PETA-WIN_009993, and PETA-WIN_010073—reinforced the opinions and conclusions expressed in my original expert report.

## Methodology

When performing a veterinary examination of an animal, I base my assessment on observations of the animal and its environment.

Performing a visual examination for zoo animals is often done non-invasively and relies heavily on observation. The physical exam includes the signalment (species, age, weight, sex, identification), a history including known disease conditions, medications, vaccinations, diet, weight history, food and water intake, fecal and urine output, and any other information regarding presenting concerns.

The subjective, objective, assessment, and plan (SOAP) exam format, upon which I have relied here to the extent appropriate, is commonly used by veterinarians. It has widespread acceptance in the veterinary medical field and is a reliable method for evaluating animal health.

The SOAP method includes the following:

1. Subjective assessment of the animal—its attitude, activity, responsiveness, and hydration status.

2. Objective measures to evaluate all body systems, including the eyes; ears; mouth and teeth; integument; musculoskeletal system; heart and lungs; assessment of fecal and urine quality and quantity; neurologic assessment; and genitourinary organs.

3. Assessment, which is made based on the subjective and objective information obtained, is an overall impression of the animals' health, and includes a list of differential diagnoses to be ruled out by further observations and or diagnostic testing.

4. Plan is how you intend to confirm or rule out your differential diagnoses and or what treatments you will be administering.

When inspecting a facility to evaluate animal health, I follow the GFAS Site Inspection protocol. This includes evaluating the following:

1. Housing, which includes the condition of the enclosures, animal groupings, safe containment, ventilation, light and heat, sleeping areas, cleanliness and sanitation, enrichment items, and furnishings.

2. Physical Facilities, which includes tools and equipment, drainage, electricity, lighting, heating, emergency measures, security measures, insect and rodent control, transportation, and protective barriers such as perimeter fencing.

3. Nutrition, including water sources, diets and record keeping, feeding protocols, monitoring individual animal consumption, food storage, and sanitation.

4. Veterinary Care, including the Program of Veterinary Care, staff number and expertise, veterinary facilities, quarantine and isolation areas, biosafety measures, medical supplies and storage, controlled substance security and logs, medical records, anesthetic records, laboratory reports, animal identification, weight records, and necropsy reports.

5. Well-Being and Animal Handling, including overall animal appearance, activity, responsiveness, animal groupings, enrichment provided and enrichment plan/calendar, and human-animal interactions.

6. General Staffing, including sufficient quantity to provide adequate care, appropriate training, evaluation of staff and volunteer policies, access to emergency information, staff supervision, contact with animals, training programs or employee manuals, and Standard Operating Procedures employees/volunteers follow.

7. Safety Policies, Protocols and Training, including how they work with dangerous animals (alone or as a team), security of enclosures, locking mechanisms, double gates,

safety zones around animal enclosures, Personal Protective Equipment in use, communication systems, animal escape plans, evacuation routes, emergency training records, security of firearms if kept on site, first aid kits, eye wash stations.

In this litigation, it was not possible to perform a full and complete veterinary examination on the big cats at issue. Rather, I have reviewed extensive video, photographic, and record evidence of the Defendants' facility and big cats, including the records produced by the Defendants, the sworn deposition testimony of witnesses in this case, and the sworn testimony of U.S. Department of Agriculture veterinarians who have inspected the Defendants' facility, among the other sources described above. A full and complete veterinary examination of each big cat would have required, among other things, sedation, blood work, radiographs, palpating the animals, and close visual and physical inspection. The site inspection that was agreed to by the Defendants did not allow for such activities, which would have taken several days, if not weeks, to perform properly. Rather, a videographer was allowed to record the big cats from a vantage point accessible to members of the public who visit the Defendants' facility, and to record additional big cats toward the rear of the facility who are not on public display.

Nonetheless, with this information, I am able to formulate opinions in this case to a reasonable degree of certainty. As described below, it is my opinion that Defendants have created a likelihood of injury to the big cats in their possession by disrupting their normal behaviors, have actually injured the big cats, and have contributed to the deaths of multiple big cats. For this reason, it is my opinion that to prevent this conduct in the future, the big cats should be moved to an appropriate sanctuary.

## Summary of Opinions[2]

[2] The opinions express in this report are held to a reasonable degree of veterinary certainty.

1. The Defendants declaw big cats, for reasons unrelated to medical necessity, which wounds them by cutting skin, connective tissue, tendons, nerves, and blood vessels, and amputates each digit at the distal phalanx, in violation of the applicable standards of care outlined in this report.

2. The Defendants prematurely separate big cats from their mothers to hand-rear them for reasons unrelated to medical necessity, namely inappropriately to use the cubs for direct contact with members of the public, in violation of the applicable standards of care outlined in this report.

3. The Defendants fail to provide the big cats with appropriate nutrition, in violation of the applicable standards of care outlined in this report.

4. The Defendants fail to provide the big cats with adequate enrichment and social grouping, in violation of the applicable standards of care outlined in this report.

5. The Defendants fail to provide the big cats with appropriate veterinary care, in violation of the applicable standards of care outlined in this report.

6. The foregoing deficiencies wound or injure the big cats or create a high likelihood of injury or death resulting from disruption to their normal behaviors. In certain instances, the deficiencies have contributed to the deaths of multiple big cats.

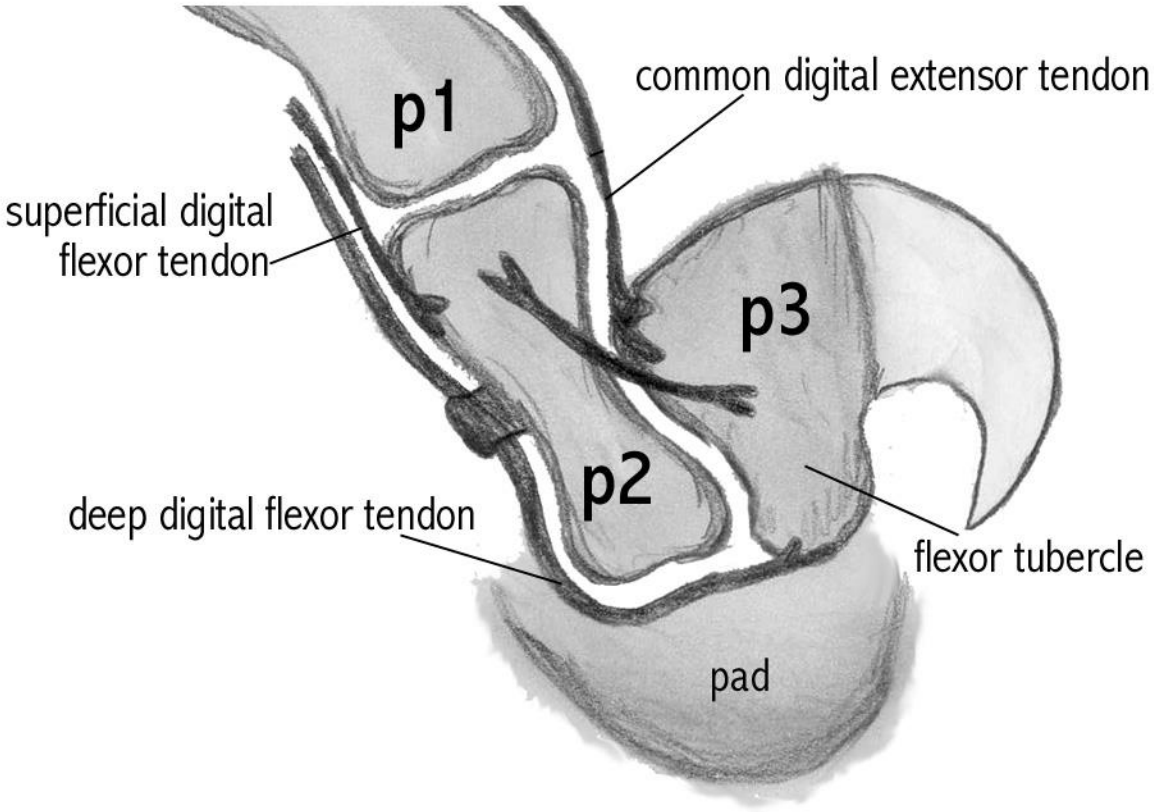
**Opinions on Declawing of Big Cats**

7. Based on the information I reviewed, I conclude, to a reasonable degree of veterinary and scientific certainty, that the big cats housed at Defendants' facility have been declawed for reasons wholly unrelated to medical necessity, which is generally defined as removing anatomical pathology in a toe, and that this surgery, used to modify a healthy animal for the owner's convenience, constitutes a significant injury to the animal that will likely result in long-term impairment to the animals' abilities to engage in normal behaviors including, among other

things, walking with a normal gait and without pain, scratching, and climbing. Defendants' declawing of big cats is not a generally accepted practice of animal husbandry and violates the standard of care for the treatment of big cats.

8. Declawing is a surgical procedure, also called onychectomy, in which the animal's distal phalanges are partially or fully amputated. Declawing is more accurately described as "deknuckling." In humans, the nails grow from the skin, but in cats, claws grow from the bones, thus necessitating removal of all or part of the third phalanx, or p3. When big cats are declawed, the last bone of each of their digits is fully or partially amputated so the claw cannot regrow.[3] In addition to the bone, the tendons, nerves, and ligaments that enable normal function and movement of the digit are severed, as are the blood vessels. This is a reference drawing I made to show normal claw position in a big cat.

[3] The following link contains a video that I prepared which demonstrates the declawing procedure on the big cats: https://youtu.be/WmLEmyL2L1o . A copy of this video was previously delivered to this Court and to counsel for the Defendants.



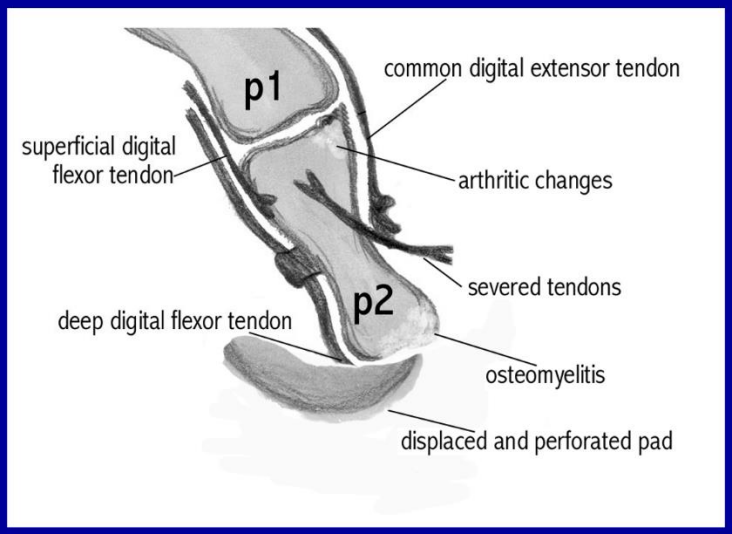

9. While the amputation of healthy bones, declawing, constitutes an immediate, unnecessary, and severe injury and some animals (including multiple big cats specifically identified in USDA inspections of Defendants' facility and in videos and photographs taken by the Defendants and by PETA's confidential informant) will have immediate complications from the procedure, it may be many months or years before other effects caused by the damage of declawing become obvious. It is my opinion that declawing these big cats likely will result and has already resulted in permanent lameness, arthritis, abnormal standing conformation, and other long-term complications.

10. Big cats normally walk with the distal interphalangeal joint bearing the weight of their bodies; each step is cushioned by the digital pad under this joint. The third phalanx, p3, sits up against the second phalanx, p2, so that the cat doesn't walk on the claw itself. The cat can therefore walk almost silently and when it needs its claws, they are sharp. In my opinion, declawed big cats have the potential to suffer lifelong severe pain. Indeed, domestic cats are declawed in clinical trials for pain studies precisely because declawing is known to cause severe pain.

11. There are four surgical methods of declawing a cat.

a. The first method is a disarticulation surgery. It is the complete amputation of the third phalanx (p3). This method often results in the untoward complication of a hammertoe of the second phalanx (p2). When declawing with a complete disarticulation, the digital extensor tendon is severed, as is the deep flexor tendon, because they both attach to p3; however, the superficial flexor tendon attaches to p2, and since its function is now unopposed by an extensor tendon, it pulls the second phalanx into the hammertoe position. Other complications from this surgery include that the digital pad atrophies and is pulled proximally (toward the back of the paw) where it can no longer serve as a cushion for the animal's footsteps. This can result in the distal portion of the second phalanx poking through skin causing infection, including bone infection, osteomyelitis. The animal often compensates by shifting its weight off the toes, walking on the back of the paw or carpus or tarsus; in more severe and particularly heartbreaking cases, the mutilation of declawing may cause so much tenderness or pain that the animal can move only by walking on its hyperextended carpi or tarsi- this makes the animal appear to have flat feet, often causing it back pain and arthritis in the limbs.

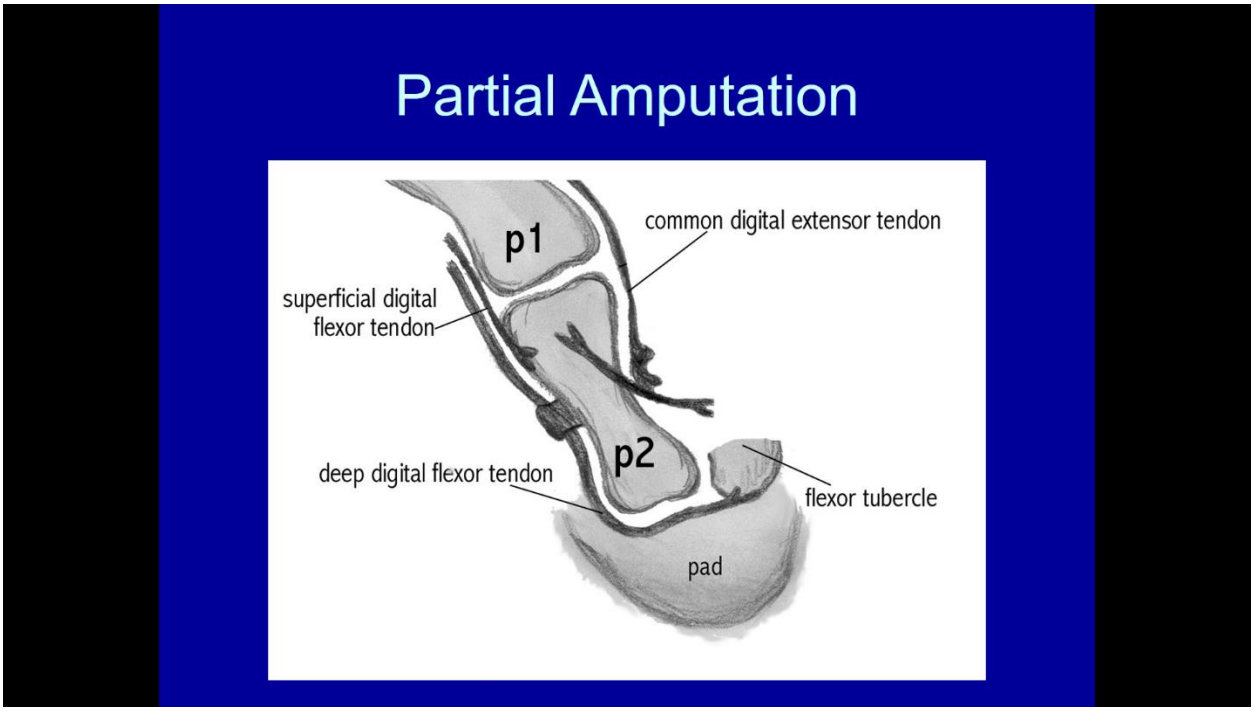
# Disarticulation Technique

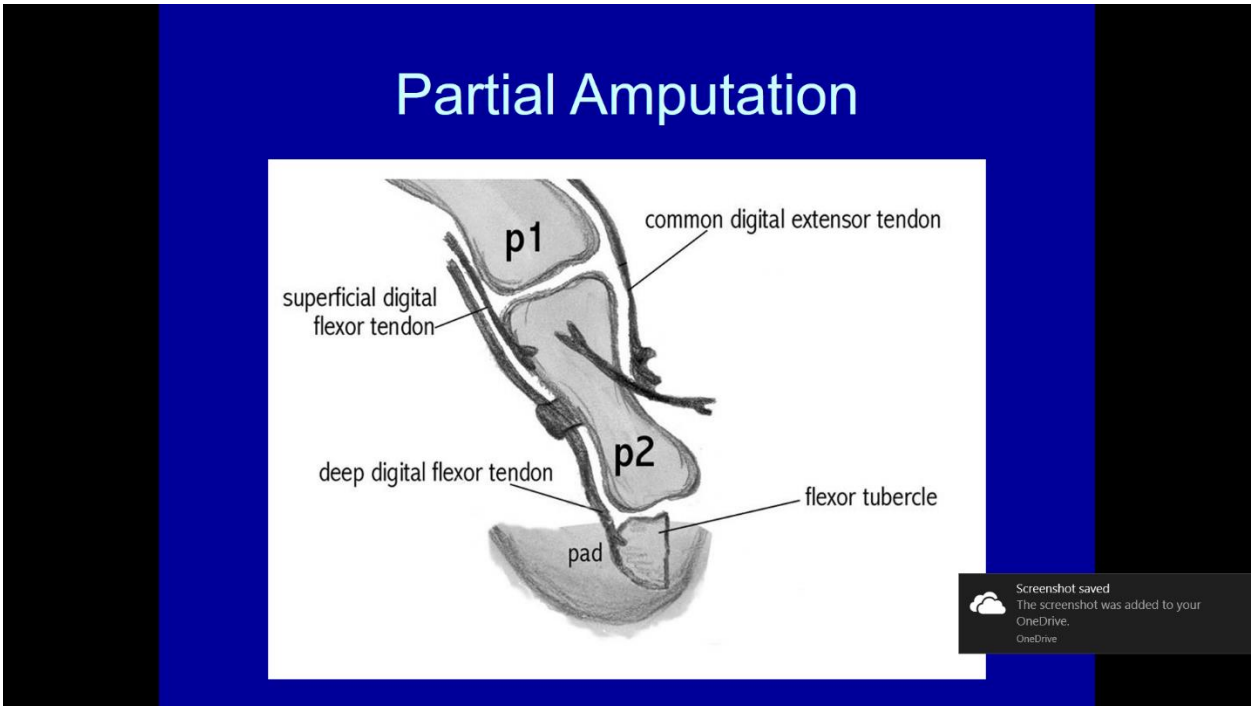



Bobcat declawed via disarticulation has P2 perforating skin, which resulted in osteomyelitis. Note pad atrophy


b. In order to try to lessen the detrimental effects (hammertoe) of complete disarticulation (method 1), another surgical option, called partial amputation, is sometimes used. In this method, the third phalanx bone is cut at the flexor tubercle. The deleterious sequelae of this method is usually one of two outcomes: one, the flexor tubercle, which is the remaining bone fragment of p3, with its deep digital flexor tendon attached (but now its action is unopposed by the extensor tendon that was attached to the dorsal aspect of p3), is pulled under the second phalanx causing a phenomenon I call "a pebble in the shoe." It forces the animal to walk on a sharp bone shard on already tender paws. The second common sequela of the partial amputation method is that the flexor tubercle is large enough that it remains in its normal anatomical position because the extensor tendon is not cut or the fragment is too big to be moved backward. With the larger fragment left, we very often see claw regrowth under the skin—claw grows from germinal tissue within p3. These fragmented claws and bones are often infected and a source of tremendous pain. Based on Dr. Pelphrey's testimony, he customarily has declawed the Defendants' big cats by

complete or partial amputation. *See* Pelphrey Dep. Tr. 186:16-187:24 (describing taking off the "articular space at the top" of the third phalanx, removing "the end of the third phalanx").




Partial amputation in jaguar with massive infection secondary to nail regrowth

Partial disarticulation in leopard with malpositioned p3 remnants. Pad displacement led to perforation of p2 and subsequent osteomyelitis



c. As mentioned above, the internal portion of the third phalanx has the germinal tissue from which the claw grows. The third method of declawing is to try to ablate the germinal tissue using electrocautery or another method (e.g., vaporizing laser) to destroy the tissue. In my

experience, this method never works and often results in osteomyelitis of the third phalanx, abnormal and infected claw regrowth under the skin, and a tremendous amount of pain in the paws.

d. The fourth method of attempting to render a cat's claws useless is called tendonectomy. This method involves surgical incisions at each toe to cut the flexor tendon so that the third phalanx becomes floppy and is useless to the animal as it no longer can flex the third phalanx. Although this method leaves the claws, it has the potential to cause a lifetime of trouble because the animal can no longer hone its claws and therefore, the claws can grow back into the paw until they penetrate the digital pad causing pathology in the paw.

12. The existing veterinary consensus surrounding the cruelty of declawing big cats was fortified by the AVMA condemning the declawing of these cats in 2013. The AAZV likewise condemns declawing big cats. Indeed, the USDA's Animal Care Program in August 2006 declared "declawing or the removal of canine teeth (fangs) in wild or exotic carnivores . . . is no longer considered to be appropriate veterinary care. . . These procedures are no longer considered to be acceptable when performed solely for handling or husbandry purposes since they can cause considerable pain and discomfort to the animal and may result in chronic health problems." *See* Plt's Ex. 8B (USDA Information Sheet on Declawing and Tooth Removal). Accordingly, the USDA has considered declawing of big cats to violate the AWA. *Id.*; *see also* Plt's Ex. 8A (USDA Animal Care Policy Manual). In my opinion, declawing physically injures the big cats, psychologically harms them, creates a likelihood of further injury to them, and annoys them, by significantly disrupting their normal behavioral patterns. It is not a generally accepted animal husbandry practice, and fails to meet the minimum humane veterinary care and treatment standards. Simply

put, declawing condemns big cats to immediate and severe wounding and a risk of lifelong chronic pain and suffering.

13. Declawing has no benefit for the cat. It is the unnecessary amputation of each distal phalanx (toe bone) in big cats' paws. It is a nontherapeutic, elective surgery and is most-often performed as a misguided management tool, in hopes of "disarming" the cat. Neither the convenience of the owner, nor the monetary capability of the owner to pay for declawing, ever constitutes medical necessity. (In contrast, phalangectomy, the necessary surgical removal of the toe bone to correct anatomical morbidity and pathology in the toe, is done only on an as-needed, per-digit basis. For example, a big cat might present with a nail bed tumor or irreparable traumatic injury to the digit, and removal of the toe bone would benefit the well-being of the cat.)

14. Further, when big cats are declawed for the convenience of the owner and in an attempt to disarm them, as I understand is the case in this litigation, such actions fail, in my opinion, to make human interaction with the animals safe or appropriate. To the contrary, based on my experience, declawing big cats generally makes them *less* safe for human interaction. Declawing these cats often gives the human handler a false sense of security. In my experience, declawed big cats are far more likely to bite or attempt to bite their human handlers. They are more temperamental and less predictable than big cats with intact claws, likely because of consequential complications; they are in intermittent or chronic pain as a result of the ten to eighteen toe bone amputations (ten digits on the front paws and eight on the hind paws). These big cats also suffer as the unnecessary surgery interferes with the animals' normal behavior by removing the animals' primary defense mode and making them resort to biting as their only form of protection.

15. Defendants declaw big cats for their own convenience. It appears from the medical records that the removal of toe bones has never been performed because it is medically necessary

for the animals undergoing the surgery. According to a March 17, 2017, USDA inspection report, Mr. Stark stated that he declaws big cats because he "has money," and "it's easier." Plt's Ex. 8F. As noted above, Mr. Stark has conceded to practices that are both against regulations and in violation of generally accepted husbandry and veterinary practices, including those recognized by the American Veterinary Medical Association, American Association of Zoological Veterinarians, and USDA. In addition, it is well-known in reputable big cat sanctuaries that declawed big cats are more dangerous to work with than clawed big cats. It is often said that if someone has to maim them to tame them, they have no business having big cats. As examples I offer two cases: A declawed tiger is responsible for the killing of a teenage girl, Haley Hilderbrand, in Kansas (2005). According to her father, she was assured that cat was safe to take a photo with because it was declawed. Declawed tiger, Montecore, was responsible for biting magician Roy Horn, of Siegfried & Roy (2003).

16. Tim Stark testified that it is his prerogative to declaw big cats. Stark Depo. Tr. 139:10-15. He reaffirmed what he previously told USDA inspectors, that he declaws big cats because, "it's easier." Stark Dep. Tr. 143:7-14. He testified that big cats living in captivity do not need their claws, Stark Dep. Tr. 144:7-9, though he could point to no learned basis for that opinion. Stark Dep. Tr. 150:7-18. That opinion lacks medical or other support. In truth, big cats, whether in captivity or in the wild, need their toe bones and claws to walk normally and to maintain the structure of their paws. Amputating the distal phalanx changes the way big cats walk and will negatively affect them the rest of their lives. They need their claws to climb, to groom themselves, to grasp objects, to hold onto feeding bones or other enrichment items in captivity. I have seen a declawed big cat struggle to remove food that was stuck to the cat's palate probably because it was unable to hook the meat with a claw; a big cat with claws would have had no trouble pulling the

stuck food out of the mouth. Declawed big cats routinely display what I refer to as a lack of confidence—I can think of no better way to put it.

17. Tim Stark testified that declawing big cats makes it easier to play with them. Stark Dep. Tr. 143:21-145:23. In a video taken November 3, 2016, Tim Stark states that he declaws big cats "not for safety—I just don't like the damn claws. They hurt." PETA-WIN_004710. He adds that big cats weighing 400 pounds can roll and "snag you." PETA-WIN_004711. In my opinion, Tim Stark declaws big cats to benefit himself. It also shows his inability to train the big cats to allow claw trimming. This is accomplished by conditioning the cat to stand against the fence and as the claws come through the chain link, trimming their pointed tips off. This is how I check the claws of my big cat patients.

18. Tim Stark testified that declawing "is not an amputation of anything," Stark Dep. Tr. 150:19-151:3, that this is "a bullshit theory made up be PETA, by your animal rights activists, [and] by Jennifer Conrad," Stark Dep. Tr. 153:10-15, and that cats' claws are "not actually connected" to the bone. Stark Tr. 154:1-9.[4] These beliefs are false. As discussed above, declawing

[4] *See also* M. Stark Tr. 111:4-15 ("Q. Yesterday I asked Tim -- I'm sorry, two days ago, I asked Tim, 'Do you think that taking off a cats claws do not involve amputating a cats toe at the last minute?' He said, 'I don't believe that, no. I think it's [a] theory made up by PETA.' Do you agree with Tim? A. Yeah. All my cats have their toes. I have no cat that's been declawed that is missing a chunk of foot. My toes are all there. If I do a finger print in a casting of clay, my cat's going to look like your cat. Mine is, the claw is all that's missing. The pads, the toes, it's all there."); *id.* at 111:16-24 ("Q. Do you agree with Tim's testimony that declawing is not an actual 'amputation'? A. . . . [O]ur cats . . . and other cats that I've had declawed, I promise you, they all have their toes. There's not a part amputated or missing."); *id.* at 111:25-112:8 ("Q. Two days ago I asked Tim, question, 'Are cats claws connected directly to the bone?' He said, "Are they connected to the bone, no. It's not actually connected.' Do you agree with that? A. I have no clue. All I know is looking on the outside of what I've experienced. My cats all have their toes. It would be like me having my fingernail. My fingernail may not be there, but I still have my finger. But it's a nail. . . . It's not amputated. If I -- if it was amputated, then I wouldn't have my nail at all and then my fingers would not look -- I wouldn't have the tip. My cats are not missing any part of their foot."); *id.* at 112:14-16 ("Q. So to sum up, do you believe that a cat's claws are comparable to fingernails? A. I do.").

a big cat fully or partially amputates the distal phalanges. Whereas, Dr. Pelphrey, the veterinarian who performed the majority of the declawing procedures for Defendants, testified:

Q Does declawing big cats remove the distal phalanx of the digit at the interphalangeal joint?

A It does.

Pelphrey Dep. Tr. 186:13-14. Again, one cannot remove the claw of a big cat without amputating the distal phalanx. This is because the germinal tissues of the claw, the cells that make the claw grow, lie deep within the bone itself. Tim Stark falsely testified that the claw is not connected directly to the bone. Stark Dep. Tr. 154:1-5. However, even he acknowledges that if declawing is an amputation (which it truly is), "It would alter the way you grip, the way you hold, the way you maneuver, everything about it." Tim Stark Dep. Tr. 153:2-6.

19. Tim Stark testified that declawed big cats are not at any disadvantage when housed jointly with big cats whose claws remain intact. Stark Dep. Tr. 154:10-21. In my experience, this is false. Big cats know when they are missing their claws, and accordingly they do not defend themselves by swiping. A big cat's ability to swipe is its primary defense mode; swiping allows a big cat to keep another big cat away, at arm's length. When the claws are removed, the big cat's defense mechanism is to resort to biting, which means the animal's head has to go in toward the aggressor big cat, thereby putting the declawed big cat at a clear disadvantage by making the animal's body, including the head and neck, in close proximity to the aggressor, and therefore becoming more vulnerable to serious injury. Additionally, big cats mask pain, including the pain that results from the morbid sequelae of declawing. Mr. Stark refers to this fact multiple times, Stark Tr. 198:23-199:2,) but the same time he is adamant that his cats are not in pain. Stark Tr. 200:3-4 ("There's not a damn thing wrong with them. They still function."). In my experience, big

cats are very hard to read, but I have concluded that they were in pain after I took them out of pain and saw the difference in the animal. In addition, such masking of pain increases stress levels. Big cats with increased stress levels will demonstrate a great difference in what I refer to as their confidence level, and can become submissive to aggressor big cats. In addition, stress aggravates pain perception and then, in turn, increased pain perception aggravates stress. This escalating cycle can continue unabated.

20. Tim Stark contradicts his position that declawed big cats are not at any disadvantage when housed jointly with big cats whose claws remain intact when he testifies that the declawed animals know when they are housed with a clawed big cat and adjust their behavior accordingly. Stark Dep. Tr. 154:22-155:9. He gives as an example a declawed tiger who he has housed and bred with two separate female tigers, both of whom had claws. *Id.* Starks says, "He knows they have claws. So pretty much it's simple. Happy wife, happy life. He ain't stupid enough to go over and stir up shit with her because she's got claws." *Id.* This demonstrates that declawed big cats adjust their behavior around, and are at a disadvantage with, clawed big cats, and that Tim Stark knows it. Declawed big cats are likely less secure, more submissive, (or possibly inappropriately aggressive to overcompensate), and suffer more stress than cats whose claws remain intact.

21. Declawing big cats is an irreversible procedure that permanently removes all or part of the distal phalanx and severs nerves, ligaments, tendons, and blood vessels. There is no surgical procedure that can restore what has been amputated from declawed paws. The animals will never have the full, normal function in their paws as they would with intact claws, and years of abnormal function may have caused, and likely will cause, irreversible arthritic changes and/or chronic pain.

22. Since the year 2000, I and other veterinarians, working with the Paw Project, have performed declaw revision surgery on over 70 lions, tigers, cougars, leopards, a jaguar, and

multiple smaller wild and exotic cats who had been victims of amputations. Following reparative surgery, declawed big cats, who before could only hobble after a few agonizing steps, regain some of their ability to leap, run, and play. In cases where part of the distal phalanx remains, the partially amputated bone is exposed during surgery, the infected tissue and nail remnants are cleaned out (debrided), and the fragment is then grasped with surgical clamps to mobilize the deep digital flexor tendon. The fragment is removed, and a heavy suture is placed in the remaining digital flexor tendon and attached into the extensor tendon on the top surface or periosteum of the second phalanx (p2). Before the suture is secured, any cartilage remaining on the distal end of the second phalanx is removed and the end of the bone is recontoured. Tightening the suture will reposition the pad nearer to its proper anatomical position. The incision is closed, and pressure wrap bandages are placed over the digits up to the carpus or tarsus. In cases where the third phalanx has been completely amputated, the surgical technique is similar except that the tendons may be more difficult to find. The second phalanx, often in a hammertoe position, is recontoured and the pad is repositioned as described above. If the cat had all four feet declawed, which is often the case, including in this litigation, two to four separate surgical procedures are required. It is considered unsafe to subject a big cat to general anesthesia for the amount of time required to treat all eighteen toes. The reparative surgery takes up to forty minutes per toe, and a six-hour surgery to repair two feet is not uncommon. The front feet usually are repaired first, often one at a time. The procedure is costly and cannot fully restore a big cat to his or her normal condition. I presented a paper, Jennifer Conrad, et al., *Deleterious Effects of Onychectomy (Declawing) in Exotic Felids and a Reparative Surgical Technique: A Preliminary Report*, describing a reparative surgical technique for declawed big cats at the annual meeting of the American Association of Zoological Veterinarians on October 7, 2002. This paper was the basis by which both the AVMA and the

Canadian VMA came out against declawing of wild and exotic cats. It is also the basis for the State of California banning declawing of wild and exotic cats in 2005.

23. Defendants' veterinarian admitted that he did not give big cats pain medication after they were declawed. Pelphrey Dep. Tr. 139:10-141:23. In my opinion, this constitutes a gross failure to meet the accepted standards of medical care, in which post-operative pain medication is provided, even more so in a surgery recognized as causing severe pain. By failing to give pain medication following declawing, one of the most painful procedures known to veterinary medicine, Defendants' veterinarian likely has fallen below the standard of care and, in my opinion, should face admonishment or discipline by the governing veterinary board(s). The failure to provide pre-peri-post-operative pain medication exposes the animal to a potential for neuropathic pain, pain from cutting the nerves and not even attempting to prevent the permanent pain that will cause. Setting the animal up for neuropathic pain, often what causes phantom pain as described by amputees, is another permanent complication from declawing that also harms and harasses the big cats and is below accepted standards of veterinary medical care.

24. Dr. Pelphrey also describes that he used only ketamine and xylazine as anesthesia. Pelphrey Dep. Tr. 141:24-142:16. This is woefully inadequate for such a painful procedure as declaw surgery. Ketamine works as a dissociative drug and is related to PCP. Dissociatives work by making the patient feel disconnected from the body. Xylazine is a drug that causes vomiting and hypotension. It, too, does little for preventing pain. This drug combination is known to cause significant cardiovascular depression within minutes of intramuscular injection. Cardiac output, heart rate, stroke volume, systolic, diastolic, and mean-arterial blood pressure are all decreased in felids with this drug combination. Xylazine itself is a powerful emetic and it seems to make the animals feel "seasick." When used in combination with ketamine, it imposes a significant danger

of causing vomiting while the cat has lost its ability to control its epiglottis (swallow reflex) and this increases the chances of aspirating stomach contents into the lungs. This threat is further exacerbated by the lack of an endotracheal tube, a tube inserted into the trachea that blocks particles of food or liquid from entering the lungs. This tube also acts as a secure way to make sure the lungs are getting oxygen. Because the cat is under heavy sedation/light anesthesia, the cat can't protect its airway nor can it cough to try to clear its lungs from the stomach contents. Dr. Pelphrey chooses not to intubate these cats so he has no way to protect their airways when/if they begin to vomit. He has no way to administer oxygen if the animals slow their breathing rate so much that they are in danger of asphyxiating or becoming anoxic. He has no oxygen tanks or anesthetic gas machine with him either. This is below standard of care in my opinion. I understand that the argument may be made that short (5 min), mildly pain-inducing, routine, simple, and necessary procedures in domestic cats might be performed without intubating. The difference here is that declawing is well-known as causing severe pain, it is not a simple, short procedure, and Dr. Pelphrey is unequipped with an emergency kit including endotracheal tubes or rescue drugs. In addition, the use of xylazine, a powerful emetic, and ketamine would not be considered adequate anesthetic medications for even domestic cats undergoing a short, routine procedure. This combination can induce emesis, gastric reflux, hypersalivation, all which threaten the unprotected airway of the patient. Arguments for not intubating cats are no longer valid due to advances in medicine. Standard practice for a highly-invasive surgery, in my opinion, would involve gas anesthesia, intubation, a constant rate infusion to decrease the probability of creating chronic pain, or wind up, and pain medications before, during, and after the surgery, and this would be for a necessary surgery. Unnecessary and nontherapeutic surgeries should simply not be performed.

25. Dr. Pelphrey does not have a way to monitor the blood chemistries in his tiny patients. He has no catheter and cannot/does not therefore assess if they are becoming hypoglycemic (low blood sugar), a common occurrence in young animals, especially ones who have been recently fasted to undergo this eighteen-toe amputation surgery. Intravenous access via a catheter would allow better monitoring and access would allow for rapid correction of cardiovascular crises like cardiac arrhythmias, hypotension, and metabolic acidosis. All of these can significantly increase mortality during surgery, and they can affect the outcome of the surgery. This is below standard of care.

26. Dr. Pelphrey does not use any objective monitoring equipment for an elective surgery. The primary goal of monitoring equipment is to assess the level of anesthesia in a patient. It is important to know how the cardiovascular system is responding to the anesthesia. He chooses not to use a pulse oximeter that would tell him how well the babies are oxygenating their tissues, meaning $O_2$ saturation in peripheral tissues, or a capnometer, which measures $CO_2$ in the exhalation to tell him if they are hypoventilating and not perfusing their lungs to the extent they should be. This is below standard of care. These are simple, inexpensive machines that are often portable.

27. Dr. Pelphrey does not have an objective way of monitoring the heart. An ECG reading would allow him to judge his patients' plane of anesthesia and know whether or not they could feel the pain he was causing them or if they were in the correct plane, deep enough not to feel the amputations while they were happening. He does not monitor blood pressure, a necessary indicator to assure the kidneys, and other critical organs are getting the blood they need. The injury to these organs might manifest later on in life, affecting well-being or even contributing to premature death.

28. Dr. Pelphrey makes no mention of having emergency drugs or even the xylazine reversal agent, yohimbine, ready for the patients. He makes no mention of having fluids, catheters, IV sets there in the event of cardiovascular collapse. If they are not there, he is helpless to rescue a crashing cub and that is below the standard of care.

29. Dr. Pelphrey makes no mention of monitoring his patients' body temperatures. Baby animals can lose heat very quickly because their surface area to mass ratio is still very high, meaning they have a lot of surface to lose heat from while their mass, or what keeps their internal temperature warm, is very low.

30. Dr. Pelphrey testifies that he gave these baby big cats a long-acting corticosteroid as an anti-inflammatory after declawing them. Pelphrey Dep. Tr. 139:3-9. This medication is similar to the stress hormone, cortisol, and usually lasts several weeks in the animals' bodies. It is known to decrease the animals' ability to mount an immune response, while delaying the animals' ability to heal. This combination of side effects might set the animals up for contracting dermatophytosis (ringworm) and for extended chronicity of their open wounds. They also might not be able to fight bacterial infections that could be in their open surgical sites. Long-acting corticosteroids can decrease immune health, and can cause gastric ulcers (especially in fasted or stressed animals), kidney injury (a high risk for patients recovering from anesthesia where renal profusion is poor and hypotension can go undetected), and decreases the tissue's ability to heal. They do not provide the appropriate type of pain management needed in these surgeries. If there were an incident of aspiration into the lungs, the body cannot fight it very well with these steroids limiting immune response. This is below the standard of care.

31. WIN personnel with responsibility for big cats, Jessica Amin and Max Strong, both admit that they have no medical training and yet they are left to manage surgery cases and medical

cases including the three-week-old cubs who just had a major surgery, were not given adequate pain control, and had their recently amputated paws wrapped in tight bandages. Amin Dep. Tr. at 34:19-35:2; 79:4-6; 144:1-15; 151:21-152:2; 156:10-22; 174:14-25; Strong Dep. Tr. at 15:3-17:8.

32. Dr. Pelphrey compares declaw surgery to circumcision. Pelphrey Dep. Tr. 139:10-25. This is inapt. This case has to do with big cats and declaw surgery is eighteen separate toe bone amputations and is widely recognized as causing severe pain in the cat. In addition, cats must walk the rest of their lives on their amputation sites.

33. To my knowledge, there is no evidence or literature that supports the proposition that laser declawing procedures are pain-free or disability-free, or that post-operative pain medications are not necessary for declawing, whether or not a laser is used in the procedure. Laser declawing is still an amputation of all or part of the third phalanx bone. I agree with USDA inspectors that post-operative pain management is critical for the well-being of big cats, *see* Plt's Ex. 8E, regardless of the method used in declawing. Laser declawing risks fourth-degree burns (burning of the bone) to the second phalanx, which not only causes acute pain but may result in osteomyelitis, or necrotic bone tissue. Furthermore, the short- and long-term complications of declawing may be observed in big cats declawed by any method.

34. According to Dr. Pelphrey, he declawed multiple big cats for the defendants using a scalpel or a guillotine; he testified that he did not use a laser. Pelphrey Dep. Tr. 136:21-138:20. Mr. Stark testified that he has had big cats declawed by laser as well, by another veterinarian. TRO Hr'g Tr. 28:25-29:2.

35. According to USDA inspectors, at least twenty wild felines on Defendants' premises at the time of the March 17, 2017, inspection, including many of the big cats at issue in this litigation, had been declawed. Plt's Ex. 8F. These animals included weeks-old lion and tiger

cubs, juvenile tigers and lion-tiger hybrids, two adult tigers, and other wild felines. *Id.* The vast majority of the big cats have been declawed within the last four years while under the care of and at the direction of Defendants. *Id.* Dr. Pelphrey testified that he declawed ten or twelve big cats for the Defendants. Pelphrey Dep. Tr. 196:8-197:4. Dr. McDonald declawed a further five big cats in the summer of 2017, well after the USDA directed Mr. Stark to stop declawing big cats. PI Hr'g Tr. 6:25-7:23. To his credit, Dr. McDonald testified that he was wrong to declaw big cats in the first place for the Defendants, that he had failed to research the issue before doing it, and that he had no intention of doing it again. PI Hr'g Tr. 7:24-8:23.

36. During the March 17, 2017, inspection, the USDA noted one orange and one white tiger cub, then approximately five or six weeks of age, who had been declawed approximately two weeks earlier. Plt's Ex. 8E. According to the inspection reports, Mr. Stark attempted to conceal these cubs from the USDA inspectors. Plt's Ex. 8E, 8F. The reports state that the two tiger cubs were brought outside to a deck in a crate that was approximately 24 inches long by 18 inches wide. Plt's Ex. 8E, 8F. Neither animal would walk from the crate onto the wooden deck for inspection, and they had to be physically removed from the crate. Plt's Ex. 8E. Each cub had one leg that was bandaged and Mr. Stark told inspectors that there were open wounds under the bandages. *Id.* Their affected paws were significantly swollen, spotting blood, and the cubs were struggling to walk, appearing very sore. *Id.* These descriptions by the USDA inspectors are consistent with photos and videos of these two tiger cubs that I have reviewed, discussed below. They also appear to be consistent with video that I reviewed, taken by Defendants during the inspection and provided to PETA in this litigation, a file named 3-17-17.MP4.

37. Both tiger cubs appeared distressed, vocalizing nearly the entire time they were on the deck. Plt's Ex. 8E; 3-17-17.MP4. The orange tiger cub immediately lied down on the deck and

then, after persuasion, moved slowly for only short periods of time before resting in front of the inspectors. Plt's Ex. 8E; 3-17-17.MP4. After each step, there were spots of blood left on the deck from the front paws. Plt's Ex. 8E; 3-17-17.MP4. The white tiger cub was very reluctant to move, walking only when prompted, and exhibiting severe lameness, dragging a hind limb and only occasionally bearing very little weight on it. Plt's Ex. 8E; 3-17-17.MP4. This cub consistently lied down and appeared to be suffering throughout the inspection. Plt's Ex. 8E; 3-17-17.MP4. Mr. Stark told the inspectors that the declawing of these cubs was "botched," and that he concealed the cubs from the inspectors because he was afraid that he would get in trouble, according to the USDA inspection reports. Plt's Ex. 8E, 8F. According to Dr. Pelphrey, these cats might be considered lame or unable to walk because they are young, and they might want to just lie down because they had just been fed. Pelphrey Dep. Tr. 180:17-181:17. This seems highly unlikely to me. Moreover, Dr. Pelphrey was not present at the inspection and admits he has no evidence to support his position that the white tiger cub was not severely lame. Pelphrey Dep. Tr. 181:12-17. The inspectors, as I read it, are describing serious pain and generalized malaise. My experience with cubs of this age is that they play and wrestle and sleep, but that 5-6 week-old cubs are coordinated enough that no experienced veterinarian would interpret their gait as lameness unless it was really lameness.

38. According to the March 17, 2017, USDA inspection report, the big cats are declawed while on Wildlife in Need premises, rather than at a dedicated veterinary surgical site. Plt's Ex. 8E. No big cat receives pain medication following the amputations because, as Mr. Stark told the USDA inspector, he does not believe that the animals are in pain. *Id.* Mr. Stark did not provide inspectors with records of pain management or antibiotics, or any written post-operative care. *Id.* These facts are consistent with Dr. Pelphrey's testimony that he declawed Defendants' big cats at their facility in a non-sterile room, and that he does not prescribe post-operative pain

medication following the declaw procedures. Pelphrey Dep. Tr. 193:15-194:14; 195:13-196:7. In my opinion, declawing procedures should never be done, but any amputation surgery, especially one as invasive as declawing is, should be performed in a dedicated surgical suite to reduce the risk of infection, have proper monitoring equipment, and proper anesthesia equipment, including oxygen tanks. Big cats who have undergone the declawing procedure surely experience severe pain following the eighteen separate amputations and the standard of care requires that they be administered long-term, proper post-operative pain medication. Baby animals do feel pain and in fact, they might feel pain more profoundly than older individuals because baby animals' developing neurons have not yet learned to modulate pain and therefore cannot dampen the extreme pain of amputating eighteen toes with a guillotine that most likely crushes/cuts the third phalanx mid bone. Pain experienced in pediatric patients may alter the way the body perceives pain and can lead to chronic pain throughout a lifetime. When a nerve is cut, its viable end will sprout out like a cauliflower looking to reattach itself. This cauliflower is called a neuroma. Neuromas are implicated in chronic, intractable pain and even phantom pain, as described by amputees.

39. The tiger and lion cubs discussed by the USDA in the March 17, 2017 inspection report surely would have been in pain—and did in fact display behaviors commonly associated with pain, such as avoiding putting weight on paws, lameness, hesitancy to walk, and the other behaviors noted by inspectors—in the weeks following the declawing procedures. In fact, the open wounds to the orange and white tigers' legs still had not healed by the date of the inspection and would have been a source of excruciating pain to these cubs.

40. According to the Defendants' records provided to PETA in this litigation, which I have reviewed, the orange and white baby tigers discussed above—those observed by USDA

inspectors on March 17, 2017—were born at the Defendants' facility on February 10, 2017. Dr. Pelphrey declawed them on March 3, 2017, at just three weeks old. The USDA inspection occurred two weeks later, at five weeks old. PETA's confidential informant, whose report I reviewed, took photographs of the declawed orange and white tiger cubs on March 7, 2017, four days after the declawing procedures, and videos on March 10 and March 17, 2017.

41. Pictures of the white tiger cub taken March 7, 2017, reveal that the declawing procedure or the aftercare caused substantial swelling to the left hind paw and leg, with the pads under each toe enlarged and spreading much farther apart than normal. PETA-WIN_000093-98. Additionally, there appears to be a lesion to the left leg, that may have been caused by a bandage wrapped too tightly or by a tourniquet placed to stop blood flow during the procedure. *Id.*

42. A picture of the white and orange tiger cubs together, taken March 7, 2017, reveals that the orange tiger cub's front left leg and paw are substantially swollen following the declaw procedure. PETA-WIN_000099. Additionally, there is a patch of hairlessness on the orange tiger cub's front left leg. *Id.*

43. A video of the orange tiger cub, taken March 10, 2017, reveals that the wound to the front left leg is full-thickness and severe. PETA-WIN_000100. In the video, the cub licks her open wound, which is deep enough that I can see fatty tissue, tendons, and ligaments. *Id.* The wound is open while the cub lies on shag carpet, exposing the wound and deep tissues to potential life-threatening infection. *Id.*

44. Another video of the orange tiger cub, taken March 10, 2017, shows the cub from behind, struggling to walk on all fours and heavily favoring the front left leg and paw, putting little weight on the limb. PETA-WIN_000101. The cub takes only a few tentative steps before lying down. *Id.*

45. Two videos of the orange tiger cub, taken March 10, 2017, show the orange tiger cub from the front, struggling to walk, and folding the front left paw underneath the leg possibly in an attempt not to put weight on the toes or to stress the open wound on the leg. PETA-WIN_000102-103. After a few short steps, the cub lies down, revealing damage to the pads under each toe from the declawing procedure. PETA-WIN_000103.

46. Another video of the orange tiger cub, taken March 17, 2017, shows the orange tiger cub from behind, now bandaged on the front left leg, again struggling to walk on all fours and taking only a few tentative steps before lying down. PETA-WIN_000104.

47. The orange and white tiger cubs, in my opinion, required emergency surgical intervention to repair the wounds to their legs and paws. According to Defendants' records and Dr. Pelphrey's testimony, this was never done. USDA inspectors attempted to return to Defendants' facility two weeks later to check on the tiger cubs, but Tim Stark met them at the gate to the property, visibly wearing a holstered sidearm, and refused the inspection, itself a violation of AWA regulations. PETA-WIN_002589. The USDA cited Tim Stark for this incident. *Id.* I have reviewed their report and video of the incident, posted by Tim Stark on Facebook, and I agree with the report as written; USDA inspectors felt that Tim Stark intended to intimidate them, and I agree that a reasonable person would have been intimidated by his conduct during the attempted inspection.

48. Additional big cat cubs possessed by Defendants have been declawed, including two lion cubs exhibited during public encounters throughout April, 2017, who were also examined during, and discussed by, the March 17, 2017 inspection reports. Plt's Ex. 8E, 8F.

49. According to Defendants' records, these two lion cubs were donated from "Beth Corley" to Tim Stark on February 7, 2017. According to deposition testimony in this litigation,

Beth Corley is a USDA licensee who was unaware of these transfers; in truth, Joe Maldonado-Passage transferred big cats on and off her license without her knowledge. Maldonado Dep. Tr. 17:3-16; 73:20-24; 89:11-14; Lowe Dep. Tr. 69:23-70:5; 71:19-75:17. Indeed, the Defendants' own animals-on-hand paperwork denotes that these lion cubs actually came from Mr. Maldonado-Passage. According to Defendants' records, Dr. Pelphrey declawed these two lion cubs on February 17, 2017. The Defendants used the two lion cubs extensively in Spring 2017 Tiger Baby Playtime events; they appear in dozens of videos, which I reviewed, taken by attendees at those events. According to records provided by Defendants in this litigation, these lion cubs are named Nera and Mauri.

50. PETA's confidential informant took photographs of Nera and Mauri on February 28, 2018, less than two weeks after they were declawed, and March 2, 2017. The photographs show open wounds on the lions, while they lie without bandages on a shag carpet. PETA-WIN_000072-74; PETA-WIN_000090-92. The photos at PETA-WIN_000072-73 depict a declawed lion cub, either Nera or Mauri, with significant swelling to the front left paw and leg. The declawing wounds are consistent with the partial disarticulation technique described by Dr. Pelphrey, and appear to have been caused by a guillotine, which would have been used to basically crush the tissue and bone. PETA-WIN_000072-74. The photo at PETA-WIN_000072 reveals a particularly severe wound to the pad of a toe on the front left paw, caused by Dr. Pelphrey's method of partial disarticulation, approached from the articular space at the top of the distal phalanx. The photo at PETA-WIN_000088 reveals similar wounds to two toes on a rear paw of one of these lion cubs, with similarly severe damage to the pads. The photos at PETA-WIN_000089-90 reveals that the soft tissue around the distal phalanx of toes on the front right paw of one of these lion cubs likewise has been crushed, resulting in wounds that still were open when the photos were taken on

March 2, 2017. Likewise, the photos at PETA-WIN_000091-92 reveal open wounds to the toes of the rear paws of this lion cub, with damage to the pads, caused by the declawing.

51. Defendants used Nera and Mauri in Tiger Baby Playtime events throughout Spring 2017. I reviewed dozens of videos featuring these two lion cubs, among other big cats, including tiger and hybrid cubs, in Tiger Baby Playtime events in Fall 2016 and Spring 2017. PETA-WIN_000114-405; PETA-WIN_000443-471; PETA-WIN_000507-605; PETA-WIN_000622-001188; PETA-WIN_001204-001438. WIN staffers or volunteers confirmed during some of these Tiger Baby Playtime events that Nera and Mauri were declawed. PETA-WIN_000815; PETA-WIN_000964; PETA-WIN_001386; PETA-WIN_001392. According to a WIN staffer or volunteer, big cats that are to stay at WIN and be used for Tiger Baby Playtime events are declawed, while big cats who are to be sent elsewhere keep their claws. PETA-WIN_000954.

52. Complications from declawing may cause death, as admitted by Wildlife in Need's veterinarian, Dr. Pelphrey, who stated during the March 17, 2017, USDA inspection that one of the tiger cubs had a fifty percent chance of dying from complications resulting from the procedure. Plt's Ex. 8F. Indeed, the white and orange tigers declawed in March 2017 never recovered from the wounds inflicted by the declawing surgeries and after care before their deaths in May of 2017, according to the Defendants' medical treatment logs provided in this litigation and Dr. Pelphrey's testimony. Pelphrey Dep. Tr. 256:22-25. Defendants' medical treatment logs indicate that both tiger cubs were treated with Sea-Clens wound cleanser for their paws in the days and weeks prior to their deaths. Dr. Pelphrey asserts that the wounds to the tigers' legs resulted from a bandage wrap by the brand name Animalintex, which he prescribes for horses. Pelphrey Dep. Tr. 137:13-22; 160:11-166:19. Dr. Pelphrey is a race horse veterinarian who testified that he spends less than one percent of his practice administering to Defendants' big cats. Pelphrey Dep. Tr. 47:25-48:10;

79:16-80:7. Had he reviewed any literature on Animalintex before merely applying a treatment for horses to felids, including the product's website, https://www.3m.com/3M/en_US/company-us/all-3m-products/~/3M-Animalintex-Poultice/, he would have seen that it is not recommended for use in cats. In fact, the main active ingredients in Animalintex are boric acid as an antiseptic and tragacanth as a poultice agent: both these ingredients are toxic to cats. Dr. Pelphrey blames his application of Animalintex for swelling and for tissue on these tigers' legs turning necrotic and sloughing off. This application of a horse treatment to felids is wholly inappropriate. In treating exotic animals, it is often true that a veterinarian has to extrapolate doses and treatments. We generally look at the animal's teeth, digestive system, and feet to decide which domestic animal to extrapolate from. For instance, to treat a giraffe, one would see that this animal has grinding teeth, multiple stomachs, and two toes, therefore it would be best to consult cow textbooks on appropriate medications for giraffes. After that, it would be a good idea to ask for help from veterinarians who also treat giraffes. A rhinoceros has three toes, a single stomach, and grazing teeth, therefore it is like a horse, a well-described odd-toed ungulate. Horse medications and treatments are generally acceptable for rhinos. In this case, Dr. Pelphrey did not do his due diligence in relating big cats to their closest domestic, and very well-described cousins, the domestic cats; instead he chose to treat them as horses, an animal whose feet, digestive system, and teeth are wholly unlike a cat's. These complications he inflicted on these big cats were avoidable and the result of lack of consulting before treating. After losing patients, he acknowledges his lack of expertise in a text message to Defendant Melisa Stark, writing, "I wish I was more help. . . . If you guys had horses I could do better work for you." Defendants' Second Amended Production re. 1-134, at 36.

53. According to Defendants' medical treatment logs, multiple big cats at the facility suffered from ringworm (dermatophyte infection), apparently recurrently and probably from re-

infecting each other or getting re-infected from the uncleaned environment, and at least four big cats seem to have died from declawing complications or from being given an improper type and dose of medicine to treat ringworm. There are no necropsy reports to confirm this diagnosis despite Dr. Pelphrey recognizing that the deaths were unexpected. The two tiger cubs discussed above, who were declawed in March, 2017, and died in May, 2017, suffered from diffuse ringworm infections before they died. It is highly likely that ringworm, which is an opportunistic infection, breached the skin barrier via the open wounds that remained from the declawing procedure at the time the infections began, and which never fully healed prior to the tigers' deaths. The infection might have been more virulent because the cubs had been immunosuppressed by the long-acting corticosteroid injection Dr. Pelphrey gave them immediately post-declawing. Pelphrey Dep. Tr. 139:3-9. Ringworm must be timely diagnosed and properly treated; if treated properly, it is highly unlikely to be fatal. Any animal with ringworm should be quarantined from other non-infected animals and from the public until the condition is resolved. Ringworm is highly contagious, both between animals and between animals and humans. Exhibiting big cats with direct contact with members of the public, as appears to have happened throughout Spring, 2017, based on these treatment logs and the photographs and videos I reviewed, put the public at high risk of zoonotic fungal infection.

54. I am gravely concerned that Defendants are unable to control and properly treat such outbreaks, and that the risks presented by ringworm may be further increased in big cats with open wounds and physiological stress from declawing. Ringworm is transmitted through open wounds or anomalies in the skin barrier. Ringworm is easily transmitted from an infected animal to a human who has a paper cut, mosquito bite, or other common break in the skin. No animal presenting with ringworm should be subjected to elective surgery until the condition has cleared.

Ringworm is often found in the animal's toes and therefore, can be spread by the declawing instruments. Dr. Pelphrey admits that he only uses cold sterilization (chlorhexidine) in between amputation surgeries. Pelphrey Dep. Tr. 194:25-195:2. While, in my opinion, this is below the standard of care for any major surgery, it is also inadequate sterilization because chlorhexidine by itself does not kill ringworm.

55. Dr. Pelphrey chose to treat these animals with ketoconazole and griseofulvin, two medications that are not recommended for cats. According to Plumb's Veterinary Drug Handbook, a formulary widely in use by veterinarians, ketoconazole use "is controversial and some clinicians recommend that ketoconazole not be used in cats because of its toxic potential. . . . Gastrointestinal effects, e.g., lack of appetite, vomiting, are the most likely side effects seen, especially in cats. . . . Gastrointestinal signs of anorexia, vomiting, and/or diarrhea are the most common adverse effects seen with ketoconazole therapy and are more prevalent in cats." Plumb's Veterinary Drug Handbook, "Ketoconazole" (9th ed. 2018). Griseofulvin, as a treatment for ringworm, is "extra-label, not recommended for use in cats due to potential for serious adverse effects. . . . Griseofulvin can cause anorexia, vomiting, diarrhea, anemia . . . . Cats, particularly kittens, may be more susceptible to adverse effects, e.g., bone marrow depression, than other species." Plumb's Veterinary Drug Handbook, "Griseofulvin" (9th ed. 2018). Mr. Stark says that he doesn't make decisions on what drugs to use, Tim Stark Dep. Tr. 208:7-18, but then remarks that Dr. Pelphrey is under Tim Stark's "supervison." Tim Stark Dep. Tr. 245:22-246:2. Ringworm, in felids, is treated with a medication called itraconazole. I would not prescribe ketoconazole or griseofulvin for big cats because of the potential for serious adverse effects in felids. Dr. Pelphrey testified that he does not recall consulting a drug formulary before prescribing griseofulvin off-label for the Defendants' big cats. Pelphrey Dep. Tr. 266:16-18. He did not consult a drug formulary before

prescribing ketoconazole for the Defendants' big cats, only consulting a formulary after the fact of their deaths. Pelphrey Dep. Tr. 271:2-272:11.

56. I reviewed video footage of the site inspection conducted at WIN as part of the discovery process in this litigation. At seven minutes (00:07:00) in this footage, there are two African lionesses and an African lion, Chief, in one enclosure and a liger in another. The lionesses appear declawed, though the lion, who is pacing and has an abnormal conformation, has his claws. After reviewing this footage, I reviewed Tim Stark's deposition transcript; he testifies that the two lionesses are named Nera and Mauri and they are indeed declawed, and that the lion Chief is not declawed. Tim Stark Dep. Tr. 189:7-10. Defendants house Chief together with Nera and Mauri in hopes that he might breed with them. Tim Stark Dep. Tr. 189:11-20. In my opinion, housing a clawed male with declawed females for breeding puts the females at a physical disadvantage. They will be less capable of fighting off unwanted mounting behavior because declawed.

57. At 00:11:22 in the video, the white lioness SnowLei's left front digital pads appear abnormal, likely from having been declawed. Indeed, after viewing this footage, I was able to confirm by inspecting Defendants' records produced in this litigation that SnowLei was declawed by Dr. McDonald. At 00:12:07 and 00:16:30, the white lioness is holding her left ear down and shaking her head as if she is in discomfort. SnowLei again is seen holding down her left ear and shaking her head at 02:27. At 02:29, I observe that she has pad atrophy, likely from having been declawed, and a callus formation of the right D4 toe pad. Dr. McDonald also declawed Mako. At 02:23, I observed Mako with a boomer ball stuck in his teeth. He struggles to remove the ball, an action that would be a simpler matter if he still had his claws.

58. At 00:21:19, a hybrid big cat, HeDaBomb, appears to have been declawed, from an abnormal pad shape. This is evident again beginning at 00:56:32. After viewing this site inspection

footage, I reviewed Defendants' records to confirm that in fact HeDaBomb was declawed. HeDaBomb appears hesitant to jump on top of his den box and also hesitant to lie down on top of it.

59. At 01:08:37 in the site inspection footage, there is a lioness, Mauri, with atrophied pads of the rear paws. This is a permanent injury resulting from being declawed. The pad atrophy I observe here is consistent with the photographs I observed of Mauri in the days and weeks following the declaw procedure. In fact, the pad atrophy represents a further—and an expected—deleterious effect of the declawing. As big cats walk on their pads, the atrophy will cause gait abnormalities that may further lead to arthritis or other future harms and certainly is causing this lioness present pain. At 01:12:35, I observe pad atrophy in the rear paws of the other lioness in this same enclosure, Nera. At 01:15:18, Mauri is seen limping on her right front leg, in an attempt to avoid putting weight on the limb and causing it pain. This, too, is a deleterious effect of the declawing, indicating ongoing pain. Beginning at 01:18:20, I observe hyperextension of the carpus (wrist) in the front right leg on Mauri. This is likely a result of an unwillingness to bear weight on the pads of the front right paw because of the declawing. Hyperextension occurs because of an inability to bear weight on the toes, which is the natural anatomical position for walking, so the animal rolls back off the toes, causing the supporting ligaments to become strained, weakened, and stretched. The ligament is no longer able to maintain a proper position. At 01:18:41, Mauri is unable to bear weight on the front right limb as she lies down.

60. At 02:14:08, I observed the tiger named Hurricane with dry and abnormally shaped pads on his paws; he appears to be declawed. There are no medical records on Hurricane that I might reference to confirm the declawing. Hurricane is lying on gravel near a pile of what looks like old feces.

61. At 02:37, the hybrid big cats Amitola and Adamma, who were both declawed by Dr. Pelphrey, are housed jointly with Bennett, who is not declawed. As discussed above, jointly housing declawed and clawed big cats puts the declawed big cats at a physical and psychological disadvantage. At 02:41, the water receptacle for these big cats is empty. Of course, water should be readily available at all times.

62. At 02:46, the hybrid big cat Kahari and the other tiger, Tabby, both declawed by Dr. Pelphrey, are housed jointly with the hybrids Nafasi and Kubwa, who have their claws. Again, the declawed big cats in this enclosure, which is too small to house four adult big cats and lacks spaces to retreat other than a single den box, are at a disadvantage compared to the big cats who have intact claws. This enclosure, like most of the others, has a gravel substrate often called DG for decomposed granite. There is feces and parts of prey animals throughout; the gravel is likely too difficult to keep properly clean. At 02:51, Tabby has feces in the fur; this is something that big cats typically do not permit to occur. At 02:52, this enclosure contains animal blood and feces in multiple places.

63. According to a spreadsheet prepared by Defendants in response to the Plaintiff's discovery requests in this matter, an orange male tiger was born at the Defendants' facility on April 1, 2016 and declawed by Dr. Pelphrey on April 17, 2016. Source: filename "Big Cats- updated 2.19." Defendants used this tiger in their "Tiger Baby Playtime" sessions in 2016. *Id.* They then transferred him to Joseph Maldonado-Passage on February 8, 2017. *Id.*; Defs' Second Amended Prod. at 154. Maldonado-Passage produced to the Plaintiff an inventory, which demonstrates that the orange male tiger was transferred on December 4, 2017 to The Wild Animal Sanctuary. PETA informs me that it facilitated this transfer to The Wild Animal Sanctuary. Its counsel in this matter, along with its expert witness, Jay Pratte, visited the orange male tiger at The Wild Animal

Sanctuary and a videographer shot footage of that visit, which I have reviewed. PETA-WIN_008041. The Wild Animal Sanctuary has named this declawed orange male tiger Larry. By my observations, Dr. Pelphrey and the Defendants declawed Larry's every digit on all four paws, as they commonly did for each big cat they declawed. This has led to gait and standing conformation abnormalities; Larry is presently flat footed, suffering from hyperextension of the carpus as a likely result of the declawing.

64. Tacova, a male tiger transferred from Jeff Lowe to Tim Stark to Joseph Maldonado-Passage, and ultimately to the Wild Animal Sanctuary, since renamed Thomas, might be suffering from metabolic bone disease, which is generally indicative of poor nutrition at an early age. He has an unkempt coat of hair, is small of stature, and has alopecia. He is duck footed: the ends of his feet are angled outward as he walks, though his claws are intact. If I were his veterinarian, I would seek to rule out metabolic bone disease as a possible explanation for his symptoms.

65. It is my professional opinion, to a reasonable degree of scientific certainty, that declawing physically injures the big cats, psychologically harms them, creates a likelihood of further injury to them, and annoys them, by significantly disrupting their normal behavioral patterns. Additionally, Defendants have actually killed big cats by declawing them and providing wound care far below the standard of care.

**Opinions on Premature Maternal Separation and Exhibition**

66. Wildlife in Need routinely exhibits big cat cubs who have been permanently and prematurely separated from their mothers at its Tiger Baby Playtime events, charging an admission fee and an optional additional photo opportunity fee, and bringing big cat cubs into direct contact with the public, including young children. Using big cat cubs in public-handling sessions such as these contravenes generally accepted husbandry practices and exposes the big cats to constant

stress and the risks of zoonotic disease transfer (e.g., ringworm), thereby psychologically harming them, creating a likelihood of further injury to them, and annoying them by significantly disrupting their normal behavioral patterns, including feeding and learning from their mothers, sleeping, retreating from public view, and other species-specific behaviors.

67. For captive animals such as Wildlife in Need's big cats, proximity to, or contact with, humans is a potential source of stress and can be extremely harmful to the animals' well-being. Chronic, unabated stress in animals causes physiologic change that can ultimately compromise immunity, impair coronary health, alter brain structure and function, stunt growth, reduce body weight, shorten lifespan, decrease homeostasis, potentiate pain, and increase abnormal behaviors.

68. I reviewed dozens of videos featuring big cats used in Tiger Baby Playtime events occurring in Fall 2016 and Spring 2017. PETA-WIN_000114-405; PETA-WIN_000443-471; PETA-WIN_000507-605; PETA-WIN_000622-001188; PETA-WIN_001204-001438; PETA-WIN_004662-004733. Mr. Stark and his staff of untrained volunteers routinely agitate the cubs by, among other things, shaking, biting, and rubbing them, restraining them, pulling on their tails, dropping them suddenly onto unsuspecting members of the public, making growling sounds at them, and pulling their tongues during photo opportunities while they sit in an abnormal position in the laps of members of the public. According to the USDA inspection reports, Mr. Stark has gone so far as to instruct customers to hit the animals if they express distress or react negatively to public handling, and to direct employees and volunteers to hit cubs with riding crops. PETA-WIN_002367-002369. Tim Stark confirmed this by his testimony. Stark Dep. Tr. 167:16-19; *id.* 168:14-170:2. The unstructured, free roaming direct contact between big cats and the public that I saw in the videos of Fall 2016 and Spring 2017 Tiger Baby Playtime events is consistent with the

descriptions of such events published in the Defendant's USDA inspection reports. PETA-WIN_001974-001990; PETA-WIN_002361-002362; PETA-WIN_2363-002366; PETA-WIN_002367-002369; PETA-WIN_002581-002588; PETA-WIN_002589-002591.

69. Such agitation increases the likelihood of physical and mental injury to the cubs, thereby harassing them. This conduct significantly disrupts the animals' normal behavioral patterns by making it impossible for them to hide or otherwise seek shelter from fear-inducing stimuli, and not only causes them psychological injury, but is so distressing that it also places the animals at significant risk for physical injury. These species of big cats are clearly not domesticated or trained and are therefore likely not to perform as domesticated animals might. Being hit by human hands or struck by riding crops, they still cannot know what behavior is expected of them by their human handlers, resulting in confusion and thus further psychological harm. Not only does the direct public contact harm and harass the cubs, but the practice of giving visitors access to a "playroom" and denying the cubs an opportunity to retreat to an area in which they can escape from the public can cause significant distress to captive big cats.

70. Further, given that big cats normally spend over three-quarters of their day resting and sleeping, physical contact with members of the public forces them to stay awake for far more hours than a young cub should. This deprives them of needed sleep. This severe reduction in resting time is inherently disruptive to their normal behavior. The big cat cubs are exhibited often multiple times per day, on the hour, every hour from 10:00 AM to 10:00 PM. PETA-WIN_002413-002416; PETA-WIN_003151-003295. The constant use of these cubs in "Playtime" events without periods of sufficient rest between hourly exhibitions exhausts the animals, and most likely impedes their growth and immune response. The cubs often appear lethargic or even exhausted and will attempt to sleep despite members of the public surrounding and handling them. I also reviewed several

dozen videos of the Tiger Baby Playtime events during which big cat cubs sleep on the floor or on members of the public while being petted, poked, prodded, or pulled. This creates a likelihood of injury because it disrupts normal sleep and rest behaviors, which are essential to natural development and physical health, thereby harassing and annoying the animals in violation of the ESA.

71. Wildlife in Need also allows public contact with big cats who have open wounds, the severity of which may be exacerbated by allowing bacterial or fungal transmission from roomfuls of people who handle the animals, thereby further wounding and harming them in violation of the ESA. In several videos of Tiger Baby Playtime sessions that I reviewed, likely ringworm lesions are visible on the skin of the big cats while roughly thirty members of the public at a time incessantly handle them. For example, the lion cubs, Nera and Mauri, appear to have had lesions to their legs, likely caused by ringworm infection, during Tiger Baby Playtime sessions held in April and May, 2017. PETA-WIN_000558; PETA-WIN_000559; PETA-WIN_001390-001393. According to Defendants' medical treatment logs, multiple big cats suffered from a ringworm outbreak during this period and into the fall of 2017.

72. The big cat cubs are separated from their mothers as neonates, well before they are naturally weaned, causing distress to the cubs and their mothers, and other physical and psychological health problems. In the wild, lion cubs nurse for an exceptionally long time, having been observed suckling at up to fifteen months of age, albeit with decreasing frequency after the first six to eight months of age. Tigers typically wean at approximately six months. At Wildlife in Need, the big cat cubs are separated from their mothers within days or weeks of birth and not allowed to nurse naturally, instead being bottle fed formulated milk, often that which is used for domestic kittens, that is nutritionally inadequate for these animals. In fact, the milk formulated for

dog puppies might be better tolerated in these big cat species. The problem is that it lacks other vital nutrients and therefore, the dog formula needs expert formulation to be nutritionally appropriate. Of course, natural mother's milk is always better than formula.

73. On January 17, 2014, the USDA found that Mr. Stark had willfully violated numerous AWA regulations, including 9 C.F.R. § 2.131(c)(1), by allowing the public to come into close proximity to tiger cubs who were too large, too strong, and too aggressive to have direct contact with the public with minimal risk of harm to the animals and the interacting public. The citation came after inspectors observed injuries that uncontrolled big cat cubs inflicted on the public. The USDA again cited Mr. Stark for this violation on August 20, 2014, and again on September 13, 2015. Despite being cited and sued by the USDA, Defendants have continued to allow the public, including untrained volunteers, to make physical contact with big cat cubs who grow too large, too strong, and become too aggressive to have direct contact with members of the public without risk of injury to the public and the animals. I observed these same conditions in the Tiger Baby Playtime videos I reviewed, in which big cats up to 20 weeks of age and up to approximately 50 pounds bite, nip, scratch (the few big cats who Defendants allowed to keep their claws), and otherwise behave aggressively or defensively toward members of the public. PETA-WIN_000131 – PETA-WIN_000405; PETA-WIN_443 – PETA-WIN_000471; PETA-WIN_000507-605; PETA-WIN_000622 – PETA-WIN_001188; PETA-WIN_001204 – PETA-WIN_001438; PETA-WIN_007739; PETA-WIN_007784 – PETA-WIN_007786; PETA-WIN_007788.

74. The USDA's observations underscore that public contact harms and harasses the animals. Indeed, several cubs were observed vocalizing, a well-recognized sign of psychological distress and suffering, while forced to come into direct contact with the public. Furthermore, in the

several dozen videos I reviewed of the Tiger Baby Playtime events, this vocalizing was present and indicates to me that the big cats are quite possibly suffering from psychological distress and trauma during these public encounters.

75. Forcing these predators to interact with humans, denying them the opportunity to escape from public interaction, and prematurely separating cubs from their mothers violates AWA regulations and is not a generally accepted animal husbandry practice. This practice harms the animals, creates a likelihood of injury to them, and annoys them, by significantly disrupting their normal behavioral patterns.

**Opinions on the Big Cats' Lack of Adequate Nutrition**

76. Careful balancing of important protein and carbohydrate ratios are necessary to provide optimal nutrition to the developing cubs. High carbohydrates, lactose in particular, as might be found in Kitten Milk Replacer (KMR) can cause some cubs GI distress and diarrhea. The addition of human baby food with meat, especially turkey or chicken, is indicated, and has been done, however imprecisely, by WIN. Cats have a requirement for taurine, an amino acid (found in high amounts in mice and other rodents). Without it being properly balanced in their diets, the big cats are subject to taurine-deficiency-induced heart disease and blindness. Minerals like calcium must be included and balanced to ensure that the milk substitute does not cause permanent bone deformities for these cubs. They require calcium supplementation in order to ensure that their rapidly growing bones and teeth have enough material to be strong and avoid metabolic bone disease, common in roadside zoo cubs. There is a very delicate balance between minerals, vitamins, trace molecules, fats, proteins, and carbohydrates that must be maintained and is not best accomplished by the cavalier attitude of giving the cubs some of this and some of that and hoping for the best. There are very clear USDA guidelines on formulas that are appropriate for cubs and

there is even a textbook, Hand-Rearing Wild and Domestic Mammals by Laurie J. Gage, DVM, that provides explanations for these carefully considered diets. Indeed, USDA officials testified that the Defendants do not feed an appropriate amount of meat to big cat cubs, OALJ Tr. 1469:1-15 (Dr. Kirsten testimony), and that allowing the general public to bottle feed these cubs puts them at risk of aspiration pneumonia. OALJ Tr. 2114:14-2116:10 (Dr. Gage testimony).

The success of a diet is measured in the cub's continued weight gain. Nowhere in the Defendants' records did I see a weight chart monitoring progress in body mass each day as is imperative for assessing baby animals. Failure to thrive in a cub often shows up first in the inability to gain weight when compared to sibling cats. Feeding the cats bottles with nipples that might flow too profusely puts them at risk for aspiration and aspiration pneumonia. As we know from one of the few necropsy reports we have, one cat died with evidence of food in its lungs. Defs' Second Amended Prod. at 195-97. Feeding milk substitutes puts the babies at constant risk for GI upset and diarrhea. Diarrhea can cause the cubs to become so dehydrated that veterinary intervention becomes necessary. I did not see any daily records of the cubs' stool quality ever. In raising neonates, we usually have a chart of what went in and what went out. Maternal separation alters the cubs' normal feeding behaviors and other natural behaviors that, had they been allowed to remain with their mothers, the cubs would have learned. This creates a risk of injury in the form of weakened immune systems and abnormal physical and behavioral development. Mr. Stark is very confident that he knows the nutritional needs of cats, T. Stark Dep. Tr. 261:1-5, but yet feels compelled to beat a baby leopard to death with a baseball bat (after having it for 5 weeks) because it had metabolic bone disease, a condition that should have been correcting itself in the young, growing animal if the animal had been finally getting proper nutrition and supplementation. Either

he didn't recognize this condition, the leopard didn't have it, or the leopard wasn't getting proper nutrition under Tim Stark's care.

**Opinions on the Big Cats' Conditions of Confinement**

77. In the wild, tigers' territories range from 7.72mi$^2$ to 154.44mi$^2$, depending on the availability of prey. Ass'n of Zoos and Aquariums, Tiger (*Panthera tigris*) Care Manual, at 6, *available at* https://www.speakcdn.com/assets/2332/tiger_care_manual_2016.pdf. Within these ranges, tigers are free to engage in natural behaviors such as swimming, climbing, stalking, and hunting. *Id.* at 11. They occupy a variety of habitats, typically comprising dense vegetative cover, sufficient prey populations, and access to water. *Id.* at 6-7. Tigers are generally solitary; however, they are known to come together for breeding, feeding, and sometimes, especially known family members, will socialize and travel in groups. *Id.* at 6, 28.

78. Given their natural needs, tigers require large, environmentally rich, natural spaces that allow them to express a wide range of behaviors. *Id.* at 11-13. Captive environments that do not provide the environmental and behavioral enrichment necessary to promote the expression of a full range of species-typical behaviors have a detrimental effect on the animals' physical and psychological well-being. *See id.* (describing spatial requirements to meet physical and psychological needs of tigers in captivity). Indeed, big cats in barren environments like the one at Wildlife in Need experience long periods of inactivity or mindless inactivity, which results in permanent long-term changes to the brain, musculo-skeletal, and endocrine systems. ECF No. 55 (Pratte Decl. at 13 (citing Foy et al., 1987; Boe et al., 1968; Bacon, 2015)). Environmental and behavioral enrichment are necessary to deter harmful coping behaviors arising such as self-mutilation and stereotypical behaviors such as pacing, Ass'n of Zoos and Aquariums, Tiger (*Panthera* tigris) Care Manual, at 72-73, which has been observed in big cats at Wildlife in Need.

Harmful behaviors such as self-mutilation and pacing, in addition to evidencing psychological distress, can lead to other physical injuries, especially when declawed animals pace on inappropriate substrates. In the wild or in a reputable sanctuary, a big cat would have the ability to exercise, explore, and engage in other species-typical behaviors on appropriate substrate.

79. Enrichment plans for captive carnivores, including tigers, are difficult to develop due to these animals' natural feeding and hunting behaviors and spatial needs. *Id.* at 72-73. In inadequate captive conditions, thwarted hunting prospects alone appear to cause carnivores like tigers to suffer stress, which causes physical and psychological injury. *See id.* (providing ideas for encouraging natural stalking behaviors to improve tiger welfare). Accordingly, enrichment plans should include natural and complex enclosures and environmental enrichment including safe whole-carcass feeding, novel toys/objects, scratch logs, introduction of new smells, enclosure rotations, pools, and adequate space to run. *Id.*

80. In the wild, a lion's habitat includes open lands, thick brush, scrub, and tall grassy areas. Ass'n of Zoos and Aquariums, Lion (*Panthera* leo) Care Manual, at 40, *available at* https://www.speakcdn.com/assets/2332/lion_care_manual_20121.pdf. Ideal habitats provide sufficient cover to facilitate hunting and denning. *Id.* at 11, 20. Wild lions mainly hunt at night, traversing distances ranging from one to eight miles each night, depending on the availability of food. *Id.* at 11. Female lions do most of the hunting in cooperative social groups by stalking and ambushing prey, frequently taking prey much larger than themselves. *Id.* at 12.

81. Lions are highly social and live in large groups called prides. *Id.* at 12, 34. For African lions, a typical pride structure includes five to nine related adult females and their offspring plus two to six males who are unrelated to the females but frequently related to each other. *Id.* at 12. Female lions typically stay in their natal prides their entire lives and often develop preferred

groupings between close relatives such as mother/daughter or siblings. *Id.* Despite their social nature, however, lions need to be able to leave a social structure and choose their social groupings. *Id.* at 12, 34.

82. Meeting the physical and psychological needs of captive lions requires providing them with the opportunity to socialize with compatible lions, and providing them with necessary environmental enrichment so that they are able to express a full range of natural behaviors. *Id.* at 34-38, 97-104.

83. The Association of Zoos & Aquariums (AZA), the nation's premier zoological accrediting organization, recommends that captive lions be provided with "large spacious enclosures designed to encourage species appropriate behaviors such as resting, walking, [simulated] hunting, stalking, grooming, playing, breeding, etc." *Id.* at 18 (citing Schaller, 1972). All enclosures should allow lions to retreat from conspecifics and provide visual privacy from humans "through the use of visual barriers, such as rock outcroppings, hills, and foliage, without limiting an animal's access to food, water, heat, or shade." *Id.* According to the AZA, the majority of lion exhibits are over 10,000 square feet, which should be considered the minimum size for new exhibits, and the typical tiger exhibit is between 2,500 and 10,000 square feet, with an average of 5,500 square feet. *Id.* at 18-19.

84. In addition to providing social privacy, enclosures should provide shade and include "various substrates, surfaces to mark, deadfall for scratching, and other aspects in their enclosure that will change their pathways and create complex behavioral opportunities." *Id.* at 18.

85. Defendants harm the big cats, create a likelihood of injury to them, and annoy them by significantly disrupting their normal behavioral patterns by confining them to small, barren enclosures, denying them appropriate, natural and complex housing, and frustrating their natural

instincts. The enclosures lack enrichment and force big cats to walk and rest upon inappropriate gravel substrates.

86. Specifically, Defendants have injured and create a likelihood of injury to the big cats by allowing them to eat gravel, and indeed have killed two big cats this way. Radiographs and medical records that I have reviewed demonstrate that three big cats owned by the Defendants were treated for stomachs "full of rocks." Plt's Ex. 14B. Two of the animals did not survive the exploratory surgery that was necessary to remove the rocks. *Id.* These deaths were caused by the Defendants creating conditions of confinement, including gravel substrates, that are inappropriate for these species of big cats. In the wild, of course, big cats do not eat gravel or rocks of any kind, and the cubs' interest in eating rocks at the Defendants' facility may have been triggered by inadequate nutrition (volume or quality), boredom, displacement behavior, or by the Defendants leaving carcass remains to mix with the gravel substrate, which cannot be sanitized after feedings. Displacement behavior would be perhaps where a cat is frustrated by not getting fed while other cats are getting fed and the cat takes it out on the rocks. I have never known big cats to crave salt as Mr. Stark characterizes why the cubs ate the rocks. Tim Stark Dep. Tr. 102:4-7.

87. Throughout the site inspection footage, there are several big cats housed with gravel as the only substrate. In my opinion, this is completely inappropriate. Big cats require a natural substrate and the ability to get away from pooling water. At 00:49:29, there are portions of meat left on the gravel, illustrating that it is difficult to keep this substrate clean and that the big cats who ate rocks possibly had been eating meat off the gravel and swallowed rocks with it.

88. At 01:34:13 in the site inspection footage, there is a declawed tiger lying on gravel. In my opinion, the gravel is an inappropriate substrate both for walking and for resting. Tigers should have access to natural substrates, such as dirt or grass, on which to lie. At 01:48 and onward,

a white tiger, named Avalanche, also is lying on gravel. At 02:01, the tigers Jomba and Babuva also are lying on gravel.

89. Throughout the site inspection footage, I saw no evidence of any feeding platform or chutes, and very few platforms for the big cats to get off the gravel. Most of the enclosures have a single den box, and apart from the roof, there are no platforms to climb, jump, or escape the gravel substrate.

90. Defendants also deprive the big cats of adequate enrichment. Inadequate enrichment thwarts the expression of a range of natural behaviors, including, for example, predatory and investigatory behaviors.

91. The enclosures at Wildlife in Need do not encourage the big cats to engage in instinctual and species-specific behaviors, including simulated natural hunting behaviors such as stalking and predation, and are therefore inadequate to provide for the animals' physiological and psychological well-being.

92. Some of the big cats at Wildlife in Need also have been denied appropriate shelter from the elements. The AWA requires that animals be provided with adequate shelter from inclement weather, 9 C.F.R. § 3.127(b), and sufficient shade from direct sunlight, *id*. § 3.127(a). The USDA has cited Wildlife in Need for failing to provide big cats with adequate shelter from winter temperatures and weather. According to a USDA inspection report, "The lack of wind breaks, or shelters that protect the animals from the rain, sleet, direct sun, and snow can cause possible health issues and discomfort to the . . . animals, that in nature would be able to find appropriate shelter from the elements if able." PETA-WIN_002356-002360. The inspector noted that snow and rain were blowing into an enclosure and that the temperature had been between seven and twenty-one degrees Fahrenheit for the week prior to the inspection, with two to three

inches of snow on the ground during the inspection. *Id.* This is consistent with current conditions I observed at WIN in the video of the site inspection conducted during the course of this litigation.

93. Failure to provide big cats with adequate protection from the elements creates a likelihood of injury, including hypothermia and illness, by denying them the ability to engage in normal behaviors such as hiding, resting, and sheltering without exposure to inclement weather, or choosing to find a more suitable location, thereby harassing them.

94. The big cats' outdoor enclosures also do not provide them with adequate shade from the sun, contrary to generally accepted animal care standards and AWA regulation. *See* 9 C.F.R. § 3.127(a). Denying captive big cats necessities such as appropriate shelter physically harms them, and significantly disrupts their normal behaviors, including sheltering and resting behaviors, in a way that puts their physical and psychological well-being at risk of injury.

95. Despite the established authority on the environmental needs of big cats, *see* Ass'n of Zoos and Aquariums, Lion (*Panthera* leo) Care Manual; Ass'n of Zoos and Aquariums, Tiger (*Panthera tigris*) Care Manual, Defendants continue to confine them in inappropriate and unsafe environments, without enrichment, and therefore wholly fail to meet their physical, social, and psychological needs. These inadequate conditions cause the big cats to suffer psychological injury. The conditions further harm the big cats' physical and psychological health by depriving them of the ability to express a full range of natural behaviors such as simulated predatory behaviors, investigatory behaviors, and social avoidance behaviors, including the autonomy to choose to engage with or avoid others, which are central to their physical and psychological well-being. Further, Defendants deprive big cats of the ability to express simulated natural hunting behaviors such as stalking and predation, creating a likelihood of injury to them by annoying the big cats to such an extent as to significantly disrupt normal feeding behavioral patterns.

96. The lion, Chief, is seen pacing at 00:21:30 and again at 01:09:45 of the site inspection video for several minutes continuously. He does not stop pacing before the camera moves on. Each time the camera returns to Chief—for example, around 01:14 and again at 01:16:10—he is still pacing. I also observed Chief pacing in several videos shot during the Spring 2017 Tiger Baby Playtime event season, on several different days. PETA-WIN_000133 (March 25, 2017); PETA-WIN_000181 (March 25, 2017); PETA-WIN_000298 (April 29, 2017); PETA-WIN_000464 (April 22, 2017); PETA-WIN_000985 (March 26, 2017); PETA-WIN_001175 (May 12, 2017). Pacing is a particularly alarming stereotypic behavior because it can indicate severe psychological distress. In Chief's case, his pacing is likely brought on by a lack of enrichment and concomitant boredom over prolonged periods of time, and/or a reaction to years of living in a small and barren enclosure. Chief's enclosure has an inappropriate gravel substrate, though he paces along a concrete path that runs alongside the fence. Chief has an abnormal gait and body conformation. Though he has claws, I suspect his tentative steps are a result of metabolic bone disease, arthritis, or a similar condition. If I were responsible for Chief's care, I would take radiographs to properly diagnose him. I would put him on pain medication and observe how his gait might improve once he is no longer in pain. Chief's pain is likely worsened by the gravel substrate and the concrete path in his enclosure—these are the only materials he has to walk on. Additionally, Chief is very small for an adult male lion. This indicates a lack of proper nutrition at a young age or some other condition, possibly inbreeding, that stunted his growth.

97. At 00:08:32 in the site inspection footage, there are two cages on the left that house one white male tiger, Simonduwa, and one orange female tiger, Fettie, in one cage, and a white lioness, SnowLei, and a white tiger, Mako, in the other cage. These cages are too small and the den boxes are not suited for snow or hard rain. The animals are on wood chips, which are hard to

clean, and there is no roof structure so that the cages are exposed. The cages appear to lack a cinderblock night house, leaving the animals exposed to harsh weather.

**Opinions on the Big Cats' General Lack of Adequate Veterinary Care**

98. My review of the Defendants' records revealed no veterinary records regarding the provision of preventive or routine veterinary care to the big cats. Rather, the Defendants consult with a veterinarian, if at all, only when a medical issue becomes beyond their comfort level to treat themselves. There is no veterinarian providing preventive or routine care, and it appears that there never has been. In my practice, working with big cats, I provide routine care like vaccines, deworming, and generalized inspections, at a minimum of a twice yearly basis. There is no evidence in Defendants' records of these services having been performed ever, let alone on a regular basis. The annual visit required of an "attending veterinarian" by USDA regulations does not suffice as a replacement for preventive or routine veterinary care in this case, because Dr. Pelphrey, the attending veterinarian from 2013 through the outset of this litigation, only walked through the property on an annual basis. He performed necessary veterinary services only upon the request of the Defendants. Defendants' latest attending veterinarian, Dr. Oliver, testified at his deposition that he has treated only one big cat for the Defendants—at their request only after the animal presented with a medical issue, not as a preventive or routine matter—and has never performed even the annual walkthrough required by the USDA.

99. Despite Defendants losing multiple big cats in recent years, they have not sought—and their veterinarians have not performed—necropsies to determine the causes of death. For example, Dr. Pelphrey testified that the orange and white tiger cubs died from hepatotoxicity—acute liver failure—caused by an overdose of ketoconazole that he prescribed. Necropsies would have confirmed or debunked that theory; however, he neither ordered nor performed necropsies,

and Defendants did not otherwise pursue necropsies. Pelphrey Dep. Tr. 214:2-18; 236:22-238:14; 238:22-240:16. Although Dr. Pelphrey also prescribed griseofulvin, another antifungal drug that is toxic to cats and has well-known gastrointestinal adverse effects, to those same tiger cubs, he did not attempt to determine whether either it or ketoconazole caused or contributed to their deaths via necropsy; rather, he testified that he knew it was the ketoconazole, and not the griseofulvin, that caused their deaths because of "common sense." Pelphrey Dep. Tr. 272:24-273:3. Defendants' records reveal that additional big cat cubs were given griseofulvin but not ketoconazole. Some of these cubs died after experiencing gastrointestinal distress. For example, a white male tiger and an orange male tiger began courses of griseofulvin on August 13, 2017; after four days on griseofulvin, the patients began suffering from diarrhea. PETA Ex. 14C at 21-26. The diarrhea persisted until the cubs' deaths—despite this devastating side effect, neither Dr. Pelphrey nor the Defendants took the cubs off griseofulvin. The orange tiger cub was put on fluids on August 20, 2017 and died the next day. *Id.* at 24-25. The white tiger cub was finally taken off griseofulvin on August 27, 2017. That day, Dr. Jill Cook came to the Defendants' premises to examine the cub and put him on fluids; the cub died two days later. *Id.* at 21. There was no necropsy done on either tiger cub. Under these circumstances, a necropsy is called for to properly determine a cause of death and to adjust veterinary care accordingly. The griseofulvin might have caused the deaths but there are other potential causes of death, like distemper virus, that would affect the rest of the big cat population and therefore, in the interest of preventing further deaths, necropsies are always indicated.

100. Throughout the litigation, the Defendants and their attending veterinarians have made clear that all defer to Tim Stark to determine when, whether, and to what degree a big cat will receive veterinary care. Dr. Pelphrey's testimony, and the records provided by the Defendants,

makes plain that Dr. Pelphrey provided veterinary care and advice via text message correspondence, at times without even examining the big cat in question. According to Dr. Oliver's testimony, Defendants did not explain to him what is required of an attending veterinarian under the Animal Welfare Act, nor did he know what those responsibilities are; he believed he was to be available as needed for consultation. Such hands-off veterinary care reflects, in my opinion, an inability or unwillingness to provide adequate veterinary care to the big cats. Big cats in captivity require hands-on, attentive, knowledgeable, and expert veterinary care. Defendants lack such qualifications and so do the veterinarians they have engaged.

101. In fact, the Defendants have entirely lacked an attending veterinarian on more than one occasion and has even falsified the signature of a veterinarian, Dr. Gough, on their USDA Program of Veterinary Care form, to make it appear as if Dr. Gough was acting as their attending veterinarian, when in fact he was not. OALJ Tr. 383:23-384:7. On another occasion, Defendants attempted to pass off Dr. Pepin as their attending veterinarian, although she did not intend to fill this role for them; initially, Defendants had been excited about Dr. Pepin because they believed she would defer to their judgment as to what veterinary care would be appropriate for the big cats. OALJ Tr. 447:1-13; OALJ Tr. 455:8-456:6. According to the USDA's testimony at Tim Stark and WIN's enforcement action hearing, Dr. Pepin "was very clear" that she "didn't intend to agree to be the attending veterinarian for the facility," and "that she did not fill out the pages that reference big cats and exotic animals." OALJ Tr. 455:8-456:6. Dr. Pepin testified that she does not see big cats or exotic animals in her practice, and that she told Tim Stark the same. OALJ Tr. 40:11-15; 55:3-7; 60:19-2082:3-4.

102. Dr. Gough testified that Tim Stark was angered when Dr. Gough would not sign a form to permit tiger cubs to travel across state lines for a commercial photo shoot because Dr.

Gough was not knowledgeable as to exotic animals, resulting in the termination of their relationship. OALJ Tr. 979:3-980:6. Dr. Gough lacked expertise to provide veterinary care to big cats or other exotic animals. OALJ Tr. 956:12-13; 959:18-22. He testified that Tim Stark would not follow his veterinary medical advice, including leaving without taking medications or without scheduling advised follow-up care with a specialist, including an incident where Tim Stark failed to procure treatment for a broken bone in a lion cub's leg. OALJ Tr. 960:1-7; 960:8-961:3; 964:24-965:13; 966:1-5 ("[O]ften [Tim Stark] didn't believe when you tried to diagnose it. He – he didn't want to believe what you ever told him. He wanted to – he wanted to only believe in himself and what he thought he knew."); 970:4-8 ("[I]f you want to sum up Tim Stark, he does what he wants. And that's [what] I always saw out of him. He wanted to do what he wanted to do. He might listen to what you had to say but not very well."); 983:21-984:6.

103. Dr. Gough testified that someone, presumably Tim Stark, forged his signature on the Program of Veterinary Care form. OALJ Tr. 970:23-972:12; 972:17-24. Dr. Pepin testified that multiple sections of the Program of Veterinary Care form were completed by someone else and were not completed when she signed, and that Tim Stark never discussed those sections with her. OALJ Tr. 55:16-22; 65:6-10; 66:12-23; 67:18-24; 77:8-19; 126:12-21; 127:15-18; 130:22-131:1; 132:20-133:1.

104. Dr. Pepin testified that Tim Stark asked her to supply her with ketamine, and she declined because it is a controlled substance and should be administered by a veterinarian, not by a lay person. OALJ Tr. 60:5-12. Ketamine is commonly known as a party drug, and Stark's request to be supplied it is particularly alarming and outside the bounds of drug law that is well known in the veterinary community. Similarly, Dr. Gough testified that he was troubled by Tim Stark's request for ketamine and other controlled substances, and that someone else's handwriting

appeared on the section of the Program of Veterinary Care form where ketamine and other drugs used to perform euthanasia are listed. OALJ Tr. 968:17-969:6; 972:7-24.

105. Similarly, Dr. Pelphrey testified that the Program of Veterinary Care form he signed included someone else's handwriting for the controlled drugs listed in the section on euthanasia; he could not read the handwriting. Pelphrey Dep. Tr. 116:4-118:18.

106. Similarly, Dr. Oliver testified that he was not aware he had agreed to become the attending veterinarian for Defendants, that he was unaware of the statutory and regulatory duties of an attending veterinarian under the Animal Welfare Act, and that his understanding of his role was to consult on an as-needed basis, as Defendants brought veterinary issues to his attention. Oliver Dep. Tr. 16:24-21:7. Dr. Oliver testified that he is not always available to the Defendants in the event of a veterinary emergency, and that he had not discussed emergency planning with the Defendants. Oliver Dep. Tr. 23:23-25:14. He is not in regular communication with the Defendants regarding animal care and has provided no guidance to Defendants' personnel regarding animal care, such as handling, immobilization, anesthesia, analgesia, tranquilization, and euthanasia. Oliver Dep. Tr. 25:15-27:5. Dr. Oliver testified that he does not know if a medical issue arising at the Defendants' facility would even be brought to his attention. Oliver Dep. Tr. 27:6-17. He does not understand that attending veterinarians have responsibilities beyond walking through the facility once annually, and he does not consider himself to be responsible for overseeing the health and wellbeing of the animals there; he did nothing to verify his responsibilities as attending veterinarian. Oliver Dep. Tr. 27:18-29:8. He has no experience with, or expertise in, treating big cats or the other wild or exotic animals exhibited by the Defendants. Oliver Dep. Tr. 30:6-32:2. This is woefully inadequate to fulfill the duties of an attending veterinarian, and it appears that Defendants merely seek a veterinarian to sign a form to pass off to the USDA inspectors as if they

have an attending veterinarian. Dr. Oliver's signature on the Program of Veterinary Care form is in fact meaningless, given these facts; it is as if Defendants have no attending veterinarian at all.

107. According to Defendants' recent filings in this litigation, Dr. Oliver is no longer responsive to their communications, suggesting that in fact there is presently no attending veterinarian to provide necessary and regulated medical services to this USDA-licensed facility. Given the testimony regarding Drs. Gough and Pepin's experiences with Defendants during the recent enforcement action that seeks to revoke Defendants' Animal Welfare Act license, this is not the first time Defendants have operated without an attending veterinarian or acted as if a veterinarian's signature on the Program of Veterinary Care form—whether real or forged—is sufficient to meet their Animal Welfare Act requirements.

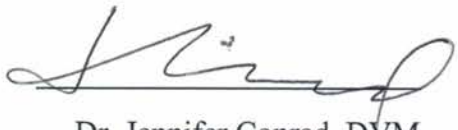
108. The record demonstrates that metabolic bone disease has afflicted animals at Defendants' facility, including a leopard that Tim Stark beat to death with a baseball bat. Blunt force trauma to the head, including by way of baseball bat, is not an AVMA-approved method of euthanasia for the species. This incident gives me great pause that Defendants are incapable of adequately caring for their big cats, including by providing appropriate euthanasia when necessary.

## Conclusion

It is my professional opinion, held to a reasonable degree of veterinary certainty, that the Defendants' conduct as described in this report falls below the standard of care for the Big Cats and that the best interests of the animals requires that they be transferred to a reputable sanctuary.

Pursuant to 28 U.S.C. § 1746, I, Dr. Jennifer Conrad, DVM, hereby declare that under the penalty of perjury the contents of the foregoing reports are true and correct to the best of my knowledge.

EXECUTED on this 19 day of February 2020.



Dr. Jennifer Conrad, DVM