OMB Control No. 1018-0093 Expires 08/31/2023

E. IMPORT/EXPORT/RE-EXPORT OF BIOLOGICAL SPECIMENS (CITES/ESA) FOR SCIENTIFIC RESEARCH

General Information

This application covers activities involving CITES and ESA-listed animal specimens used for scientific research, including any readily recognizable parts, products, or derivatives unless otherwise noted in the Appendices.

Review this application carefully and **provide complete answers to all of the questions**. If you are applying for multiple species, be sure to indicate which species you are addressing in each response. **If more space is needed, attach a separate sheet with your responses numbered according to the questions.**

Please allow at least 90 days for the application to be processed.

How do I determine whether the species is protected under CITES and/or the ESA?

CITES	ESA
To determine whether an animal species is protected under CITES, when the species was listed, or whether exemptions apply to your requested activity, see the <u>list of CITES species</u>	To determine whether an animal species is protected under the ESA, please review the list of ESA-listed species in the Code of Federal Regulations.
	Please be aware that any permit request involving an ESA endangered species must be published in the Federal Register for a required 30-day public comment period.

- If applying as an individual or institution please note that you will have to pay the appropriate permit fee.
- If applying as an **institution** that is (or is acting) on behalf of a Federal, Tribal, State, and/or local government agency, no permit fee is required. Provide fee exempt documentation with your application materials.
 - The individual signing the permit must have legal authority to do so if applying on behalf of the institution.

Questions

If you have any questions regarding an action you are requesting authorization for please contact the Division of Management Authority at managementauthority@fws.gov.

Please note: for renewal or amendment of a multi-use permit being requested **within the 5 year** Federal Register public notice period, use application <u>3-200-52</u>

This form should NOT be used for:

- Captive Bred Wildlife Registration (use application 3-200-41)
- ESA Plants (use application 3-200-36)

Electronic Information Submission

<u>Electronic submission of inventories, photographs, and receipts:</u> For hard copy applications, if you wish to provide information electronically, please include a flash drive containing this information with your physical application.

FWS Form 3-200-37e (Rev. 01/2020) U.S. Department of Interior

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All Applicants Must Complete

1.	Name and address where you wish the permit to be mailed, if different from physical address . If you would like expedited shipping, please enclose a self-addressed, pre-paid, computer-generated, courier service airway bill. If unspecified, all documents will be mailed via regular mail through the U.S. Postal Service.
2.	Point of contact if we have questions about the application (name, phone number, and email).
3.	Have you or any of the owners of the business (if applying as a business, corporation, or institution), been assessed a civil penalty or convicted of any criminal provision of any statute or regulation relating to the activity for which the application is filed; been convicted, or entered a plea of guilty or nolo contendere, for a felony violation of the Lacey Act, the Migratory Bird Treaty Act, or the Bald and Golden Eagle Protection Act; forfeited collateral; OR are currently under charges for any violation of the laws mentioned above?
	No Yes If you answered "Yes" to Question 3, provide: a) the individual's name; b) date of charge; c) charge(s); d) location of incident; e) court, and f) action taken for each violation. Please be aware that a "Yes" response does not automatically disqualify you from getting a permit.
	Proposed Activity
	☐ Import
	□ Export□ Re-export (e.g. export of a specimen that was previously imported into the United States)
4.	The current location of the samples (if different from the physical address provided):
	Name:
	Address:
	City:
	State/Province:
	Postal Code:
	Country:

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5. Recipient/Sender:

- If export or re-export, provide name and physical address of the recipient in the foreign country.
- If **import**, provide name and **physical address** of the exporter/re-exporter in the foreign country.

Name:	
Address:	
City:	
State/Province:	
Postal Code:	

Country:

- 6. Information on the type of **biological samples** involved in the import/export/re-export, provide for **each species** (you may use the table located below):
 - a. Scientific name (genus, species, and, if applicable, subspecies);
 - b. Common name;
 - c. Number and type of sample(s) (e.g. 10 blood samples, ear clips, etc.)
 - d. Source (wild or captive-born)
 - e. Approximate date of collection (MM/YYYY)
 - f. Description of packaging (vials, slides, envelopes, etc.)
 - g. Total # of all samples in shipment.

a. Scientific name (genus, species, and, if applicable, subspecies)	b. Common Name	c. Number & type of sample/part	d. Wild or Captive born	e. Approximate date of collection (mm/yyyy)	f. Description of packaging (vials, slides, envelopes, etc)
EXAMPLE: Pan troglodytes	Chimpanzee	10 blood samples; 4 hair samples	W	08/2015	Vial Envelope
				g. TOTAL # of all samples in the shipment:	

Source of Specimen

- 7. For **each biological sample taken from a captive-born/captive hatched animal(s)**, provide a signed and dated statement from the breeder or appropriate documentation (e.g. Species 360 report) that includes the following:
 - a. Scientific name (genus, species, and if applicable, subspecies),
 - b. Common name,
 - c. Name and address of the facility where the animal was bred and born;
 - d. Birth/hatch date (mm/dd/yyyy),
 - e. Identification information (studbook #, microchip, leg band, etc.),
 - f. Name and address of facility where the parental stock is located; and
 - g. A statement from the breeder that the animal was bred and born at the breeder's facility (including the facility's name and address), and
 - h. If not the breeder, documentation demonstrating the history of transactions (e.g., chain of custody or ownership of the sample(s), *if applicable*).
- 8. For each biological sample taken from an animal in the wild, provide:
 - a. Scientific name (genus, species, and if applicable, subspecies),
 - b. Common name,
 - c. Specific location (e.g., county, state, province, country) where the samples were taken from the wild,
 - d. The name of the individual(s) who collected the animal/samples and their authorization to do so including (but not limited to) copies of foreign and domestic (Federal, State, and/or Tribal) government collecting permits, licenses, contracts, and/or agreements.
 - e. Method of collection: sampling protocol, approximate length of time held in captivity, any injury and/or mortality experienced during collection, transport, or holding;
 - f. Information related to any remuneration, either financial or in-kind, provided for acquiring the sample(s);
 - g. Efforts to use captive specimens (e.g., captive-born, captive-held) in lieu of taking samples from wild animals.
- 9. For **each biological sample being re-exported** (e.g., exporting a specimen that was previously imported into the United States), provide:
 - a. A copy of the **canceled** CITES export or re-export document issued by the appropriate CITES office in the country from which the wildlife was imported;
 - A copy of your Declaration for Importation or Exportation of Fish or Wildlife (Form 3-177), cleared by USFWS
 Office of Law Enforcement.
 - c. A copy of the ESA permit that authorized the original import.
 - d. If you did not make the original import, please provide documentation outlining chain-of-ownership since import, including:
 - i. A copy of the importer's CITES, ESA, and declaration documents (a, b, & c above) and,
 - ii. Subsequent invoices (or other documentation) showing the history of transactions leading to your ownership of the sample(s) after import (provenance).

Description and Justification For Requested Activity

- 10. Describe the purpose of the scientific research and include:
 - a. A copy of the research proposal (outlining the purpose, objectives, methods),
 - b. How long the research has been (or will be) conducted,

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- c. Detailed information on sampling methods including:
 - who will be taking the samples
 - ii. equipment and methods used
 - iii. measures taken to prevent injuries and mortalities during collection
- d. A copy of the study's Institutional Animal Care and Use Committee (IACUC) form (if applicable),
- e. Peer-reviewed scientific papers published from this research (if applicable),
- f. An explanation of whether similar research has already been conducted or is currently being conducted.
- 11. Please provide a detailed description on how the proposed activities will **enhance or benefit the wild population within its native range** (e.g., direct or indirect **conservation efforts**) and provide documentation (e.g., signed memorandums of understanding) demonstrating your commitment to supporting the program and how the program contributes directly to the species identified in your application.

Technical Expertise & Authorizations

12. CV or resume outlining the technical experience of the researchers and field technicians collecting the samples, as it relates to the proposed activities, including experience with other similar species.

Shipment Information

- 13. Please indicate if this is a one-time shipment or if you anticipate needing to import/export/re-export samples multiple times within one year or over multiple years.
- 14. How will the samples be imported or exported (e.g., personally carried or shipped)?
- 15. If personally carried, please specify the individual(s) who will be transporting the samples.

All international shipment(s) must be through a designated port. A <u>list of designated ports</u> (where an inspector is posted) is available. If you wish to use a port not listed, please contact the Office of Law Enforcement for a Designated Port Exemption Permit (form 3-200-2).

CITES Appendix I & Marine Mammal Species

- For export of a CITES Appendix I-listed species, provide a copy of the CITES import permit, or evidence one
 will be issued by the Management Authority of the country to which you plan to export the specimen(s). In
 accordance with Article III of the CITES treaty, it is required that import permits are issued before the
 corresponding export permit.
- For **import** of **CITES Appendix-I listed species**, provide information to show the import is not for primarily commercial purposes as outlined in <u>Resolution Conf. 5.10 (Rev CoP15)</u>.
- For import of CITES Appendix-I marine mammal samples, please provide a copy of your FWS or NMFS Marine Mammal Protection Act (MMPA) permit or authorization.

7 Samples from captive animals

a. Scientific name: Lycaon pictus

- b. Common name: African wild dog
- c. Name and address of the facility where the animal was bred or born

87 animals contributing 178 samples:

Port Lympne Safari Park, Port Lympne Reserve, Hythe, Kent, CT21 4PD, UK

2 animals contributing 5 samples:

SafariPark Beekse Bergen, 5081 NJ Hilvarenbeek, Netherlands

4 animals contributing 15 samples:

Dublin Zoo, Phoenix Park, Dublin 8, D08 AC98, Ireland

2 animals contributing 6 samples:

Warsaw Zoo, Ratuszowa 1/3, 03-461 Warszawa, Poland

1 animal contributing 7 samples:

West Midland Safari Park, Spring Grove, Bewdley DY12 1LF, U.K.

1 animal contributing 1 sample:

Friguia Animal Park, GP 1, Aïn Rahma, 4089, Bouficha, Tunisia

1 animal contributing 1 sample:

Zoo de Pont Scorff, All. de Kerruisseau, 56620 Pont-Scorff, France

1 animal contributing 4 samples:

Ree Park Safari, Stubbe Søvej 15, 8400 Ebeltoft, Denmark

1 animal contributing 1 sample:

Zoo Duisburg, Mülheimer Str. 273, 47058 Duisburg, Germany

3 animals contributing 6 samples:

Ann Van Dyk Cheetah Centre, R513, Brits, North West Province, 0251 South Africa

- d. Date of birth: Provided in Table attached as Annex 7d.
- e. Identification information: Provided in Table attached as Annex 7d.
- f. Name and address of facility where the parental stock is located: As listed in 7c above.
- g. Statement attached as Annex 7g.
- h. Origin data from Species 365 attached as Annex 7h.
- 8 NA. The samples do not originate from free-ranging animals.
- 9 NA. The samples are not being re-exported.

10 Purpose of Scientific Research

- a. A copy of the research proposal is enclosed at **Annex 10a**.
- b. These samples are to be exported as part of a two-year research project. However, the samples themselves were collected over a 20-year period, from 2001 to 2021.
- c. i Blood samples were all collected by Jane Hopper, Head of Veterinary Services for the Howletts Wild Animal Trust.
 - Blood samples were collected in the course of routine animal care, including when animals were captured for vaccination, health checks, microchipping, and transfers between enclosures. Adult wild dogs were chemically immobilised by darting, while small pups were captured with nets.
 - iii Great care was taken to avoid injury or death of these animals in the course of handling. All darting was conducted by an experienced zoo veterinarian, assisted by experienced zoo keepers familiar with the individual animals' behaviour. PPE was worn to minimise the risks of pathogen transmission from animals to humans and humans to animals. Animals were kept quiet and calm at all times, and drugs, and drug doses, were chosen to minimise induction and recovery times.
- d. A copy of the study's Ethical Review form (UK equivalent of IACUC form) is enclosed at **Annex 10d**. Note that permission was granted for a captive trial, but when the team learned of this existing bank of wild dog serum, no trial was needed, but no further ethical review was required.
- e. A peer-reviewed paper from the study is enclosed at **Annex 10e**.
- f. There has been very little research on the effectiveness of CDV vaccination on African wild dogs. One recent paper (Wahldén *et al.* (2018) *Hosts and Viruses* 5, 26-34) considered this question, likewise using samples collected opportunistically in a zoo setting. Our study uses a different vaccine, which we plan to test subsequently in a field trial. Our study also provides a larger sample size and should provide better data on the duration of effectiveness of the vaccine.

11 How will this study enhance or benefit the wild population within its native range? This project is specifically designed to support the conservation of free-ranging African wild dogs.

The African wild dog is a globally endangered species, with fewer than 700 packs remaining in the wild. In the past five years, six separate fatal outbreaks of Canine Distemper Virus (CDV) have been recorded across Africa¹⁻⁴, with the worst all but wiping out the largest population in the northern hemisphere. Conservation managers throughout Africa urgently need guidelines on effective ways to manage this disease.

Previous research shows that CDV cannot easily be controlled by vaccinating domestic dogs⁵⁻⁷. However, simulation modelling suggests that, where CDV risks are most acute, vaccinating wild dogs themselves could greatly reduce extinction risks⁸. Unfortunately, recent attempts to implement CDV vaccination in Kruger National Park, using a recombinant vaccine, provoked minimal immune responses⁹. This project involves trialling a different (modified live) vaccine, widely used on domestic dogs and likely to be both safe and effective in wild dogs¹⁰. The samples included in this shipment are from captive-born wild dogs vaccinated at a zoo in the course of routine animal husbandry. We plan to follow this captive work with a field trial in Kruger National Park. The results of the captive and field data collection will be used to parameterise a simulation model linking wild dog management to

extinction risk⁸, to evaluate different vaccination strategies and identify the most cost-efficient. These results will then be used to develop guidelines for CDV management in free-ranging African wild dogs, under the auspices of the IUCN/SSC Canid Specialist Group.

¹Loots, AK *et al. PLOS ONE* **13**, e0199993 (2018); ²Du Plessis, C. *Canine distemper virus inoculations at HIP*. (https://wildlifeact.com/blog/canine-distemper-virus-inoculations-hip/, 2016); ³Grumeti Fund. *Wild dog report*. (https://www.grumetifund.org/blog/updates/wilddog-report/, 2018); ⁴Mutinda, M *et al. Canine distemper outbreak in wild and domestic carnivores in Laikipia ecosystem of Kenya*. (Kenya Wildlife Service, 2017); ⁵Viana, M *et al. Proceedings of the National Academy of Sciences* **112**, 1464-1469 (2015); ⁶Prager, KC *et al. EcoHealth* **9**, 483 (2013); ⁷Woodroffe, R *et al. PLoS One* **7**, e30099 (2012); ⁸Smallwood, T. *Modelling multi-host viral pathogens for African wild dog conservation*. (PhD thesis, Imperial College London, 2020); ⁹van Schalkwyk, L *et al. Health survey and targeted vaccination of the Kruger National Park African wild dog population south of the Olifants River*. (Final Report to SANParks, 2019); ¹⁰Woodroffe, R. *J Zoo Wildl Med.* **52**, 176-184 (2021).

Technical Expertise & Authorizations

Samples included in this shipment were collected by Jane Hopper, Head of Veterinary Services for the Howletts Wild Animal Trust, which owns Port Lympne Reserve where the wild dogs were held in captivity.

A CV for Jane Hopper is enclosed at Annex 12.

Shipment Information

- This is a one-time shipment. We anticipate applying for a second permit in 2022 to cover samples collected in the field, but these will come from a different origin country and will come from free-ranging animals rather than captive animals.
- 14 The samples will be shipped.
- 15. NA



Vet Dept
Port Lympne Reserve
Aldington Road
Lympne
Nr. Ashford
Kent
CT21 4PD

Telephone: 01303 234175

16 October 2021

Dear Sir

Cornell University Import Permit Application

Cornell University is applying for permission to import blood samples collected from animals born in captivity in the United Kingdom.

I confirm details of these samples as follows

- a Scientific name: Lycaon pictus
- b Common name: African wild dog
- Name and address of the facility where the animal was bred and born:
 Port Lympne Safari Park, Port Lympne Reserve, Hythe, Kent, CT21 4PD, UK.
 Animals marked * were captive bred at another institution and their place of birth is recorded in the attached documents (Species 360 reports).
- d Birth date: Listed in the table below
- e Identification information: Listed in the table below
- f Name and address of facility where the parental stock is located: Port Lympne Reserve, Port Lympne Reserve, Hythe, Kent, CT21 4PD, UK.

As Head of Veterinary Services for the Howletts Wild Animal Trust, which owns and runs Port Lympne Reserve, I confirm that the information is correct.

Yours faithfully,

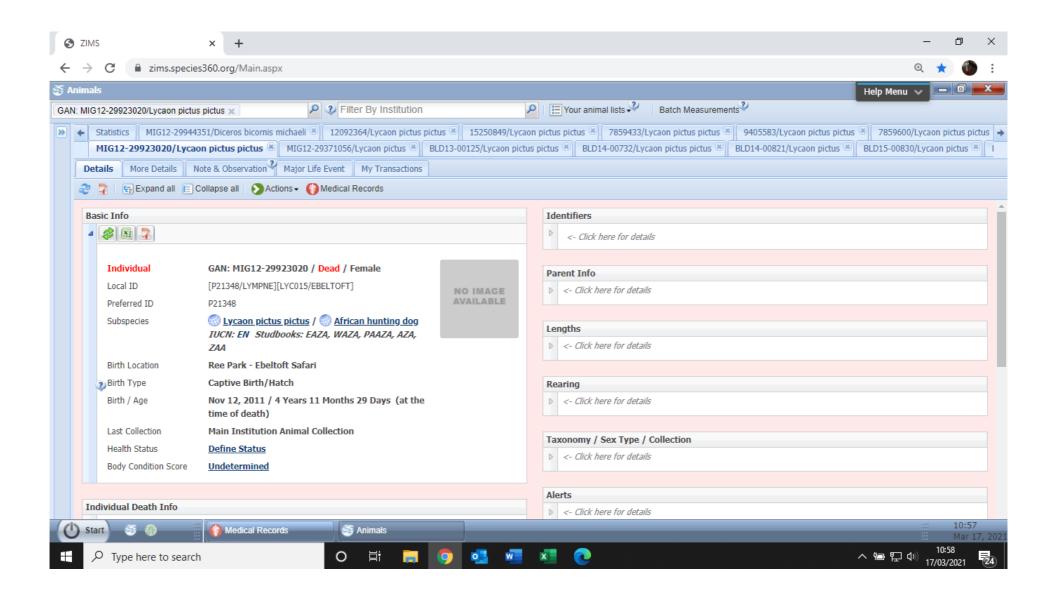
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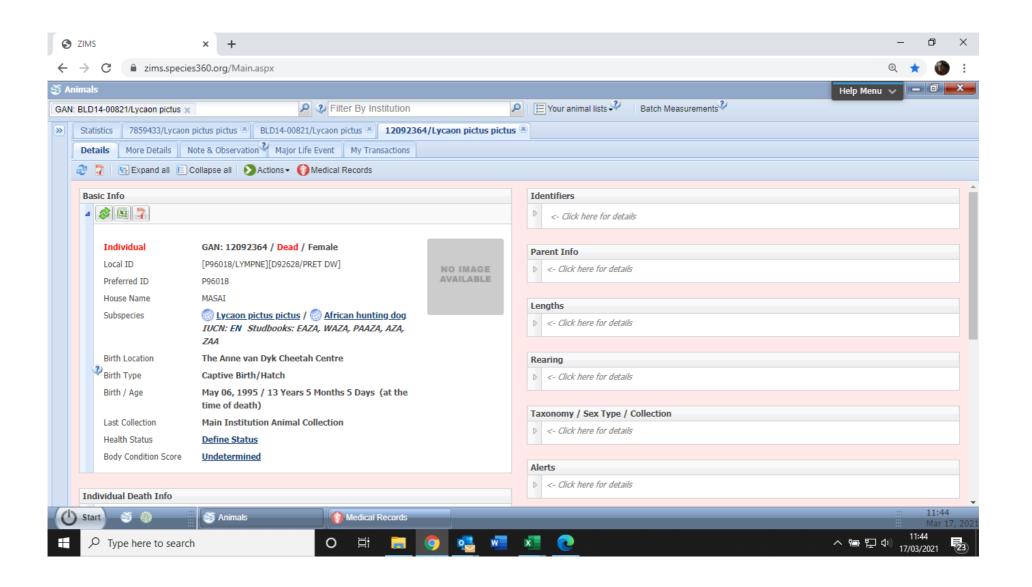
Jane Hopper

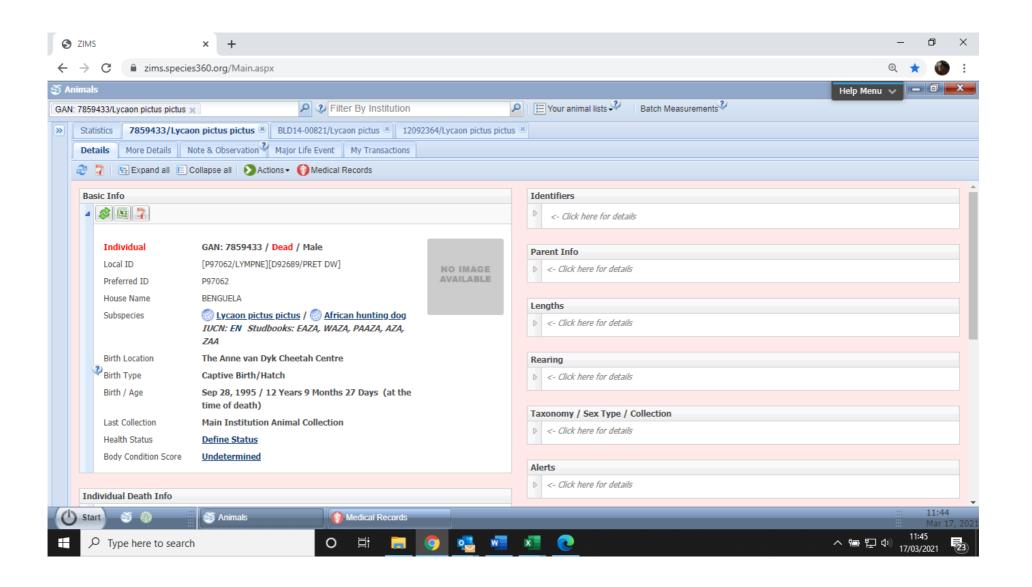
Table – Details of the identities of all African wild dogs represented in the set of samples to be exported. Addresses of places of birth: A SafariPark Beekse Bergen, 5081 NJ Hilvarenbeek, Netherlands; B Port Lympne Safari Park, Port Lympne Reserve, Hythe, Kent, CT21 4PD, U.K.; C Dublin Zoo, Phoenix Park, Dublin 8, D08 AC98, Ireland; D Warsaw Zoo, Ratuszowa 1/3, 03-461 Warszawa, Poland; E Friguia Animal Park, GP 1, Aïn Rahma, 4089, Bouficha, Tunisia; F West Midland Safari Park, Spring Grove, Bewdley DY12 1LF, U.K.; G Zoo de Pont Scorff, All. de Kerruisseau, 56620 Pont-Scorff, France; H Ree Park Safari, Stubbe Søvej 15, 8400 Ebeltoft, Denmark; I Zoo Duisburg, Mülheimer Str. 273, 47058 Duisburg, Germany; J Ann Van Dyk Cheetah Centre, R513, Brits, North West Province, 0251 South Africa. For animals not born at Port Lympne, rigin details are provided in the enclosed data sheets from the Species 360 Zoological Information Management System.

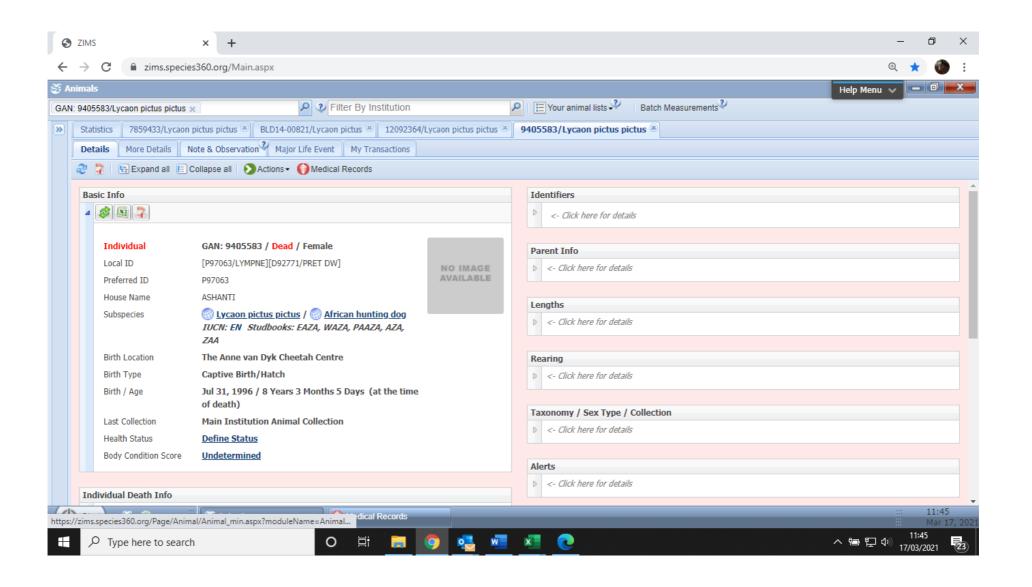
	ogical Information	place of		
ID number	name/number place birth		date of birth	number of vials
H20236	Mzungu/B790	A	08-Jan-98	2
H20237	Kassama/17AB	В	00-Jan-98 01-Mar-97	4
H20612	Rafiki	В	22-Nov-93	1
H20912	Two Socks/594302	В	04-Dec-06	5
	Blade/599654			3
H20946	Selous/053881	B C	04-Dec-06	3
P21478		C	18-Oct-09	5
P21349	Ruaha	A	17-Oct-09	3
P20047	Tsenga		08-Jan-98	1
P20053	Kippa/8DF0	В	13-Nov-00	2
P20077	Shue/B25E	В	13-Nov-00	
P20078	Rhunt/A079/97F6	В	13-Nov-00	1
P20079	Krane/D481	В	13-Nov-00	4
P20080	Depti/B94F	В	13-Nov-00	1
P20082	Spot/E1AD	В	13-Nov-00	3
P20169	Tatu	D	17-Oct-04	1
P20170	Wili	D	17-Oct-04	5
P20520	Tunis	E	15-Sep-02	1
P20572	NA (48.4886	В	04-Nov-05	1
P20573	Nyae Nyae/424326	В	04-Nov-05	10
P20574	Whitey/44543	В	04-Nov-05	1
P20680	Sandy/593476	В	04-Dec-06	2
P20684	Tango/599146	В	04-Dec-06	1
P20686	Blacky/595132	В	04-Dec-06	2
P20688	NA	В	00-Jan-00	1
P20789	956000000/847617	В	04-Nov-07	2
P20790	Spot-tail	В	04-Nov-07	3
P20791	Bandy	В	04-Nov-07	6
P20793	Teye/751	В	04-Nov-07	4
P20796	Tanny	В	04-Nov-07	5
P20799	Pirate/352	В	04-Nov-07	2
P20911	Vango	F	27-Oct-05	7
P21050	Snake/4749	В	24-Nov-01	4
P21052	Domino/E182	В	24-Nov-01	2
P21053	/000606BC5C	В	24-Nov-01	2
P21055	Neleh/FC90	В	24-Nov-01	1
P21056	Eva/88FF	В	24-Nov-01	1
P21057	E03F/0A9F	В	24-Nov-01	2
P21263	Socks	В	10-Nov-12	1
P21264	Scorpion	В	10-Nov-12	3
P21265	Splodge	В	10-Nov-12	2
P21266	Romeo	В	10-Nov-12	4
P21267	Mantler	В	10-Nov-12	2
P21268	Chevron	В	10-Nov-12	2
P21269	Kudu	В	10-Nov-12	4
P21301	Zuri	G	05-Jun-06	1
P21348	Nadifa	Н	12-Nov-11	4
P21350	Chobe	С	18-Oct-09	4
P21360	Ghost	В	15-Dec-13	3
P21361	Two spot	В	15-Dec-13	3
P21464	Sprench	В	12-Nov-14	2
P21465	Flash	В	12-Nov-14	1
P21467	Gecko	В	12-Nov-14	1
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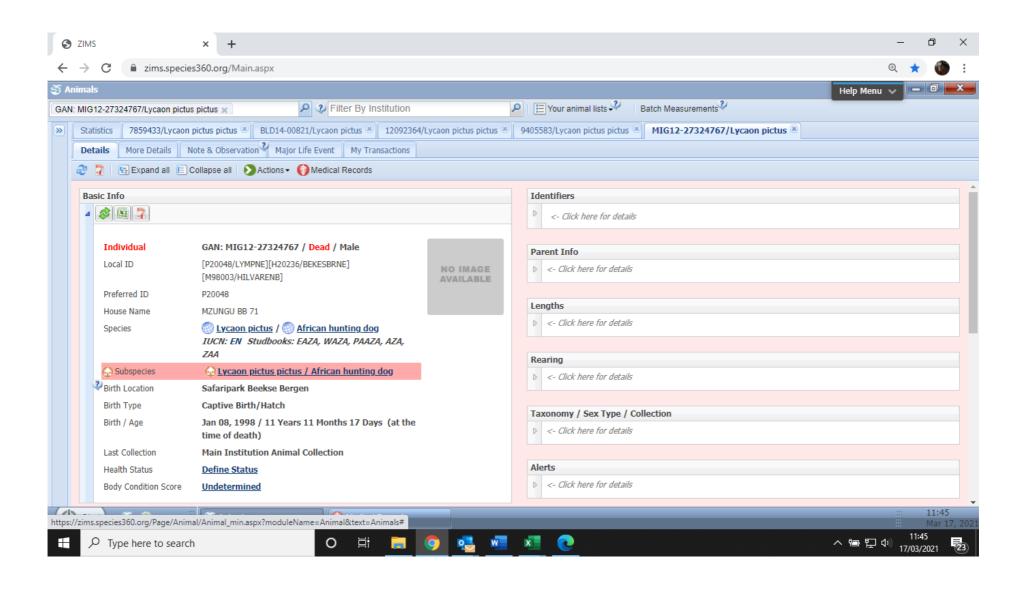
ID number	•			number of vials				
P21469	Madi	В	12-Nov-14	3				
P21470	Five	В	12-Nov-14	1				
P21471	Horseshoe	В	12-Nov-14	1				
P21477	Kruger	C	18-Oct-09	3				
P21479	Branka	Ī	27-Oct-10	1				
P21480	NA	В	29-Dec-14	2				
P21481	NA	В	29-Dec-14	2				
P21482	NA	В	29-Dec-14	1				
P21483	NA	В	29-Dec-14	1				
P21484	NA	В	29-Dec-14	2				
P21485	NA	В	29-Dec-14	1				
P21486	NA	В	29-Dec-14	1				
P21487	NA	В	29-Dec-14	1				
P21489	Ace	В	27-Nov-14	1				
P21491	Icarus	В	27-Nov-14	2				
P21492	Kamana	В	27-Nov-14	1				
P21493	Kite	В	27-Nov-14	1				
P21494	Cross	В	27-Nov-14	1				
P21495	Sickle	В	27-Nov-14	1				
P21496	Comma	В	27-Nov-14	1				
P21590	Lenny	В	27-Dec-15	1				
P21594	S	В	27-Dec-15	1				
P21598	V/Tooth	В	27-Dec-15	1				
P21990	NA	В	05-Nov-20	2				
P21994	NA	В	05-Nov-20	2				
P21995	NA	В	05-Nov-20	2				
P21996	NA	В	05-Nov-20	2				
P21997	NA	В	05-Nov-20	2				
P21999	NA	В	05-Nov-20	2				
P22045	Assegai/B50D	В	02-Nov-02	2				
P22049	Falcon/82F1	В	02-Nov-02	2				
P22050	Yella/EB07	В	02-Nov-02	2				
P22051	Kenya/138E	В	02-Nov-02	2				
P22052	Nora/140/1105	В	02-Nov-02	2				
P22053	/0006201E4A/141	В	02-Nov-02	2				
P22054	Lessa/142/B6AC	В	02-Nov-02	2				
P22055	/0006202304/143	В	02-Nov-02	2				
P22056	Psyche/02A00	В	02-Nov-02	1				
P22057	Saddle/0E42/145	В	02-Nov-02	1				
P22059	Bibi/94FE/146	В	02-Nov-02	2				
P22070	NA	В	05-Nov-20	2				
P22071	NA	В	05-Nov-20	2				
P22072	NA	В	05-Nov-20	2				
P22085	/0006205B85	В	02-Nov-02	2				
P96018	Masai	J	06-May-95	2				
P97005	Kassanga/sccsc	В	01-Mar-97	1				
P97011	Kassala/1E18	В	01-Mar-97	1				
P97062	Ben(guela)	J	28-Sep-95	3				
P97063	Ashanti/2465	J	31-Jul-96	1				
P98044	Kang/E1F60	В	15-Jun-98	3				
P98047	Tchad/4CF2	В	15-Jun-98	1				
Total anima	als: 103	Total	samples:	224				

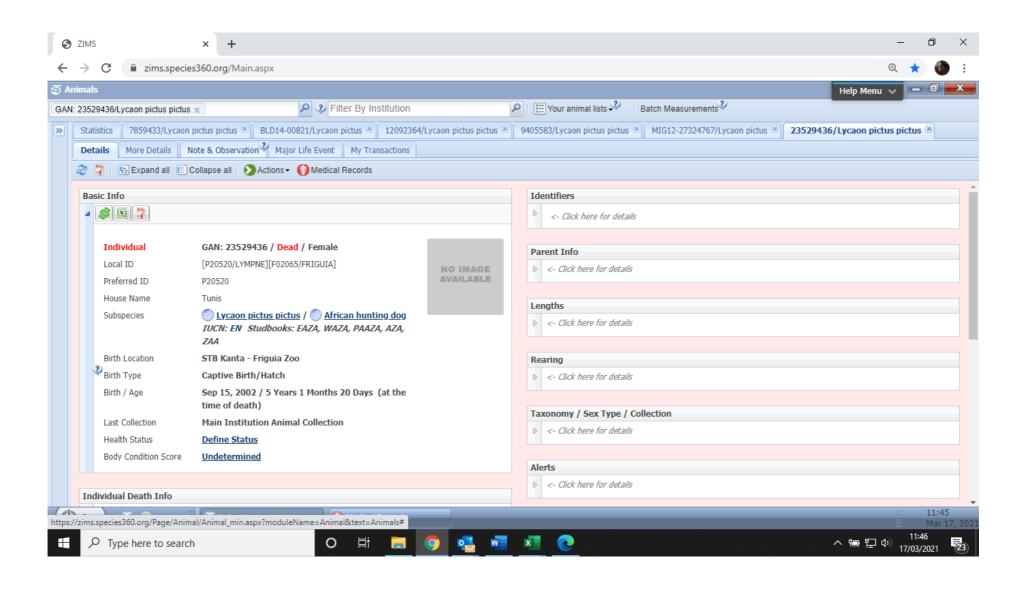


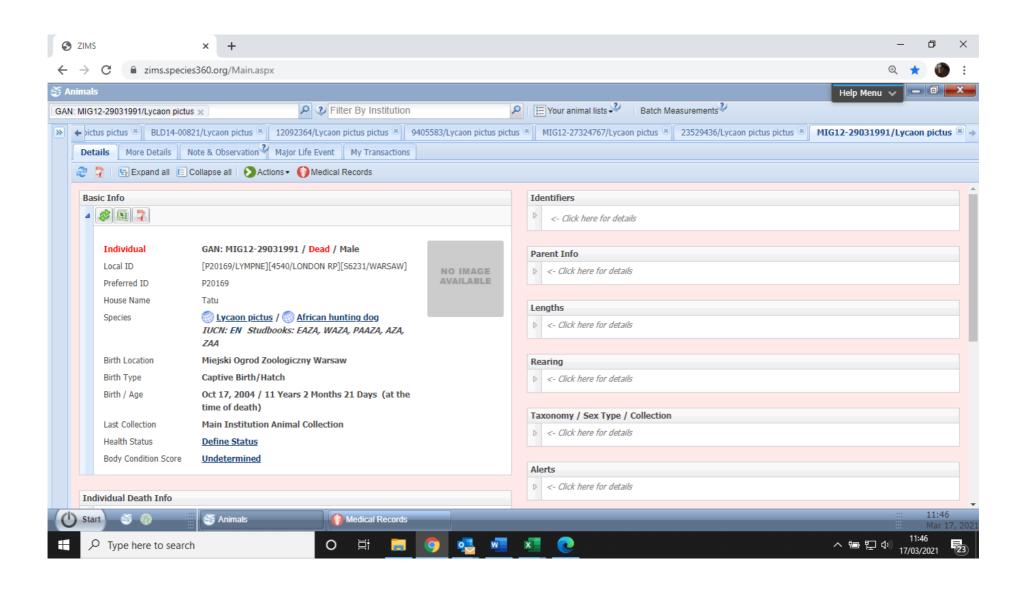


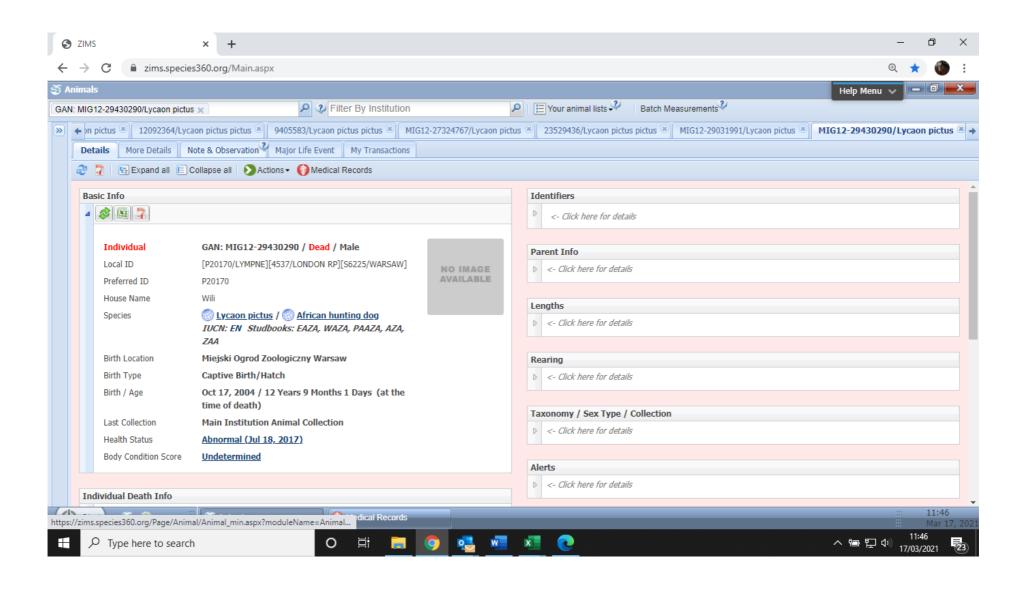


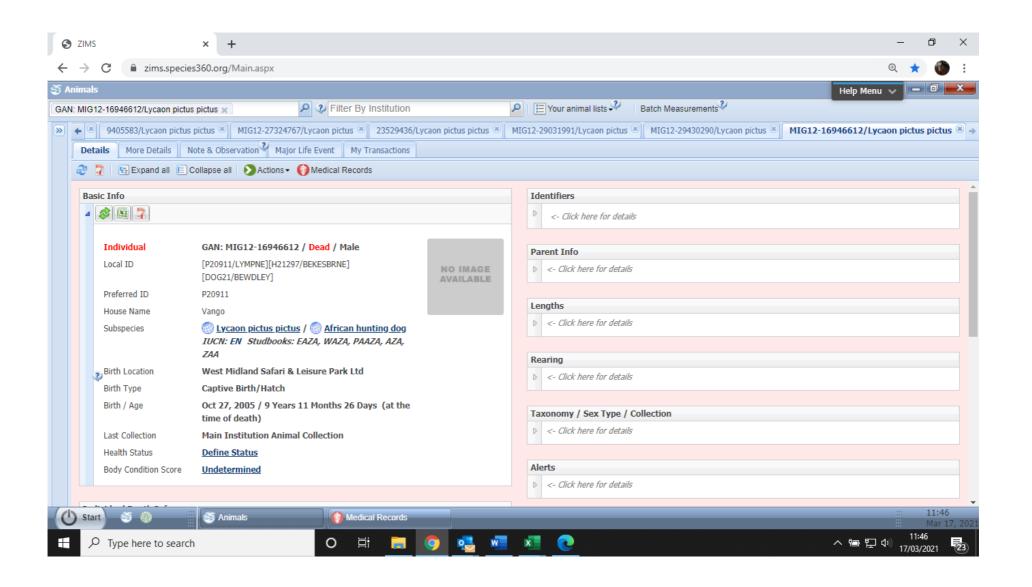


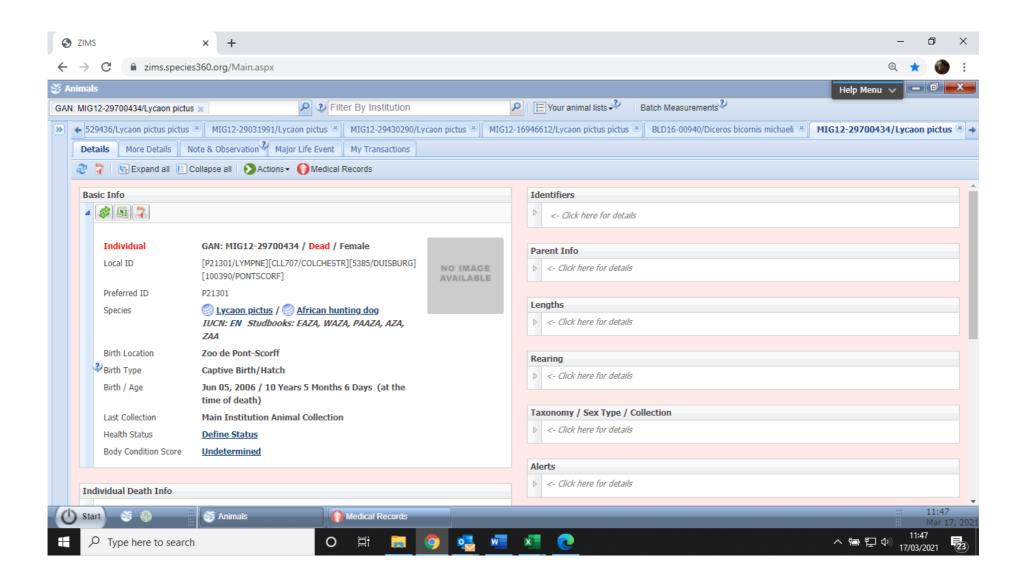


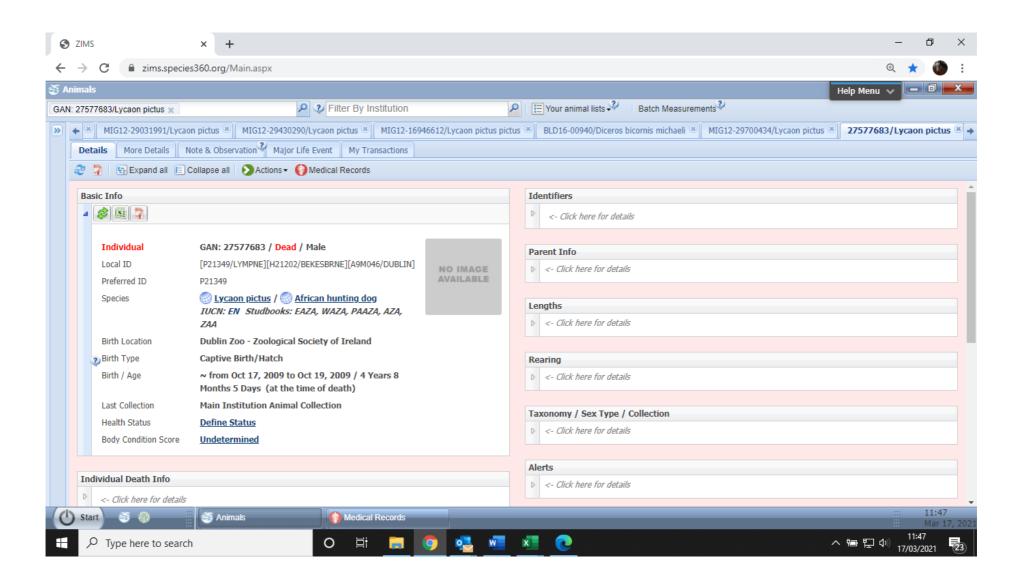


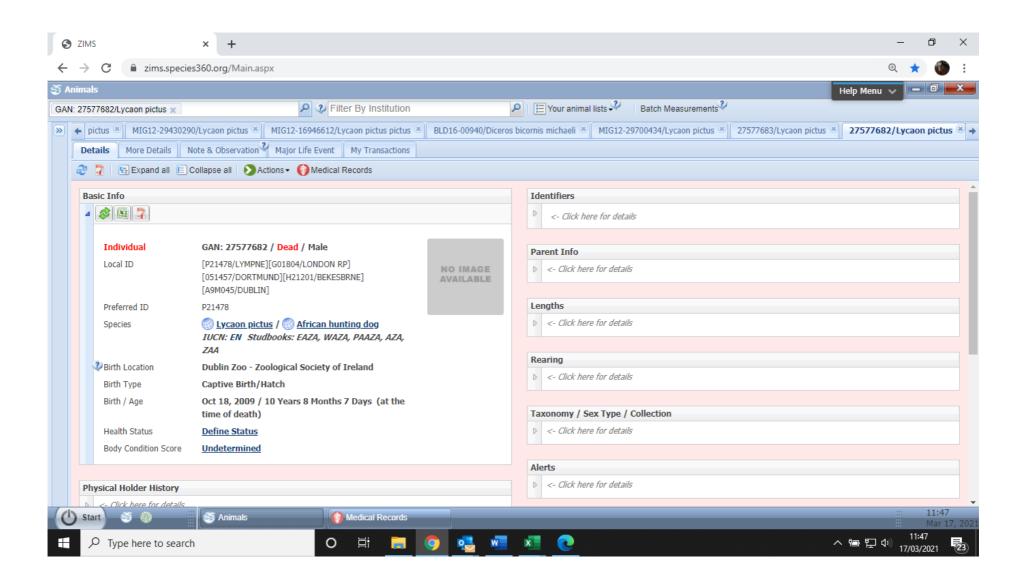


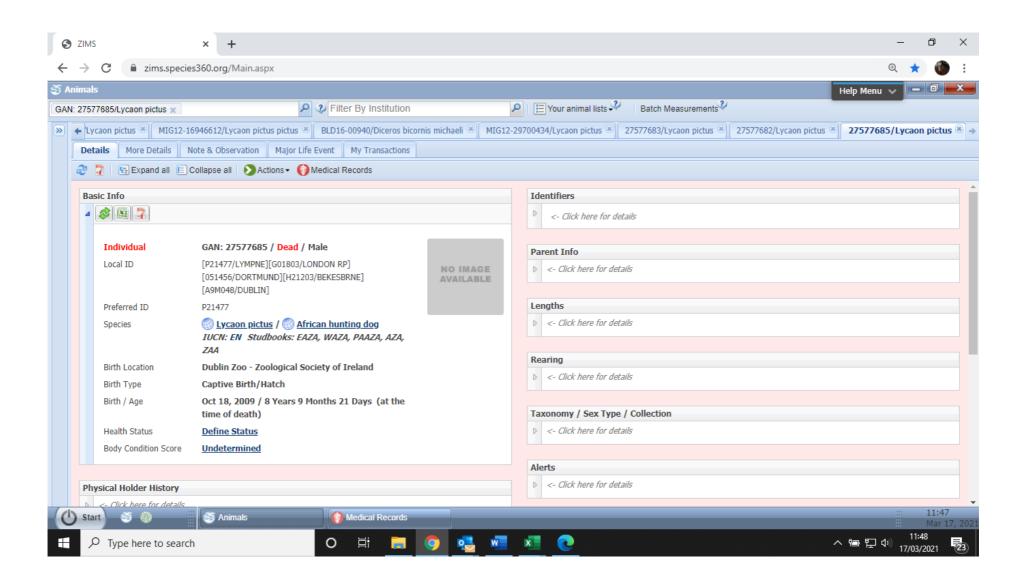


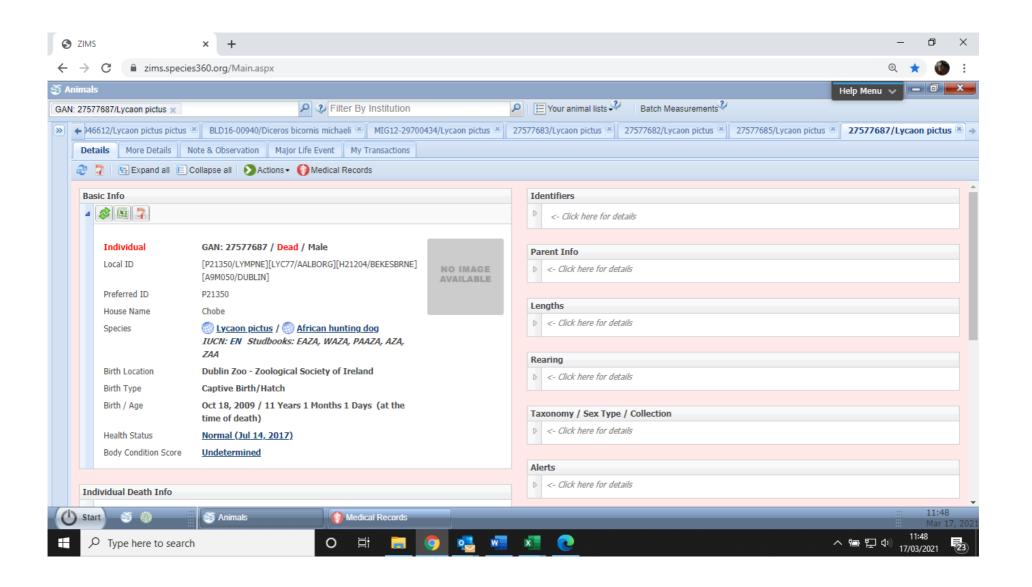


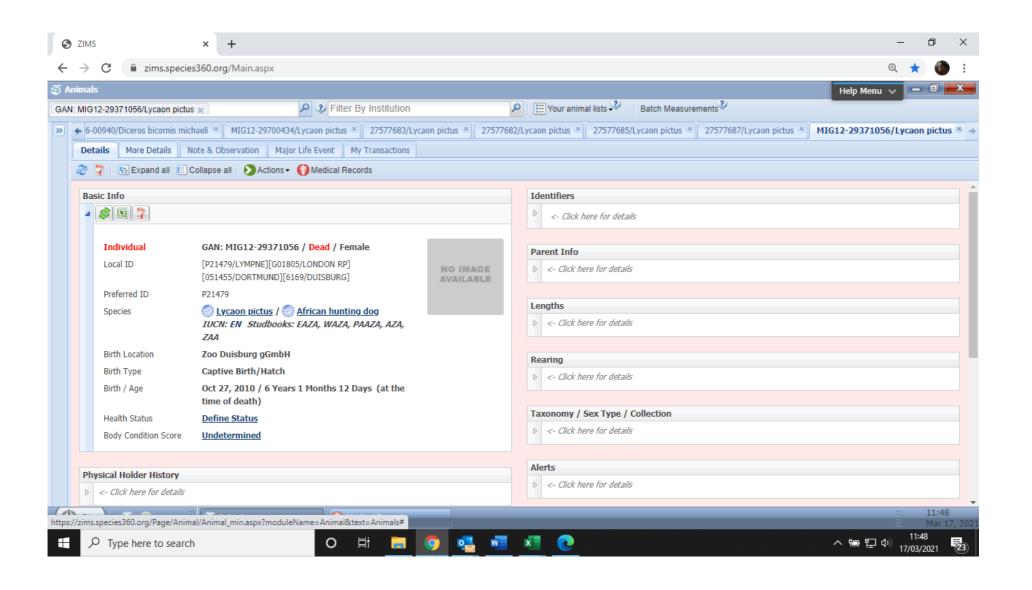


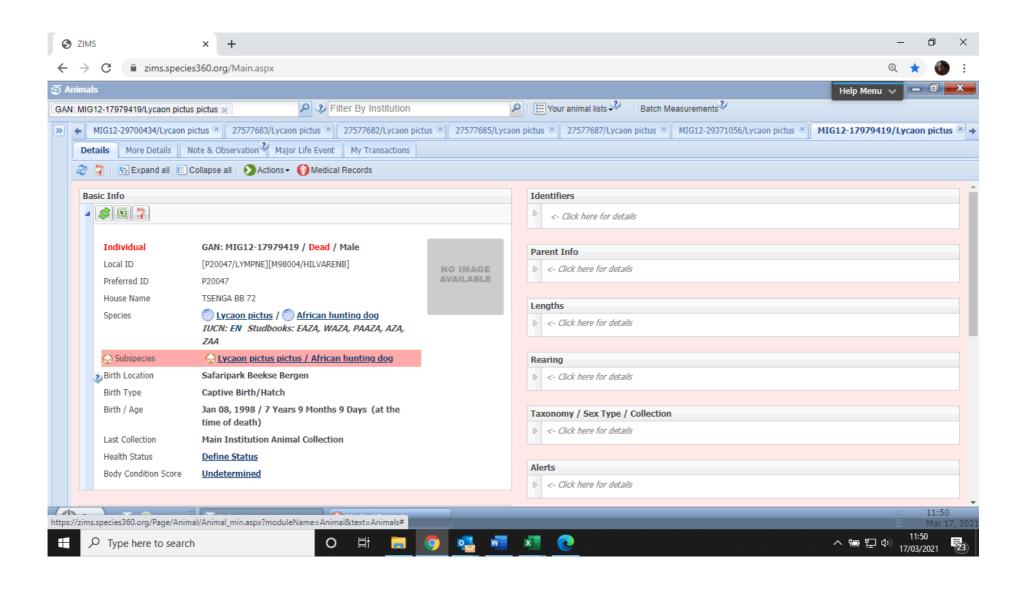












Annex 7d – Details of the identities of all African wild dogs represented in the set of samples to be exported. Addresses of places of birth: A SafariPark Beekse Bergen, 5081 NJ Hilvarenbeek, Netherlands; B Port Lympne Safari Park, Port Lympne Reserve, Hythe, Kent, CT21 4PD, U.K.; C Dublin Zoo, Phoenix Park, Dublin 8, D08 AC98, Ireland; D Warsaw Zoo, Ratuszowa 1/3, 03-461 Warszawa, Poland; E Friguia Animal Park, GP 1, Aïn Rahma, 4089, Bouficha, Tunisia; F West Midland Safari Park, Spring Grove, Bewdley DY12 1LF, U.K.; G Zoo de Pont Scorff, All. de Kerruisseau, 56620 Pont-Scorff, France; H Ree Park Safari, Stubbe Søvej 15, 8400 Ebeltoft, Denmark; I Zoo Duisburg, Mülheimer Str. 273, 47058 Duisburg, Germany; J Ann Van Dyk Cheetah Centre, R513, Brits, North West Province, 0251 South Africa.

ID	name/number	place of	date of	number
number	•	birth	birth	of vials
H20236	Mzungu/B790	Α	08-Jan-98	2
H20237	Kassama/17AB	В	01-Mar-97	4
H20612	Rafiki	В	22-Nov-93	1
H20944	Two Socks/594302	В	04-Dec-06	5
H20946	Blade/599654	В	04-Dec-06	3
P21478	Selous/053881	С	18-Oct-09	3
P21349	Ruaha	С	17-Oct-09	5
P20047	Tsenga	Α	08-Jan-98	3
P20053	Kippa/8DF0	В	13-Nov-00	1
P20077	Shue/B25E	В	13-Nov-00	2
P20078	Rhunt/A079/97F6	В	13-Nov-00	1
P20079	Krane/D481	В	13-Nov-00	4
P20080	Depti/B94F	В	13-Nov-00	1
P20082	Spot/E1AD	В	13-Nov-00	3
P20169	Tatu	D	17-Oct-04	1
P20170	Wili	D	17-Oct-04	5
P20520	Tunis	E	15-Sep-02	1
P20572	NA	В	04-Nov-05	1
P20573	Nyae Nyae/424326	В	04-Nov-05	10
P20574	Whitey/44543	В	04-Nov-05	1
P20680	Sandy/593476	В	04-Dec-06	2
P20684	Tango/599146	В	04-Dec-06	1
P20686	Blacky/595132	В	04-Dec-06	2
P20688	NA	В	00-Jan-00	1
P20789	956000000/847617	В	04-Nov-07	2
P20790	Spot-tail	В	04-Nov-07	3
P20791	Bandy	В	04-Nov-07	6
P20793	Teye/751	В	04-Nov-07	4
P20796	Tanny	В	04-Nov-07	5
P20799	Pirate/352	В	04-Nov-07	2
P20911	Vango	F	27-Oct-05	7
P21050	Snake/4749	В	24-Nov-01	4
P21052	Domino/E182	В	24-Nov-01	2
P21053	/000606BC5C	В	24-Nov-01	2
P21055	Neleh/FC90	В	24-Nov-01	1
P21056	Eva/88FF	В	24-Nov-01	1
P21057	E03F/0A9F	В	24-Nov-01	2
P21263	Socks	В	10-Nov-12	1
P21264	Scorpion	В	10-Nov-12	3
P21265	Splodge	В	10-Nov-12	2
P21266	Romeo	В	10-Nov-12	4
P21267	Mantler	В	10-Nov-12	2
P21268	Chevron	В	10-Nov-12	2
P21269	Kudu	В	10-Nov-12	4
P21301	Zuri	G	05-Jun-06	1
P21348	Nadifa	Н	12-Nov-11	4
P21350	Chobe	С	18-Oct-09	4
P21360	Ghost	В	15-Dec-13	3
P21361	Two spot	В	15-Dec-13	3
P21464	Sprench	В	12-Nov-14	2
P21465	Flash	В	12-Nov-14	1
P21467	Gecko	В	12-Nov-14	1
-				

ID number	name/number	place of birth	date of birth	number of vials
P21469	Madi	В	12-Nov-14	3
P21409 P21470	Five	В	12-Nov-14 12-Nov-14	1
P21470	Horseshoe	В	12-Nov-14 12-Nov-14	1
P21471 P21477	Kruger	С	18-Oct-09	3
P21477	Branka	I	27-Oct-10	1
P21479	NA	В	29-Dec-14	2
P21480 P21481	NA	В	29-Dec-14 29-Dec-14	2
P21481 P21482	NA	В	29-Dec-14 29-Dec-14	1
P21482 P21483	NA	В	29-Dec-14 29-Dec-14	1
P21483 P21484	NA NA	В	29-Dec-14 29-Dec-14	2
P21484 P21485	NA	В	29-Dec-14 29-Dec-14	1
P21486	NA	В	29-Dec-14	1
P21487	NA	В	29-Dec-14	1
P21489	Ace	В	27-Nov-14	1 2
P21491	Icarus	В	27-Nov-14	
P21492	Kamana	В	27-Nov-14	1
P21493	Kite	В	27-Nov-14	1
P21494	Cross	В	27-Nov-14	1
P21495	Sickle	В	27-Nov-14	1
P21496	Comma	В	27-Nov-14	1
P21590	Lenny	В	27-Dec-15	1
P21594	S	В	27-Dec-15	1
P21598	V/Tooth	В	27-Dec-15	1
P21990	NA	В	05-Nov-20	2
P21994	NA	В	05-Nov-20	2
P21995	NA	В	05-Nov-20	2
P21996	NA	В	05-Nov-20	2
P21997	NA	В	05-Nov-20	2
P21999	NA	В	05-Nov-20	2
P22045	Assegai/B50D	В	02-Nov-02	2
P22049	Falcon/82F1	В	02-Nov-02	2
P22050	Yella/EB07	В	02-Nov-02	2
P22051	Kenya/138E	В	02-Nov-02	2
P22052	Nora/140/1105	В	02-Nov-02	2
P22053	/0006201E4A/141	В	02-Nov-02	2
P22054	Lessa/142/B6AC	В	02-Nov-02	2
P22055	/0006202304/143	В	02-Nov-02	2
P22056	Psyche/02A00	В	02-Nov-02	1
P22057	Saddle/0E42/145	В	02-Nov-02	1
P22059	Bibi/94FE/146	В	02-Nov-02	2
P22070	NA	В	05-Nov-20	2
P22071	NA	В	05-Nov-20	2
P22072	NA	В	05-Nov-20	2
P22085	/0006205B85	В	02-Nov-02	2
P96018	Masai	J	06-May-95	2
P97005	Kassanga/sccsc	В	01-Mar-97	1
P97011	Kassala/1E18	В	01-Mar-97	1
P97062	Ben(guela)	J	28-Sep-95	3
P97063	Ashanti/2465	J	31-Jul-96	1
P98044	Kang/E1F60	В	15-Jun-98	3
P98047	Tchad/4CF2	В	15-Jun-98	1
Total anima	als: 103	Total	samples:	224

A. Abstract

- **i. Title:** Can vaccination protect African wild dogs from canine distemper? Addressing a conservation emergency.
- **ii. Rationale:** The African wild dog is a globally endangered species, with fewer than 700 packs remaining in the wild. Canine Distemper Virus (CDV) was assumed to pose little risk to the species, because field studies in many parts of Africa had found healthy animals with antibodies to the virus, suggesting that wild dogs often survived the disease. Then suddenly, in the past three years, six separate fatal CDV outbreaks have been recorded, with the worst all but wiping out the largest population in the northern hemisphere. Previous MAF-funded research shows that CDV cannot easily be controlled by vaccinating domestic dogs, suggesting that wild dogs themselves might need to be vaccinated where CDV risks are most acute. Unfortunately, no safe and effective vaccination protocol has been devised for use on free-ranging wild dogs. This project aims to identify such a protocol, to inform urgent conservation efforts.
- **iii. Hypothesis/Objectives:** We aim to test the hypothesis that extinction risks to African wild dog populations can be reduced by vaccination against CDV. Our project has three specific objectives:
- (1) Working with captive African Wild Dogs, *identify a protocol* for vaccination against CDV which is safe, effective, and likely to be practical for field use.
- (2) Working with free-ranging African Wild Dogs, assess the safety, efficacy, and practicality of the CDV vaccination protocol
- (3) Using an existing mathematical model, and findings from Objectives 1 and 2, quantify impacts on extinction risk to develop *guidelines for CDV management* in African wild dog populations.
- **iv. Experimental design and methods:** Our project has three components. First, a *captive trial* designed to test whether presumed protective antibody titres can be triggered on a single handling event, appropriate for field use. Second, a *field trial* in South Africa designed to evaluate whether the captive protocol is safe and likely effective for free-ranging wild dogs, and whether it can be implemented with a reasonable level of effort. Third, we shall use these new data to parameterise an existing *dynamic model of CDV dynamics* and control, to identify the management approaches most likely to reduce population extinction risks, allowing us to develop guidelines for managing the conservation impacts of this deadly disease.
- v. Preliminary Data: Attitudes to CDV vaccination of wild dogs have been shaped by a fear that modified live vaccines, widely used in domestic dogs, can kill wild dogs. Our unpublished field data suggest that an alternative vaccine type, recombinant vaccine, is safe but probably ineffective. However, zoo vaccination records suggest that the risks of modified live vaccine have been greatly over-estimated, with no confirmed vaccine-induced deaths among 135 pups given the vaccine. Our modelling suggests that, if safe and effective, CDV vaccination could reduce extinction risks by 40%. Our project therefore focuses on evaluating the safety and effectiveness of modified live CDV vaccine.
- **vi. Expected Results:** Our project is specifically designed to inform time-sensitive conservation decisions for this endangered species. As well as producing three peer-reviewed papers, we anticipate developing guidelines for CDV management to be shared with conservationists throughout Africa. Our project also showcases the way that zoo-based research can support active conservation in the field, helping to link conservation efforts in developed and developing countries.
- vii. Budget: Year 1 \$44,872 Year 2 \$48,956 Grand Total: \$93,828
- **Timeline:** Our project is carefully designed to be completed within two years, to inform conservation management decisions as rapidly as possible. Wild dog births are highly seasonal, so we can realistically complete Phase 1 of the captive trial within the first 6 months (Objective 1), allowing us to commence a 15-month field trial of the most promising vaccination protocol (Objective 2). Field and captive data can be readily integrated into an existing model of CDV dynamics and control, helping us to develop management guidelines (Objective 3) within our two-year timeframe.
- viii. Potential impact for Animal Health: Our project has enormous potential to improve both animal health and wildlife population viability. Conservation managers from Kenya and South Africa are partners on the proposal, poised to implement is recommendations as soon as they become available. Our approach could also shape disease management planning for other endangered species.

B. Title Page

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C. Resubmission

This proposal is not a resubmission of a previously-reviewed proposal.
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D Study Proposal

i. Hypothesis and Objectives

Our project aims to test the hypothesis that extinction risks to African wild dog populations can be reduced by vaccination against Canine Distemper Virus. Our project has three key objectives, thus:

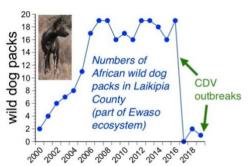
- (1) Working with captive African Wild Dogs, *identify a protocol* for vaccination against Canine Distemper Virus which is safe, effective, and likely to be practical for field use.
- (2) Working with free-ranging African Wild Dogs, assess the safety, efficacy, and practicality of the vaccination protocol
- (3) Using an existing mathematical model, and findings from Objectives 1 and 2, quantify impacts on extinction risk to develop *guidelines for CDV management* in African wild dog populations.

ii. Justification, Significance and Literature Review

The need to manage Canine Distemper risks to African wild dog populations

The African wild dog (*Lycaon pictus*) is an endangered species threatened by infectious disease, and Canine Distemper Virus (CDV) appears to be a growing threat. Habitat loss and deliberate killing

have extirpated the species across 93% of its historic range₁, and climate change now compounds these threats to the <700 packs that remain₂. Infectious disease has long been recognised as a threat to wild dog populations. The rabies-related loss of wild dogs from the iconic Serengeti National Park in 1991₃, and



several subsequent whole-pack deaths linked to rabies₄₋₇, led to rabies being considered the African wild dog dying of confirmed Canine Distemper Virus. Ewaso ecosystem, Kenya, 12 Oct 2019. Photo: Shivani Bhalla.

greatest disease threat to the species. In contrast, CDV exposure was often nonfatal, with multiple field studies reporting seropositivity in apparently healthy animals₇₋₁₁. Although sporadic whole-pack deaths were reported_{12,13}, the only major confirmed outbreak was in a captive breeding centre₁₄. However, in 2016 CDV killed whole packs at three separate sites in South Africa_{15,16}, and the following year another pack

succumbed in Tanzania's Serengeti ecosystem. In 2017 a major *CDV epidemic caused the near-extinction of the wild dog population* in the Ewaso ecosystem in Kenya, killing ≥20 packs₁₇. By 2019, three packs had re-formed from the remnants of the Ewaso population, but CDV killed one of them. Evidently, *CDV is a serious and emerging threat* to this endangered species.

Because CDV is a canine pathogen, there have been several attempts to reduce wildlife CDV risks by vaccinating domestic dogs_{17,18}. However, this approach may have limited effectiveness, since

- (i) Domestic dog populations may not act as reservoir hosts for CDV. Mass dog vaccination around the Serengeti reduced CDV incidence in dogs but not in wild lions₁₈, suggesting that the virus was persisting in wildlife. Likewise, molecular analyses suggest that CDV affecting tigers in the Russian far east came from wildlife, rather than domestic dogs₁₉. MAF-funded research within the Ewaso ecosystem showed that CDV was not persisting in local domestic dogs₂₀, and that wild dogs with greater opportunities for domestic dog contact were not more likely to have been exposed to CDV₉.
- (ii) Even if domestic dogs did act as a CDV reservoir, controlling infection would be challenging because CDV, like other morbilliviruses (e.g. measles₂¹, phocine distemper virus₂₂), may persist only on very large geographic scales, and control may require vaccination coverage of ≥95%₂₃.
- (iii) While governments are committed to eradicating dog-mediated rabies by 2030₂₄, CDV has no human health impacts, and hence no eradication strategy. For this reason, any local CDV vaccination of domestic dogs would need to be maintained by conservationists in perpetuity.

Since vaccination of domestic dogs appears to be an imperfect way to reduce CDV threats to African wild dogs, in some circumstances vaccination of wild dogs may need to be considered.

Choice of CDV vaccine

Three categories of vaccine are currently available: inactivated, modified-live, and recombinant.

Modified-live vaccines (MLVs) are highly effective in domestic dogs₂₅,₂₃, and can prompt seroconversion in captive African wild dogs₂₆. Nevertheless MLVs have occasionally induced clinical distemper in a number of nondomestic carnivores_{27,28}, including African wild dogs₂₉₋₃₁. Risks appear to be low, however₃₂, and MLVs are widely used on captive African wild dogs in Europe.

Inactivated vaccines have been used on African wild dogs in captivity to avoid all risk of vaccine-induced distemper₃₂. However, they have consistently failed to provoke serological responses_{26,33}, and failed to prevent CDV from killing 49 of 52 wild dogs in a captive facility in Tanzania₁₄.

Recombinant vaccines likewise cannot induce distemper, because they do not contain a complete viral genome. Such vaccines have induced seroconversion in African wild dogs₃₄, and other sensitive species₃₅. However, a trial in captive tigers showed that recombinant vaccines produced weaker immune responses than MLVs₃₆. Moreover, use of the recombinant CDV vaccine on free-ranging wild dogs in an outbreak situation might be difficult, because the import of GMOs is forbidden in some African countries and requires time-consuming permitting in others₃₇. Moreover, the vaccine has faced repeated supply problems_{38,39}.

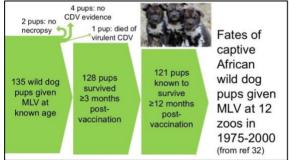
As MLV appears to be immunogenic, low risk, and widely available in Africa, it is a strong candidate for use in protecting free-ranging populations of African wild dogs threatened by canine distemper. However, there is currently no established vaccination protocol suitable for field use.

Choice of vaccination protocol

Like domestic dogs, most captive wild dogs are given their first CDV vaccinations as young puppies, although maternal antibodies may neutralise the vaccine₄₀. To ensure vaccine "take", doses are repeated at 2-4 week intervals until 16 weeks of age₄₀. However, because vaccination of freeranging wild dogs would require darting, it would have to target older animals, as darting would injure young pups. If a domestic dog receives its first vaccinations at >20 weeks, after maternal antibodies have waned, a single MLV dose is protective₄₀. If the same were true in wild dogs, MLV might be able to protect free-ranging wild dogs after a single handling event. However, this point is uncertain because wild dogs which seroconverted in published studies had previously been given MLV₄₁ or inactivated₂₆ CDV vaccine. If a single dose proved insufficient, immune responses might be strengthened by giving multiple doses simultaneously, as in rabies control_{42,43}. We anticipate that a double dose would be safe, because the dose for a 5-month pup (2ml/15.9kg₄₄ or 0.13ml/kg) would be lower than that for a 2-month pup (1ml/6.1kg or 0.16ml/kg), and that for an adult of a small domestic dog breed (e.g. adult chihuahua, 1ml/3kg or 0.33ml/kg). The monovalent MLV contains no adjuvant₄₅ which some have tentatively linked to adverse vaccine reactions in small domestic dog breed₅₄₆. It may thus be helpful to evaluate both single and double doses of MLV in African wild dogs.

iii. Preliminary Data

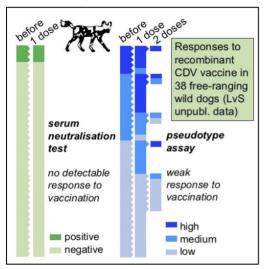
We have previously evaluated the **safety** of **modified live CDV vaccine** in **captive** African wild dogs, by requesting zoos' vaccination records for the period 1975-2000, and comparing individual



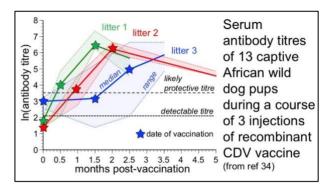
survival using studbook data_{47,48}. This work₃₂ revealed no cases of confirmed vaccine-induced distemper among 135 pups given MLV for the first time at known age, suggesting a risk of 0% (exact binomial 95% confidence interval [CI] 0-2.7%). If one pup which died in 1983 of virulent CDV (likely not a vaccine strain) and two pups with no reported cause of death are conservatively assumed to have died of vaccine-induced distemper, the risk would be 2.2% (CI 0.5-6.4%).

We have also evaluated antibody responses to **recombinant CDV vaccine** in **captive** wild dog pups, showing that this vaccine is safe and immunogenic in captivity, if delivered by a parenteral route₃₄. All pups without detectable maternal antibodies at the start of vaccination showed strong, rising titres after a single dose, although those with maternal antibodies required multiple doses₃₄.

However, our evaluation of immune responses to recombinant CDV vaccine in free ranging wild dogs showed a much less promising immune response (van Schalkwyk, unpubl. data). Wild dogs in 20 packs given recombinant vaccine in Kruger



National Park, South Africa. showed no immune



response detectable by serum neutralisation tests. A pseudotype assay on the same samples showed evidence of a weak response: only 11 of 38 individuals had high titres after a single vaccine dose, of which four had had high titres pre-vaccination (see left). These (unpublished) data raise concerns about the utility of recombinant CDV vaccine for free-ranging wild dogs.

Nevertheless, our team's *population modelling* work

Impact of

simulated wild dog

vaccination

extinction

risk, shown as odds ratio

relative to no

management

From ref 50

low CDV

55 60

wild dogs vaccinated

on population

1.7-over 50 years 1.9-1.9-1.9-

1.3 bersistence of 1.1

odds ratio ratio

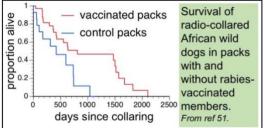
0.9

0.8

40

suggests that, if we could identify an

effective vaccination protocol, it would have conservation benefits. In a model (see right) simulating wild dog population dynamics (including within-and between-pack dynamics₄₉), vaccination was associated with >40%



reductions in extinction risk if CDV could cause high mortality 50.



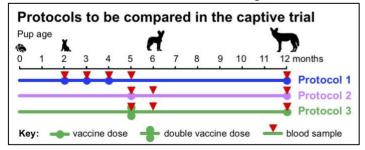
previously used *field trials* to evaluate vaccine safety. including a trial at a site in Kenya₅₁ which showed that rabies

vaccination was safe for use in African wild dogs (see left).

iv. Experimental methods and design

Objective 1: Working with captive African Wild Dogs, identify a protocol for vaccination against Canine Distemper Virus which is safe, effective, and likely to be practical for field use.

Based on the evidence presented above, we propose evaluating MLV as a tool for protecting wild dogs against CDV. For simplicity, we shall use a monovalent CDV vaccine such as Neovac-D. In captivity, we plan to compare three protocols, designed to maximise information relevant to managing CDV risks in the field, while minimising both the health risks to captive animals, and disruption to zoo



staff. The effect of each protocol will be measured using samples collected at the time of vaccination, and approximately 30 days after the end of each vaccination course. **Protocol 1** is the standard zoo protocol (doses at 2, 3, and 4 months of age, with a booster at 12 months). It is not suitable for field use, but it provides a baseline against which other protocols can be compared.

Protocol 2 is the approach recommended for older domestic dog pups (a single dose at ≥5 months with a booster at 12 months). In the field, vaccinated animals would be >10 months old, but we

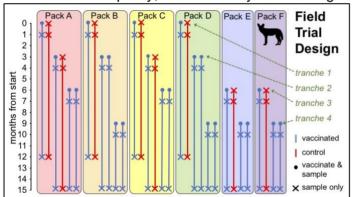
propose evaluating this protocol in captivity on pups aged 5 months, to avoid prolonging the period of CDV risk in animals which have cleared their maternal antibodies.

Protocol 3: is a modification of Protocol 2, using two simultaneous doses of vaccine at different sites. The captive trial will be conducted at a minimum of four participating zoos, coordinated by the Association of Zoos and Aquaria (AZA), with all vaccination and blood sampling performed by the zoos' own veterinary staff. In Phase 1, two litters (approximately 18 pups, based on average captive litter size₄₇) will be allocated to Protocol 1 while, in two other litters, equal numbers of pups will be randomly assigned to receive either Protocol 2 or Protocol 3 (approximately 9 pups per protocol). If no ill-effects are observed, the protocols will be repeated with another four litters in Phase 2.

Blood samples will be centrifuged on the day of collection, with serum stored at -20°C pending analysis. CDV antibody titres will be measured at the Animal Health Diagnostic Center at Cornell University, using a serum neutralization test and the Onderstepoort virus strain. A published study suggests that the proportion of adult wild dogs with protective titres after a single MLV dose (8/8₂₆) falls between 100% and 63% (exact binomial confidence interval). Our study could improve the precision of this estimate to 90-100% (Protocol 1, 36 pups) and 81-100% (Protocols 2 & 3, 18 pups each). We shall compare individuals' titres before and after vaccination using Wilcoxon signed rank tests, and will use Mann-Whitney U-tests to compare post-vaccination titres between protocols; we prefer nonparametric statistics because titre data often include values (e.g., <8, >160) which cannot be analysed using parametric statistics. Test results from Phase 1 (expected May 2021; see timeline below) will determine the protocol to be used in the field trial.

Objective 2: Working with free-ranging African Wild Dogs, assess the safety, efficacy, and practicality of the vaccination protocol

The field trial will be conducted in Kruger National Park, South Africa. It will evaluate whether free-ranging wild dogs mount a strong immune response to MLV after a single handling event, and whether vaccinated individuals survive as well as unvaccinated pack-mates. Although the vaccine will have been tested in captivity, our field study will reflect guidance on designing "first in man" trials₅₂, initially



vaccinating a small number of animals and increasing numbers if no ill-effects are found.

To measure *vaccine safety*, we plan to compare the survival of vaccinated and control animals, focusing on the first month of monitoring since all recorded cases of vaccine-induced distemper have occurred 10-22 days post-vaccination₂₉₋₃₁. Animals will be recruited to the trial in four tranches. For *tranche 1*, two yearling animals will be darted in each of four packs, with one of each pair randomly selected to receive vaccine (either single or double

dose, depending on captive trial findings) and a mortality-sensing satellite-linked GPS collar, while the other remains unvaccinated and is fitted with a mortality-sensing VHF collar. Both animals will be blood sampled on initial collaring and again 1 month and 12 months later. We shall monitor mortality daily, and will attempt visual observations every 2-3 days in the first month post-vaccination. Any signs of ill health will prompt daily visual monitoring and immediate consultation with veterinarians. Any mortality signals will trigger immediate attempts to retrieve a carcass for necropsy, and screening for CDV using histologic examination, virus isolation, reverse transcriptase-PCR, and nucleotide sequencing at Cornell. If CDV is detected, vaccinations will be paused pending discussions within the team, and with SANParks, about how to proceed. If none of the vaccinated animals dies of CDV in the first three months of monitoring, *tranches 2* (six vaccinated, two control), 3 (six vaccinated, two control), and 4 (eight vaccinated) will be recruited at three-month intervals, as illustrated above. Using continuity correction and α =0.05, this study design should provide 80% power to detect mortality increases among vaccinated animals of 35% in the first month of monitoring, and 8% in the full 312 dog-month monitoring periods3.

To measure *likely vaccine effectiveness*, we plan to compare CDV antibody titres (measured at Cornell using serum neutralisation tests) in vaccinated animals one month post-vaccination with their own pre-vaccination titres, and with simultaneous titres of unvaccinated control animals, using nonparametric statistics as for the captive trial. Our proposed sample size (24 vaccinates and eight controls) should provide 85% power to detect the difference between conservatively-estimated baseline CDV seroprevalence and the expected proportion of seropositive animals post-vaccination₅₃. We use similar methods to compare vaccine titres 6-12 months post-vaccination, providing some information on likely *duration of protection*.

To measure the *practicality of vaccine delivery*, we shall record the effort (in person-hours, vehicle mileage, and other costs) required to deliver each vaccination and each visual observation.

Objective 3: Combine the findings from Objectives 1 and 2 with ongoing mathematical modelling to develop and agree *guidelines for CDV management* in African wild dog populations

Our captive and field trials will provide data on the proportion of wild dogs likely to be protected from CDV by MLV, and the duration of such benefits. We shall parameterise our existing population model₅₀ with these new data to estimate the likely consequences for population persistence of different vaccine coverage scenarios. Our population model has the capacity to represent vaccine coverage as a function of pack encounter probability per unit effort₅₀; hence, we can use our data on the effort and costs associated with delivering each vaccination to parameterise our model. We can then compare the likely conservation gains associated with specific investments in effort.

We shall use the outcomes of this empirical and modelling work, together with our own and others' previous evidence, to develop guidelines on CDV management in African wild dog populations.

v. Timeline

Our project is designed to be completed within two years, as information for wild dog conservation is needed on the shortest practicable timescale. Specific timings reflect the seasonal nature of wild dog reproduction, with most pups born in November in the northern hemisphere 1, and the need to complete Phase 1 of the captive trial before starting the field trial.

Year:						202	21									2022	2					
Month:	っ	F	МА	М	J	J	Α	S	0	Ν	D	J	F	М	Α	М	J,	JΑ	S	0	Ν	D
Objective 1 – Captive t	ria	l oi	f CDV	/ Vá	acc	ina	itio	n														
USA pup age (months)	2	3	4 5	6	7	8	9	10	11	12	1	2	3	4	5	6 7	8	9	10	11	12	1
Protocol 1 vaccinations				_																		
Prot 2 & 3 vaccinations																						
sample screening																						
Objective 2 – Field trial	l o	f C	DV va	acc	ina	atic	n															
adult vaccinations																						
adult sampling																						
sample screening																						
monitor survival																						
Objective 3 – Develop	gu	ide	lines	fo	r C	DV	m	ana	gem	ent i	n A	\fri	cai	7 W	rild	dog	IS					
parameterise model		•						•	•	•	•	_						•				
develop guidelines																						

vi. Expected Results

Our project is specifically designed to inform time-sensitive conservation decisions for this endangered species. Co-Is Mutinda and Njoroge will use the findings of this project to decide whether to vaccinate the few wild dogs in the Ewaso population devastated by CDV. We plan to disseminate our findings to other wildlife decision-makers throughout the networks of the Rangewide Conservation Programme for Cheetahs and African wild dogs, which PI Woodroffe co-founded, and the IUCN/SSC Canid Specialist Group. We anticipate three scientific papers from this work (one per objective). We shall share project progress and findings with the public through social media (e.g. the PI has >7,500 twitter followers with up to 500,000 impressions per month, Cincinnati Zoo has >200,000 followers) and potentially through zoo signage, linking captive research to field conservation.

E. Sample size Calculation

Sample Size Estimate



If you do not believe it is appropriate for your grant application to include sample size estimates, please include a brief explanation here.

This project entails two principal statistical comparisons, comparing

- seroprevalence among vaccinated and unvaccinated animals in the field trial
- survival among vaccinated and unvaccinated animals in the field trial

For the captive trial, unvaccinated controls cannot be justified because vaccination is an important part of zoo animal husbandry. Preliminary data strongly suggest that all vaccination protocols are likely to lead to high proportions of animals seroconverting. This means that absolute differences between protocols in effect sizes are likely to be small (and irrelevant in management terms). In choosing sample sizes for the captive trial we have therefore focused on improving the precision of the estimated effect size, to better parameterize our population dynamic model, rather than focusing on comparisons between vaccination protocols.

Subsequent analyses therefore refer to the proposed field trial.

If sample size estimates are necessary for your study, continue filling out this form.

Proposed study design: Field trial of effectiveness: randomized controlled trial

Eg: case-control, randomized control trial Sample type: two groups (vaccinated vs control)

Eg: a single group, two or more independent groups, matched pairs

Analytic approach: comparison of proportions seropositive among vaccinated and unvaccinated individuals; can use chi-squared test.

Eg: t-test, logistic regression, non-parametric analysis

Statistical test: primary test will be a comparison of proportions seropositive one- and 12-months post-vaccination

Eg: a mean, a difference in means, a proportion, an odds ratio, a risk ratio

	Insert response:
Power (\Box) :	85%
Significance level (□):	0.05
Estimated (or desired) standard deviation:	NA (proportions)
Estimated detectable difference (if applicable):	42%
Estimated between (within) subject correlation (if applicable):	NA
	24 vaccinated, 8
Estimated Sample size:	controls

Please discuss any additional assumptions that went into your power calculation.

The calculation includes a continuity correction, and is two-sided. The seroprevalence in the reference group (unvaccinated controls) is conservatively estimated as the upper exact binomial confidence limit for the most recent measure of seroprevalence (3/38 seropositive without vaccination, exact binomial CI 1.7-21.4%, therefore conservatively assume baseline seroprevalence of 21.4%). The seroprevalence in the vaccinated group is conservatively estimated as the lower exact binomial confidence limit for the only estimate of seroprevalence post-vaccination (8/8 seropositive post-vaccination, exact binomial CI 63.1-100%, therefore conservatively assume a post-vaccination seroprevalence of 63.1%). This

calculation gives a conservatively-estimated expected difference of 42% between the vaccinated and unvaccinated groups. A sample size of 24 vaccinates and eight controls provides 85% power to detect such a difference.

Proposed study design: Field trial of safety: randomized controlled trial

Eg: case-control, randomized control trial Sample type: two groups (vaccinated vs control)

Eg: a single group, two or more independent groups, matched pairs

Analytic approach: Chi-squared test (over 1 month); Cox proportional hazards (over 15 months)

Eg: t-test, logistic regression, non-parametric analysis

Statistical test: primary test will be a comparison of proportions surviving one month post-vaccination, using a simple chi-squared test

More complex secondary analyses will be conducted to explore survival over long periods, using Cox proportional hazards models to account for possible effects of pack identity, age, and sex.

Eg: a mean, a difference in means, a proportion, an odds ratio, a risk ratio

Insert response: Power (\square) : 80% Significance level (\square): 0.05 Estimated (or desired) standard deviation: NA (proportions) 35% over first month 8% over full study Estimated detectable difference (if applicable): period Estimated between (within) subject correlation (if applicable): NA 24 vaccinated, 8 **Estimated Sample size:** controls

Please discuss any additional assumptions that went into your power calculation.

The calculation includes a continuity correction, and is one-sided because the field trial is designed to evaluate whether vaccination causes excess mortality, not whether it reduces mortality.

F. Animal Involvement Justification



Animal Involvement Justification Form

All studies receiving funding must adhere to MAF's Health Study Policy for Animals Involved in Research, which was written to ensure that every animal involved in a MAF funded health study receives excellent, compassionate care throughout the study. Please review MAF's Health Study Policy prior to filling out this form. Click here for the full Health Study Policy.

All MAF studies will be reviewed by MAF's Animal Welfare Advisory Board (AWAB) for adherence to MAF's Health Study Policy. All studies must be approved by the AWAB before funding can be awarded.

Note: This form must be completed in its entirety, at time of submission. Incomplete forms may result in disqualification of the proposal.

SECTION 1: This section must be filled out, regardless of animal use (including invertebrates)

A. Does this study...

- a. Involve live animals (including client-owned animals)? (yes/no) yes
- b. Utilize archived samples that were originally obtained from live animals? (yes/no) ___no
- c. Utilize samples that will be obtained prospectively from live animals? (yes/no) yes
- d. Utilize archived samples that were originally obtained from animals that died from natural causes or were euthanized for clinical reasons prior to sample collection? (yes/no) no
- e. Utilize samples that will be obtained prospectively from animals that die from natural causes or are euthanized for clinical reasons prior to sample collection? (yes/no) yes
- f. Utilize archived samples that were originally obtained from animals that were euthanized for an unrelated study prior to sample collection? (yes/no) no
- g. Utilize samples that will be obtained prospectively from animals that will be euthanized for an unrelated study prior to sample collection? (yes/no) no
- h. Utilize samples that will be obtained from animals that will be euthanized for the proposed study prior to sample collection? (yes/no) no
- i. Utilize samples obtained from a third-party vendor (yes/no) no

SECTION 2: If you answered yes to any of the above, this section must be filled out in its entirety

A. Describe, in detail, all animal involvement proposed in this study. This includes all live animal involvement (including client-owned animals), retrospective live animal involvement for sample collection and prospective live animal involvement for sample collection.

This study involves animals both in the wild and in captivity.

In captivity, all study animals will be young pups, and all will be vaccinated against CDV (albeit using different protocols) and blood-sampled for antibody screening. The way that these

procedures will be implemented are likely to vary based upon the age of the pups, the training schedule in place at each zoo, and the disposition of the pups' mother. Younger pups (2-4 months) can usually be manually held or restrained during vaccination and sampling. In older pups, training should allow vaccination and sampling to be performed without the need for chemical restraint. Many zoos perform an annual health examination under anaesthesia, and this would provide an opportunity to vaccinate and sample older animals (e.g. at 12 months of age).

In the wild, all study animals will be free ranging. All handling will be conducted in collaboration with SANParks, under provincial research permits, according to guidelines of the IUCN/SSC Canid Specialist Group, and following a protocol approved by the Ethics Committee of the Zoological Society of London.

All handling of free-ranging wild dogs will be closely overseen by experience wildlife veterinarians. For the purposes of this study, all wild dogs will be captured by darting from a vehicle. Darting is conducted using a CO₂-powered rifle (Dan-Inject JM) at distances of ≤15m, targeting the large muscle mass in the hindguarter of a stationary standing or sitting animal. No darts are fired where there is a risk of hitting another animal. Darted wild dogs typically move 10-30m away from the location where they were darted, then sit down again before becoming recumbent. Darted animals do not show any evidence of pain (e.g. vocalizing, running away, licking the dart site, subsequent avoidance of the project vehicle). Other pack members typically do not respond at all, or move a short distance with the darted animal; this behaviour regularly

allows two animals to be darted on a single event.



All immobilizations will take place between dawn and early afternoon, to avoid the risk of lion predation on disoriented dogs that could occur at nightfall. Immobilized animals are kept in the shade and cooled with water if deemed necessary.

Wild dogs are immobilized with a combination of medetomidine (Domitor; approximately 26mg/kg) and ketamine (approximately 2.6 mg/kg) which induces anesthesia within 5 minutes, lasting 45-60 minutes. Moisturizing eye ointment is administered immediately on immobilization to prevent drying of the corneas, and all immobilized animals are blindfolded throughout handling.

Once heart and respiratory rates start to rise, or 60-75 minutes following administration of the initial dose (whichever is the sooner), medetomidine anesthesia is reversed with an intramuscular or intravenous injection of atipamezole (Antisedan; 130µg/kg). This usually leads to the dog standing within 10 minutes of administration, with full coordination attained within a further 10 minutes. Dogs are monitored closely throughout this period. Other pack members usually remain nearby during



handling, and immobilized animals are often seen to be reunited with their pack within a few minutes of reversal (see left). No aggression towards recovering animals has ever been witnessed. All immobilized animals have remained with their packs for at least several weeks after handling.

< Pack members return to greet a newly radio-collared wild dog minutes after reversal drugs have been administered

During the period of immobilization, blood samples are collected from the jugular vein using vacutainers. In addition to collection of samples, full biometric measures are taken. These comprise weight (measured using a spring balance), head-body length, tail length, ear height, neck diameter, chest girth, hindfoot length, and the length of the carpals, metacarpals, radius, humerus and scapula. All animals are photographed to permit individual recognition using their individual coat patterns. Tooth wear (an approximate measure of age) is also recorded. Reproductive status

is noted (pregnancy and lactation status for females, testis length and width for males; note that females thought likely to be pregnant are never immobilized). Any injuries sustained during capture are also recorded if they occur.

All wild study animals will be fitted either with a mortality-sensing satellite-linked GPS collar (≤300g), or with a conventional mortality-sensing VHF radio-collar (≤300g), to facilitate monitoring. All collars will be removed at the end of the study.

B. If this study involves archived samples describe, in detail, the nature and origin of all proposed archived sample use.

This study does not use archived samples

C. List the <u>USDA category</u> (B, C, D, E) for pain and distress. This includes the USDA category pertaining to previous animal involvement, which yielded archived sample collection: <u>C</u>

Attention: "N/A" will not suffice as a selection.

D. State the status of your IACUC approval. If approval is pending or if IACUC approval is exempt, please explain.

Note: The entire IACUC protocol and approval letter will be required before funding can be awarded. If biological or archived samples will be utilized, IACUC approval for original sample collection, or a letter stating that the study was exempt, will also be required.

IACUC approval for this proposal is pending. However, approval from the Ethics Committee of the Zoological Society of London (equivalent to IACUC) is in place for the field trial element, which we originally intended to conduct in Kenya. The field trial element has been moved to South Africa because Canine Distemper Virus devastated the intended study population in Kenya. Ethical approval will be secured from ZSL and SANParks before the study begins. Ethical approval will also be sought from participating zoos; however, as it is not yet known precisely which zoos will have wild dog litters in 2021-2022, this approval cannot be sought until a later date.

E. Describe how all animals included in the study will be acquired (e.g., client-owned, USDA licensed breeder, institutional "herds" or "colonies", etc.). This includes describing how all animals were acquired for retrospective samples and/or will be acquired for prospective sample collection.

Animals involved in the captive study will be captive-bred wild dogs born at zoos as part of the AZA's Species Survival Program. These captive animals will remain the property of the participating zoos. In the unlikely event of an individual transferring between zoos in the course of the study (most between-zoo transfers occur at >12 months of age and our study involved animals age 2-12 months), we shall request continued participation in the study until the individual reaches 12 months of age.

Animals involved in the field study will be free-ranging in Kruger National Park, South Africa. These animals will remain in the wild for the duration of the study, being immobilized briefly for vaccination and/or blood sampling before being released the same day at the same location.

G. Describe how many animals will be included in this study. If more than one species, please explain.

We plan to involve a total of 72 animals in the captive trial, and 32 in the field trial, giving a project total of 104 African wild dogs.

H. Summarize the numerical justification of animals included in this study.

The numbers of animals included in each element of the project have been carefully chosen to balance the numbers of animal used (which should be minimized) against the sample size required to address the study questions and, hence, to inform wild dog conservation efforts (which should be maximized). In performing our power calculations (described in a dedicated section), we have therefore taken account of the need to obtain estimates with adequate precision, while minimizing the number of animals involved. We have also accounted for the fact that, in the field study especially, some individuals may die from causes unrelated to the study (such as predation), requiring a slightly larger sample size to provide adequate precision.

I. Describe how all procedures with animals will be conducted with appropriate consideration of animal welfare, including the use of anesthesia or analgesia, humane handling techniques and best veterinary practices. This includes procedures with client-owned animals and animals which occurred retrospectively during sample collection.

Considerations of animal welfare influence every element of project procedures.

When darting, animals could become injured by a misplaced dart. This is avoided by exercising extreme caution when darting, firing only at short range, and when the position of the target animal, and other nearby animals, is such that a dart which goes high or low, or is moved laterally by the wind, is likely to miss entirely rather than hit another animal or a body part which could be harmed. Darting accuracy is maintained by avoiding darting on windy days, regular practice, and frequent checking of gun sights.

When collaring, animals might be harmed if over-large collars were fitted. The collars used are similar to those used on other wild dog projects and constitute 1.2-1.5% of body weight. Animals might also be harmed during anaesthesia by a major drug overdose. This is avoided by using immobilizing drugs with a wide safety margin, using doses which have been refined through field experience to be the lowest needed to achieve immobilization, and reviewing drug doses on an ongoing basis. Reversal agents are kept on hand throughout immobilization. Animals' pulse and respiratory rates, and SpO2 where possible, are monitored through immobilization, with early reversal or administration of respiratory stimulant possible should this appear necessary. Animals might also over-heat during immobilization. This is avoided by keeping animals in the shade, and monitoring body temperature throughout anaesthesia. Animals are cooled with water (either onto the skin or by wrapping in wet towels) when temperature appears elevated.

Animals might be harmed by sampling if too large a quantity of blood were collected. The maximum volume collected on first capture (30ml) constitutes 0.1% of body weight, substantially less than the 1% suggested by guidelines as the maximum that can be removed in the course of repeated sampling.

Animals might over-heat during recovery if they move out of the shade while still somewhat disoriented. This is avoided by attempting to minimise the period of disorientation through careful choice of drug doses and administration times. The doses of immobilizing drugs have been refined to use the minimum dose of ketamine (which is not reversible) needed to achieve recumbency. Careful monitoring of pulse, respiration rate, eye position, muscle tone, blink response etc is used to assess depth of anaesthesia and to delay administration of atipamezole (the reversal agent for medetomidine) as long as possible. This means that, on removal of the effects of medetomidine at reversal, animals are left with a very low residual dose of ketamine, minimising the length of the recovery period. Animals are monitored closely during recovery to allow intervention should ill effects be detected. However, no such problems have been encountered in over 200 past immobilizations. At release, animals are often disoriented for a period of one or two hours, and might be harmed by larger carnivores (e.g. lions and hyaenas), or by people. Minimising the length of the recovery period (see above) reduces these risks. In addition, all immobilizations occur in daylight, ideally in the morning but in all cases several hours before dusk, so that recovery periods do not coincide with the (nocturnal) activity period of lions and hyaenas.

J. Describe the environment and housing conditions (quality of life) in which animals will live throughout the duration of the study (species-appropriate exercise, enrichment, socialization, veterinary care, etc.). This includes client-owned animals and animals that were retrospectively utilized during sample collection.

In the captive study, wild dog pups will live in AZA-accredited zoos. AZA-accreditation demands a high standard of care, including best practices in husbandry, enclosure design, enrichment, socialization, health care, nutrition, and health & welfare monitoring (AZA Canid TAG. *Large Canid (Canidae) Care Manual*. https://www.speakcdn.com/assets/2332/largecanidcaremanual2012r.pdf). We are therefore confident that animals in our captive study will have the best quality of life that can be provided in a captive setting.

For the field study, wild dogs will range freely with Kruger National Park. In this wild setting, they will have the opportunity to exhibit their full range of natural behaviours.

K. Describe what will happen to all animals upon completion of the proposed study. If adoption, explain the adoption plan. If other, justify the proposed plan for all animals involved. This includes animals that were retrospectively utilized during sample collection.

All animals involved in the captive trial will remain the property of the participating zoos and, on completion of the study, will continue to be maintained as part of the AZAZ Species Survival Program.

On completion of the field trial, all study animals will be immobilized for final sampling and collar removal.

L.	Does this study	induce of	or have the	potential	to induce	disease,	injury,	pain o	r distress	in	animals
	(yes/no)?	yes									

Does this study involve samples that were originally acquired as part of a study that induced or had the potential to induce disease, injury, pain or distress in animals (yes/no)? ______

If yes to either above,

a. Defend the necessity of the aspects of the experimental design that may induce disease, injury, pain or distress.

This study has the potential to induce

- disease, through a very low probability of vaccine-induced distemper. However, no cases of such disease have been reported since 1989, despite the widespread use of the vaccine in European zoos.
- injury, in the unlikely event of an accident during darting
- momentary **pain** in the course of darting free-ranging animals for anaesthesia, and in vaccinating and blood-sampling young pups in captivity without anaesthesia
- mild distress during restraint of pups in captivity.

The risk of disease or injury is very low, and the levels of pain and distress are likewise low. While efforts have been made to minimise negative impacts, these cannot be eliminated entirely. Nevertheless, we feel that these mild effects are justified, for two reasons. First, for 96 of the 104 animals to be enrolled in the study's two trials, short-term negative effects will be associated with administering a vaccine which is very likely to protect those same 96 individuals against a potentially fatal disease. Second, the project's principal aim is to develop effective conservation tools for this endangered species; hence the mild adverse effects on study animals can be balanced against the important potential conservation benefit.

b. Explain how pain and/or distress will be (or was) controlled.

The risk of inducing disease is being controlled in three ways. First, the trials outlined here are proposed only following a zoo survey which established that the risk of vaccine-induced distemper was very low. Second, the first trial is being conducted in captivity where, in the unlikely event of inducing disease, full veterinary care (and potentially euthanasia) are readily accessible to minimise any suffering. Only if the vaccination protocol proves safe in captivity will it be further evaluated in the field trial. Third, in the field trial, animals will be recruited in tranches so that, if the vaccine proves harmful, only a small number of animals will be exposed to it.

The risk of injury during darting is minimised by taking great care, to only shoot when the animal is immobile (ideally sitting down), and when there is a clear shot with no other animal behind the target.

Levels of pain and distress in captivity will be minimised by avoiding darting, instead relying, wherever possible, on either manual restraint (for young pups which are accustomed to being handled) or trained behaviour, which many zoos use to facilitate vaccination and blood sampling of wild dogs. In the wild, pain and distress will be minimised by careful approach of free ranging animals, wherever possible darting while packs are calmly resting, always approaching by vehicle rather than on foot.

c. Justify that no alternative, including clinical studies, can be used to accomplish study objectives.

This study builds upon an extensive literature on CDV vaccination of domestic dogs. However, it is apparent that responses to CDV vaccination vary between species, and even between captive and wild members of the same species. Because free-ranging African wild dog populations urgently need protection against CDV, the studies we propose can only be conducted through research on this species. However, we have sought to derive the maximum possible information from the individuals enrolled in our two trials, by using data on the individual responses of a relatively small number of animals to parameterize a simulation model representing responses in larger populations.

d. Weigh the potential benefits of this study (ie. the fact that the disease/condition to be studied is of such significance for improving the health of the species) against the potential harms to the animals enrolled in this study.

CDV has already shown its ability to devastate African wild dog populations, killing >20 packs in Kenya's Ewaso ecosystem as well as whole packs in Tanzania, Botswana, and South Africa. The major conservation benefits of finding a solution to this challenge compare very favourably with the minor harms likely to befall individual animals enrolled in this study.

M.	Is euthanasia a possible outcome in this proposed study (yes/no)?yes
	If this study involves analysis of archived samples, was euthanasia an outcome when samples were originally acquired (yes/no)?no
	8 4 3 44 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4

If yes to either above,

a. State and justify the total number of animals that will be or were euthanized.

We anticipate that no animals will be euthanized in this study. Nevertheless, we must consider the possibility that euthanasia might prove necessary if, contrary to expectation, vaccination induces clinical distemper and hence major suffering.

b. Describe the method of euthanasia.

No euthanasia of study animals is anticipated as part of the project protocol. In the unlikely event of it proving necessary to humanely dispatch a study animal in the captive trial, zoo vets would perform euthanasia by intravenous injection (https://www.avma.org/KB/Policies/Documents/euthanasia.pdf). If euthanasia were to be required in the field trial, a field veterinarian would normally dart the animal with immobilizing drugs before delivering the same lethal intravenous injection. If darting were to prove impossible under field circumstances (e.g. due to difficult terrain), euthanasia by gunshot would be considered (Longair JA, Finley GG, Laniel MA, et al. Guidelines for euthanasia of domestic animals by firearms. Can Vet J. 1991;32(12):724–726). Euthanasia by gunshot would have to be performed by a SANParks-appointed game warden with a licenced firearm, rather than by project staff.

c. Provide justification that no alternatives can be used to accomplish study goal(s).

Since Modified Live CDV vaccination has very occasionally induced distemper and death, there is a small risk of having to euthanize study animals. As described in the project proposal, alternative vaccine types have been evaluated and found to be ineffective. The only remaining alternatives are to evaluate Modified Live Vaccine, or to decide not to vaccinate wild dogs against CDV. The dangers of this latter approach became clear in 2017, when CDV killed >20 unvaccinated wild dog packs in Kenya's Ewaso ecosystem. We therefore conclude that the very low (and highly managed) risk of vaccine-induced distemper in this study compares favourably with the very high conservation benefit of developing a safe and effective protocol for protection against CDV.

d. Weigh the potential benefits of this study (ie. the fact that the disease/condition to be studied is of such significance for improving the health of the species) against the need for a terminal endpoint in this study.

This study does not involve a planned terminal endpoint. Rather, it entails a very small risk of having to implement a terminal endpoint on welfare grounds. As discussed above, the major conservation benefits of developing a safe and effective method for protecting wild dogs against CDV compare favourably with the very small risk of a terminal endpoint.

e. Provide detailed objective criteria for determining when euthanasia is appropriate or necessary.

In this study, any decision about euthanasia would be made by the responsible veterinarian (zoo-specific vets in the captive trial, co-I van Schalkwyk in the field trial) using their clinical judgement. Euthanasia would be performed only to relieve suffering and/or to prevent disease spread to other pack members.

G. Recombinant DNA/Biohazards

This project does not entail recombinant DNA m	methods or biohazards such as radiation.
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H. Facilities and Equipment

This project has all key facilities and equipment needed to deliver its three objectives.

AZA-accredited zoos within North America together support more than enough captive wild dogs to meet our research needs. As part of their AZA accreditation, all such zoos are required to keep their wild dogs with a high standard of care, and to have dedicated veterinary staff. Vaccination and blood sampling are routine parts of wild dog husbandry, so our project should add little extra inconvenience; so far, all our contacts with specific zoos about this project have met with enthusiasm.

Cornell University is supremely well set-up to screen our samples, having a world-class reputation for animal health diagnostics and for wildlife CDV diagnostics in particular.

In the field, the team in Kruger National Park will have access to key facilities including accommodation, office space, field vehicles, and internet access. Kruger has a long history of supporting wildlife research and the technician will form part of a local research community.

The population model of CDV dynamics is already constructed, requiring only updated parameters. Computing power, expertise and office space are available at ZSL's Institute of Zoology to deliver the modelling-related parts of the project.

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- ⁵⁰Smallwood, T. *Modelling multi-host viral pathogens for biodiversity conservation*. (PhD thesis, Imperial College London, in prep);
- ⁵¹Woodroffe, R *et al.* Effects of double-dose rabies vaccination on endangered African wild dogs (Lycaon pictus): a field trial. (in prep.);
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MORRIS ANIMAL FOUNDATION

J - Proposal Budget

Include subtotals, calculated indirect costs and grand totals in all applicable fields. All funds must be U.S. dollars.

Category	Year 1	Year 2	Year 3	Total	
Personnel:					
1. Technicians South African technician to help deliver field trial Salary (50% in Y1, 100% in Y2) Fringe Benefits (50% in Y1, 100% in Y2) Total Salaries & Wages	\$9,000 \$3,000 \$12,000	\$18,000 \$6,000 \$24,000	\$0	\$27,000 \$9,000 \$36,000	
Supplies, Equipment, Travel & Other Expenses:	\$620	\$590	\$0	\$1.200	
 Vaccine @ \$5: 124 doses Y1 and 116 doses Y2 Serology @ \$17: 184 tests Y1 and 200 tests Y2 Sample shipments within USA (4 in Y1, 4 in Y2) Sample shipments SA to USA (2 in Y1, 1 in Y2) GPS collars @ \$1,600 (1 per pack in Y1, 0 in Y2) GPS airtime @ \$5/month (24 mo Y1, 48 mo in Y2) VHF collars @ \$300 (18 in Y1, 8 in Y2) VHF radio receiver (1 @ \$600) Immobilizing drugs @ \$50 (40 in Y1, 56 in Y2) Other field consumables (miscellaneous) Field vehicle mileage @ \$0.5 (6000 Y1, 12000 Y2) Field accommodation @ \$12 (180 Y1, 360 Y2) Field laptop (1 @ \$800) Field cellphone @\$20 (6mo in Y1, 12mo in Y2) 	\$620 \$3,128 \$600 \$1,000 \$9,600 \$120 \$5,400 \$600 \$2,000 \$400 \$3,000 \$2,160 \$800 \$120	\$580 \$3,400 \$600 \$500 \$0 \$240 \$2,400 \$0 \$2,800 \$250 \$6,000 \$4,320 \$0 \$240	\$0 \$0 \$0 \$0 \$0 \$0 \$0 \$0 \$0 \$0 \$0 \$0 \$0 \$	\$1,200 \$6,528 \$1,200 \$1,500 \$9,600 \$360 \$7,800 \$600 \$4,800 \$650 \$9,000 \$6,480 \$800 \$360	
Total Supplies, Equipment, Travel & Other Expenses	\$29,548	\$21,330	\$0	\$50,878	
Animal Use & Care: 1. Animal Purchase Total Animal Care	\$0	\$0	\$0	\$0	
Subtotal of All Categories	\$41,548	\$45,330	\$0	\$86,878	
Indirect Costs (maximum of 8% of direct costs)**	\$3,324	\$3,626	\$0	\$6,950	
Grand Total Requested from MAF	\$44,872	\$48,956	\$0	\$93,828	

^{**} Indirect costs may be claimed only if you are charged for indirect costs by your institution for work carried out in this proposal. **You must make this calculation yourself.** If your institution charges less than 8%, claim only that amount and indicate the percentage.

K. Itemised budget justification

Our proposed budget covers only technician time, and consumables associated with data collection. We have requested no salary support for the PI or co-Is. We have also all but eliminated travel costs by avoiding international travel; we have taken this approach because our own research suggests that carbon emissions represent a major threat to African wild dog populations; however it also reduces our project costs.

We have requested 2 months of UK-based technician time (2 @ \$2,700 per month) to help coordinate the captive vaccine trial. We have also requested 18 months (18 @ \$2,000 per month) of South African field technician time, to deliver most of the monitoring for the field trial. The field technician also requires field accommodation within Kruger National Park (540 nights @ \$15) as well as a basic laptop (1 @ \$800) and cellphone charges (18 months @ \$20).

To deliver the captive trial, our principal costs are the vaccine (216 doses @ \$5), serological screening (288 samples @ \$17), and regular shipments of samples to Cornell for screening (8 shipments @ \$150).

To deliver the field trial, principle costs are GPS collars (Vectronic Aerospace, one per pack, hence 6 collars @ \$1,600) and associated airtime (\$5/collar/month, hence 72 months @ \$5). As long as there is one GPS collar per pack, other pack members can be fitted with (cheaper) conventional VHF collars (26 collars @ \$300). Monitoring collared animals also requires vehicle mileage (18,000 miles @ \$0.6), and a radio receiver (1 @ \$600). Additional costs are for immobilizing drugs, which cost approximately \$50/immobilization, hence (96 immobilizations @\$50). Vaccine is also required for the field trial (24 doses @ \$5). Serological testing for the field trial is likely to amount to (96 tests @ \$17), to be sent to Cornell in four batches (4 shipments @ \$500). Finally, we have budgeted \$500/year for miscellaneous field consumables such as notebooks, sample tubes, cool bags, etc.

L. Current and Pending Support

- i. Project ID: NE/T001348/1
- ii. Funding Source: Natural Environment Research Council
- iii. Project Title: Hot Dogs: climate change impacts in an endothermic predator
- iv. **Summary:** Climate change is contributing to the devastation of the world's biodiversity, not only through its impacts on individual species, but also by changing the way that species interact with one another. Among the world's species, large carnivores have some of the most profound ecological impacts, but are also some of the most vulnerable to climate change. Using African wild dog populations across a range of climates as our study system, our project is employing novel empirical and modelling approaches to address six specific objectives:
 - (1) Characterise how weather and climate influence individual time and energy budgets.
 - (2) Evaluate the consequences for population growth of changing time and energy budgets.
 - (3) Identify forms of phenotypic plasticity which might reduce climate change impacts.
 - (4) Investigate evolutionary adaptations which might reduce climate change impacts.
 - (5) Assess the consequences for predation of weather and climate impacts on foraging.
 - (6) Test whether insights into climate impacts on behaviour and demography alter management recommendations

Addressing these six objectives allows us to tackle fundamental questions about how individual behaviour influences population dynamics, and how climate impacts on individual species may affect ecological communities.

Project started 1 Nov 2019 and has not yet yielded results.

- v. Budget: GBP 486,223
- vi. **Timeline:** 1 Nov 2109 31 Oct 2022
- vii. **Overlap:** Although this project also concerns African wild dogs, it entails no overlap with the current proposal. There is no infectious disease element to the "Hot Dogs" project, and no overlap between study animals or study sites. This project shares a PI (Rosie Woodroffe) with the current proposal, but the two projects together account for <50% of her time.
- i. Project ID: NA
- ii. Funding Source: IUCN Save Our Species programme
- iii. **Project Title:** Recovering a globally important African wild dog population in Kenya's Ewaso

ecosystem

- iv. Summary: Range-wide conservation planning for African wild dogs was inspired by a study showing how wild dogs and people could coexist in Kenva's Ewaso ecosystem. Yet, in 2017, an epidemic of canine distemper devastated this iconic population. In the wellstudied core of the ecosystem, two solitary animals remained where 20 packs had lived just a few months earlier. As survivors and immigrants re-form tiny packs, we aim to recover this globally-important population, encouraging rapid population growth by tackling the two greatest causes of mortality: infectious disease, and deliberate killing by people. The distemper epidemic has passed, and rabies is the most immediate risk to recovery. We plan to locally eliminate rabies by mass domestic dog vaccination, protecting human health as well as wild dogs. We shall also vaccinate wild dogs against rabies, if an expert-led workshop recommends this approach. Our local outreach programme will use participatory theatre to share evidencebased advice on livestock husbandry practices known to reduce wild dog depredation, and to encourage domestic dog vaccination. We shall build support for wild dog conservation nationally using in-country print, broadcast and social media, and **build national capacity** by offering project staff opportunities to gain skills and qualifications while in-post. Project is **pending** and has not yet yielded results.
- v. **Budget:** EUR 399,307
- vi. **Timeline**: proposed 1 Oct 2019 31 Mar 2022 but grant still pending

- vii. **Overlap:** Although this project also concerns African wild dogs, it complements the current proposal rather than overlapping with it. The IUCN SOS provides grants for conservation action **not** research; hence the current proposal could provide data to support and inform the conservation action proposed to SOS. Also, the SOS proposal focuses on a specific site in Kenya while the current proposal involves captive wild dogs and those residing in Kruger National Park. PI Rosie Woodroffe is named on the SOS proposal as a technical advisor; she is not the lead applicant. Co-I Mathew Mutinda is also a technical advisor on the proposal to SOS.
- i. Project ID: NA
- ii. Funding Source: Darwin Initiative
- iii. Project Title: African wild dogs and African people – conservation through coexistence iv. **Summary:** Kenya's Ewaso ecosystem is a poster child for the *coexistence of people and* wildlife. For years, it sustained a globally-important population of African wild dogs. thriving on the lands of traditional pastoralists and commercial ranchers. Yet, in 2017, an epidemic of canine distemper killed over 95% of the ecosystem's wild dogs. As survivors and immigrants re-form tiny packs, we aim to recover this iconic population, while also improving human health and livelihoods. The distemper epidemic has passed, and rabies is the most immediate risk to wild dog recovery, and also threatens human health. We plan to locally eliminate rabies by mass domestic dog vaccination, protecting people and wild dogs alike. The second greatest barrier to wild dog recovery is deliberate killing by people, linked to livestock predation. Using participatory theatre, we shall share evidence-based advice on practices of livestock husbandry and land management known to reduce depredation, improving local livelihoods and reducing retaliatory killing of wild dogs and other large carnivores. We shall build support for wild dog conservation nationally using in-country print, broadcast and social media, and build national capacity through knowledge exchange and by offering project staff opportunities to gain skills and qualifications while in-post.
- v. **Budget:** GBP 395,090
- vi. Timeline: proposed 1 Apr 2020 31 Mar 2023 but grant still pending
- vii. **Overlap:** Although this project also concerns African wild dogs, it complements the current proposal rather than overlapping with it. Like SOS, the Darwin Initiative provides grants for conservation action **not** research; hence the current proposal could provide data to support and inform the conservation action proposed to SOS. PI Rosie Woodroffe is named on the Darwin proposal as a technical advisor; she is not the lead applicant. Co-I Mathew Mutinda is also a technical advisor on the proposal to Darwin.

M. Prior MAF support

The investigator team have no MAF support from the previous three years.

N. Biographical Data

i. Name: Prof Rosie Woodroffe

ii. Position on this project: Principal Investigator

Role: Coordination of all project activities including captive trial, field trial, data analysis and interpretation, and production of management guidelines.

iii. Current position: Professor, Institute of Zoology, Zoological Society of London

Regent's Park, London NW1 4RY, UK.

Visiting Professor, Imperial College London

iv. Education:

1992: D. Phil., University of Oxford. Thesis title "Factors affecting reproductive success in the European Badger, Meles meles L."

1989: B. A. (Hons. I) Zoology, University of Oxford.

v. **Previous positions:**

2007: Professor of Conservation Biology, Department of Wildlife, Fish & Conservation Biology, University of California, Davis.

2001-7: Assistant, then Associate Professor of Conservation Biology, Department of Wildlife, Fish & Conservation Biology, University of California, Davis.

1998-2001: Lecturer (Assistant Prof) in Ecology & Epidemiology, University of Warwick.

1994-8: Research Fellow, Gonville & Caius College, University of Cambridge.

1993-4: Research Associate, Institute of Zoology, Zoological Society of London. Visiting Research Officer, Uganda Institute of Ecology.

Selected advisory positions:

1996-present: IUCN/SSC Canid Specialist Group. Coordinator of African Wild Dog Working Group 2004-present; coordinator of Infectious Disease Working Group 1998-2004.

1996-present: IUCN/SSC Wildlife Health Specialist Group.

2005-present: Task Force on conservation and management of large carnivores, Kenya Wildlife Service.

2007-8: IUCN/SSC Species Conservation Planning Task Force.

1998-2007: Independent Scientific Group on Tuberculosis in Cattle, UK Department of Environment, Food & Rural Affairs.

2004-7: Island Fox Recovery Team, US Fish & Wildlife Service.

Selected honours and awards:

2017: Specially Commended, Maddox Prize for Standing up for Science

2015: BBC Wildlife "Power List"

2015: Winner, British Ecological Society "Science Slam" science communication event

2014: Marsh Award for Ecology, British Ecological Society.

2012: Denver Zoo Conservation Award.

2008: Chair's Citation of Excellence, IUCN Species Survival Commission.

2006-2011: Chancellor's Fellowship, University of California, Davis.

v. Selected peer-reviewed publications:

Woodroffe, R., O'Neill, H.M.K., & Rabaiotti, D. (2019). Within- and between-group dynamics in an obligate cooperative breeder. *Journal of Animal Ecology* doi: 10.1111/1365-2656.13102.

Woodroffe, R., Rabaiotti, D., Ngatia, D.K., Smallwood, T.R.C., Strebel, S. & O'Neill, H.M.K. (2019). Dispersal behaviour of African wild dogs in Kenya. *African Journal of Ecology* doi: 10.1111/aje.12689.

- Ham, C., Donnelly, C.A., Astley, K.L., Jackson, S.Y.B., & <u>Woodroffe</u>, R. (2019). Effect of culling on individual badger (*Meles meles*) behaviour: potential implications for bovine tuberculosis transmission. *Journal of Applied Ecology* doi: 10.1111/1365-2664.13512.
- McNutt, J.W., Groom, R. & <u>Woodroffe</u>, R. (2019). Ambient temperature provides an adaptive explanation for seasonal reproduction in a tropical mammal. *Journal of Zoology* doi: 10.1111/jzo.12712.
- Rabaiotti, D. & <u>Woodroffe</u>, R. (2019) Coping with climate change: limited behavioural responses to hot weather in a tropical carnivore. *Oecologia*, 189, 587-599.
- Woodroffe, R., Groom, R. & McNutt, J. (2017). Hot dogs: high ambient temperatures influence reproductive success in a tropical mammal. *Journal of Animal Ecology*, 86, 1329-1338.
- Woodroffe, R., Donnelly, C.A., Ham, C., Jackson, S.Y.B., Moyes, K., Chapman, K., Stratton, N.G. & Cartwright, S.J. (2017) Ranging behaviour of badgers *Meles meles* L. vaccinated with Bacillus Calmette Guerin. *Journal of Applied Ecology*, 54, 718-725.
- Woodroffe, R., Donnelly, C.A., Ham, C., Jackson, S.Y.B., Moyes, K., Chapman, K., Stratton, N.G. & Cartwright, S.J. (2016) Badgers prefer cattle pasture but avoid cattle: implications for bovine tuberculosis control. *Ecology Letters*, 19, 1201-1208.
- Connolly, M. Thomas, P., <u>Woodroffe</u>, R. & Raphael, B.L. (2015). Single versus double-dose rabies vaccination in captive African wild dogs (*Lycaon pictus*). *Journal of Zoo and Wildlife Medicine* **46**: 691-698.
- Woodroffe, R. & Redpath, S. (2015). When the hunter becomes the hunted. *Science* **348**: 1312-1314.
- Ford, A.T., Goheen, J.R., Augustine, D.J., Kinnaird, M.F., O'Brien, T.G., Palmer, T.M., Pringle, R.M. & <u>Woodroffe</u>, R. (2015) Recovery of African wild dogs suppresses prey but does not trigger a trophic cascade. *Ecology* **96**: 2705-2714.
- Ford, A.T., Goheen, J., Otieno, T.O., Palmer, T., Ward, D., <u>Woodroffe</u>, R. & Pringle, R. (2014). Large carnivores make savanna tree communities less thorny. *Science* **346**: 346-349
- Woodroffe, R., Hedges, S. & Durant, S. (2014). To fence or not to fence. Science 344: 46-8.
- Bielby, J., Donnelly, C.A., Pope, L.C., Burke, T & <u>Woodroffe</u>, R. (2014) Badger responses to small-scale culling may compromise targeted control of bovine tuberculosis. *PNAS* **111**: 9193-9198.
- Connolly, M. Thomas, P., <u>Woodroffe</u>, R. & Raphael, B.L. (2013). Comparison of oral and intramuscular recombinant canine distemper vaccination in African wild dogs (*Lycaon pictus*). *Journal of Zoo and Wildlife Medicine* **44**: 882-888.
- Prager K.C., Mazet J.K., Dubovi E.J., Frank L.G., Munson L., Rupprecht C.E., Wagner A. & Woodroffe R. (2012). Rabies and canine distemper virus in wild and domestic carnivores in northern Kenya: Are domestic dogs the reservoir? *EcoHealth* **9**: 483-498.
- Woodroffe, R., Prager, K.C., Munson, L., Conrad, P.A., Dubovi, E.J. & Mazet, J.A.K. (2012). Contact with domestic dogs increases pathogen exposure in endangered African wild dogs (*Lycaon pictus*). *PLoS ONE* **7**: e30099.
- Prager, K.C., Mazet, J.A.K., Munson, L., Dubovi, E.J., Szykman Gunther, M., Davies-Mostert, H., McNutt, J.W., Rasmussen, G., Terio, K., Cleaveland, S., Mills, M.G.L., Donnelly, C.A. & <u>Woodroffe</u>, R. (2012). The effect of protected areas on pathogen exposure in endangered African wild dog (*Lycaon pictus*) populations. *Biological Conservation* **150**: 15-22.
- <u>Woodroffe</u>, R. & Donnelly, C.A. (2011). Risk of contact between endangered African wild dogs *Lycaon pictus* and domestic dogs: opportunities for pathogen transmission. *Journal of Applied Ecology* **48**:1345-1354.
- Prager, K.C., <u>Woodroffe</u>, R., Cameron, A. & Haydon, D.T. (2011). Vaccination strategies to conserve the endangered African wild dog (*Lycaon pictus*). *Biological Conservation* **144**:1940-1948.

i. Name: Karen Bauman, M.S.

ii. **Position on this project:** Co-investigator

Role: Liaison with zoos to deliver captive trial; help with design and interpretation of this trial; translation of research findings into practical applications for captive population.

iii. **Current position**: Manager of Reproductive Sciences

Department of Reproductive and Behavioral Sciences

Saint Louis Zoo 1 Government Drive

Saint Louis, MO 63110 USA

iv. **Education**:

2010: M.S., University of Missouri St. Louis. Thesis title "How Does the Abundance and Distribution of Resources Affect Female Raccoon Home Ranges in an Urban Park?"

1992: B. S. Animal Science, Colorado State University.

v. **Previous positions:**

1998-2018: Laboratory Manager/Scientist, Department of Reproductive and Behavioral Sciences (formerly Research Department), Saint Louis Zoo.

1994-98: Research Technician, Research Department, Saint Louis Zoo

1993-94: Research Assistant, Research Department, Saint Louis Zoo

1993: Research Technician, BEECS Reproductive Lab, University of Florida

Selected advisory positions:

2010-present: Chair, Association of Zoos and Aquariums (AZA) Canid and Hyenid Taxon Advisory Group

2001- present: IUCN/SSC Canid Specialist Group member.

2010-present: IUCN/SSC Canid Specialist Group member. Coordinator of Ex Situ Working Group.

2001-present: Coordinator, AZA Fennec Fox (Vulpes zerda) Species Survival Plan (SSP)

2004-7: Island Fox Recovery Team, US Fish & Wildlife Service.

1993-present: Member, USFWS/Mexican Wolf SSP Reproductive Team

v. Selected peer-reviewed publications:

Asa, C.S., Fischer, M.K., Houston, E.W., Bauman, J.E., <u>Bauman</u>, K.L., Read B.W. and Plotka, E.D. Ovulatory cycle dynamics and pregnancy in Addax, Addax nasomaculatus. Biology of Reproduction, vol. **52**. pg. 65, 1995.

Asa, C.S., Fischer, M.K., Bauman, J.E., Houston, E.W., <u>Bauman</u>, K.L., Hagberg, P.K. and Read B.W. Ovulatory cycles and anovulatory periods in the Addax (*Addax nasomaculatus*). Journal of Reproduction and Fertility, **107**:119-124, 1996.

Bauman, K., Snyder, T., Asa, C. and Macek, M. The use of a telemetric egg for monitoring bird incubation. Pages 452-463 in Biotelemetry **15**: Proceedings of the 15th International Symposium on Biotelemetry, J.H. Eiler, D.J. Alcorn and M.R. Neuman (editors). Juneau, AK, USA. International Society on Biotelemetry. Wageningen, The Netherlands, 1999

Bertschinger, H.J., Asa, C.S., Calle, P.P., Long, J.A., <u>Bauman</u>, K., DeMatteo, K., Jöchle, W., Trigg, T.E., and Human, A. Control of reproduction and sex related behaviour in exotic wild

- carnivores with the GnRH analogue deslorelin: preliminary observations. Journal of Reproduction and Fertility Supplement, **57**:275-283, 2001.
- <u>Bauman</u>, K.L., Asa, C.S., Grisham, J., and Verberkmoes, W. Captive breeding: Canids in captivity and captive breeding. In D.W. Macdonald and C. Sillero-Zubiri (eds.), IUCN/SSC Canid Action Plan, pp.280-288. IUCN, Gland, Switzerland, 2004.
- Asa, C.S., <u>Bauman</u>, K.L., Callahan, P., Bauman, J., Volkmann, D.H., and Jöchle, W. GnRH-agonist induction of fertile estrus with either natural mating or artificial insemination followed by birth of pups in gray wolves (*Canis lupus*). Theriogenology **66**: 1778-1782, 2006.
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- <u>Bauman</u>, K.L. How Does the Abundance and Distribution of Resources Affect Female Raccoon Home Ranges in an Urban Park? Thesis, University of Missouri--St. Louis, 1-112, 2010.
- <u>Bauman</u>, K. L., Mekarska, A., Grisham, J., and Lynch, C., Small canid husbandry challenges: reviewing problems, with recommendations for improving captive canid programmes. In Asa, C.S. and Kitchener, A., eds., Canids and Bears, International Zoo Yearbook, **44**: 87-101, 2010.
- Boutelle, S., Lenahan, K., Krisher, R., <u>Bauman</u>, K.L., Asa, C.S. and Silber, S. Vitrication of oocytes from endangered Mexican gray wolves (*Canis lupus baileyi*). Theriogenology **75** #4:647-654, 2011.
- Asa, C.S., Bauman, K.L., Devery, S., Zordan, M., Camilo, G.R., Boutelle, S. and Moresco, A., Factors Associated With Uterine Endometrial Hyperplasia and Pyometra in Wild Canids: Implications for Fertility. Zoo Biology, 2013.
- Christensen, B.W., Asa, C.S., Wang, C., <u>Bauman</u>, K.L., Agnew, M.K., Lorton, S.P., and Callahan, M., Kinematic activity of gray wolves (*Canis lupus*) sperm in different extenders before or after centrifugation. Theriogenology **79** #6: 953-960, 2013.
- Bauman, K.L. Wildlife Technologies. In Miller, R.E., Lamburski, N. and Calle, P., Eds., Zoo and Wild Animal Medicine Volume 9. Pp. 11-16. St. Louis, MO. 2018
- Traylor-Holzer, K, Leus, K. and <u>Bauman</u>, K. (Eds) Global Integrated Collection Assessment and Planning Workshop for Canids and Hyaenids: Final Report. IUCN SSC Conservation Planning Specialist Group, Apple Valley, MN. 2018.
- Bauman, K.L., Sahrmann, J., Franklin, A., Asa, C.S., Agnew, M.A., Traylor-Holzer, K., and Powell, D.P. Reproductive Viability Analysis as a New Tool in Captive Animal Management. Zoo Biology 1:1-12. 2019.
- Traylor-Holzer, K., Leus, K.R., and <u>Bauman</u>, K.L. Integrated Collection Assessment and Planning (ICAP) Workshop: helping zoos adopt the One Plan Approach. Zoo Biology **1**:1-11. 2019.

- i. Name: Dr Diego G. Diel
- ii. Position on this project: Co-Investigator

Role: Oversee all diagnostic procedures at Cornell; coordinate collection and interpretation of serological and virological data.

- iii. **Current position**: Associate Professor, Department of Population Medicine and Diagnostic Sciences, Cornell University, College of Veterinary Medicine, Ithaca, NY USA
- iv. Education:
 - 2010: PhD, Virology, Federal University of Santa Maria, Brazil
 - 2007: MSc, Virology, Federal University of Santa Maria, Brazil
 - 2004: DVM, Virology, Federal University of Santa Maria, Brazil
- v. Previous positions:
 - 2016-19: Virology Section Leader, Animal Disease Research and Diagnostic Laboratory, Department of Veterinary and Biomedical Sciences, College of Agriculture and Biological Sciences, South Dakota State University, Brookings, SD.
 - 2014-19: Assistant Professor, Department of Veterinary and Biomedical Sciences, College of Agriculture and Biological Sciences, South Dakota State University, Brookings, SD.
 - 2013-14: Postdoctoral Research Associate, Department of Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign.
 - 2011-13 Postdoctoral Research Microbiologist, Exotic and Emerging Avian Viral Diseases Research Unit, Southeast Poultry Research Laboratory, U.S. Department of Agriculture, Agricultural Research Services, Athens. GA.

v. Selected peer-reviewed publications:

- Fernandes, M.H.V., Maggioli, M.F., Otta, J., Joshi, L.R., Lawson, S., **Diel, D.G.** 2019. Senecavirus A 3C protease mediates host cell apoptosis late in infection. *Frontiers in Immunology*, doi: 10.3389/fimmu.2019.00363.
- Ramírez, M., Bauermann, F.V., Navarro, D., Rojas, M., Manchego, A., Nelson, E.A., **Diel, D.G.**, Rivera, H. 2019. Detection of porcine reproductive and respiratory syndrome virus (PRRSV) 1-7-4-type strains in Peru. *Transboundary and Emerging Diseases*, **2019**. doi: 10.1111/tbed.13134.
- Joshi, L.R., Bauermann, F.V., Hain, K.S., Kutish, G.F., Armién, A.G., Lehman, C.P., Regg Neiger, Afonso, C.L., Tripathy, D.N., **Diel, D.G.** 2019. Detection of fowlpox virus carrying distinct genome segments of reticuloendotheliosis virus. *Virus Research*, Oct 22. pii: S0168-1702(18)30556-2.
- Fernandes, M.H.V., Maggioli, M.F., Joshi, L.R., Clement, T., Faccin, T.C., Rauh, R., Bauermann, F.V., **Diel, D.G.** 2018. Pathogenicity and cross-reactive immune responses of a historical and a contemporary Senecavirus A strains in pigs. *Virology*, Sep;522:147-157.
- Joshi, L.R., Okda, F.O., Singrey, A., Maggioli, M.F., Faccin, T.C., Fernandes, M.H.V., Hain, K.S., Dee, S., Bauermann, F.V., Nelson, E.A., **Diel, D.G.** Passive immunity to porcine epidemic diarrhea virus following immunization of pregnant gilts with a recombinant Orf virus vector expressing the spike protein. *Archives of Virology*. April 2018.
- Dee, S., Bauermann, F.V., Niederwerder, M., Singrey, A. Clement, T., de Lima, M., Long, C., Patterson, G., Sheehan, M., Stoian, A., Petrovan, V., Jones, C., de Jong, J., Ji, J., Spronk, G., Hennings, J., Zimmerman, J., Rowland, R.R., Nelson, E., Sundberg, P., **Diel, D.G.** 2018. Survival of viral pathogens in animal feed ingredients under transboundary shipping models. *PLOS One*, Mar 20;13(3):e0194509.
- Maggioli, M.F., Lawson, S., de Lima, M., Joshi, L.R., Faccin, T.C., Bauermann, F.V., **Diel, D.G.** 2018. Adaptive immune responses following Senecavirus A infection in pigs. *Journal of Virology*, 92(3). pii: e01717-17.

- Martins, M., Joshi, L.J., Rodrigues, F.S., Anziliero, D., Frandoloso, R., Kutish, G.F., Rock, D.L., Weiblen, R., Flores, E.F., **Diel, D.G.** 2017. Immunogenicity of *ORFV*-based vectors expressing the rabies virus glycoprotein in livestock species. *Virology*, 511:229-239.
- Khatiwada, S., Delhon, G., Ponnuraj, P., Chaulagain, S., Luo, S., **Diel, D.G.**, Flores, E.F., Rock, D.L. 2017. A novel poxviral virion protein inhibits NF-κB signaling early in infection. *PLOS Pathogens*, 13(8):e1006561.
- Okda, F.A., Lawson, S., Singrey, A., Nelson, J., Hain, K.S., Joshi, L.R., Christopher-Hennings, J., Nelson, E.A., **Diel**, **D.G.** 2017. The S2 glycoprotein subunit of porcine epidemic diarrhea virus contains immunodominant neutralizing epitopes. *Virology*, 509:185-194.
- Bauermann, F.V., Joshi, L.R., Mohr, K.A., Kutish, G., Meier, P., Chase, C., Christopher-Hennings, J., **Diel, D.G.** 2017. A novel bovine papillomavirus type in the genus *Dyokappapapillomavirus*. **Archives of Virology.** Jun 14. doi: 10.1007/s00705-017-3443-9.
- Van Noort, A., Nelsen, A., Pillatzki, A.E., **Diel, D.G.**, Li, F., Nelson, E., Wang, X. 2017. Intranasal immunization of pigs with porcine reproductive and respiratory syndrome virus-like particles plus 2', 3'-cGAMP VacciGradeTM adjuvant exacerbates viremia after virus challenge. *Virology Journal,* Apr 12;14(1):76.
- Joshi, L.R., Fernandes, M.H.V., Clement, T., Lawson, S., Pillatzki, A., Resende, T.P., Vanucci, F.A., Kutish, G.F., Nelson E.A., **Diel, D.G.** 2016. Pathogenesis of Senecavirus A infection in finishing pigs. *Journal of General Virology*. Oct 14 doi:10.1099/jgv.0.000631.
- Hain, K.S., Joshi, L.R., Okda, F., Nelson J., Singrey, A., Lawson, S., Martins, M., Pillatzki, P., Kutish, G.F., Nelson, E.A., Flores, E.F., **Diel, D.G.** 2016. Immunogenicity of a Recombinant Parapoxvirus Expressing the Spike Protein of Porcine Epidemic Diarrhea Virus. *Journal of General Virology*. Aug 24. doi: 10.1099/jgv.0.000586.
- Cardenas-Garcia, S., Dunwoody, R. P., Marcano, V., **Diel, D.G.**, Williams, R. J., Gogal Jr., R. M., Brown, C. C., Miller, P. J., Afonso, C. L. 2016. Effects of Chicken Interferon Gamma on Newcastle Disease Virus Vaccine Immunogenicity. *PLOS One*, 11(7):e0159153.
- **Diel, D.G.**, Lawson S., Okda F., Singrey, A., Clement T., Fernandes, M.H.V., Christopher-Hennings, J., Nelson, E.A. 2016. Porcine epidemic diarrhea virus: an overview of current virological and serological diagnostic methods. *Virus Research,* May 14. pii: S0168-1702(16)30209-X.
- Burmakina, G., Malogolovkin, A., Tulman, E.R., Zsak, L., Delhon, G., **Diel, D.G.**, Shobogorov, N.M., Morgunov, Y.P., Morgunov, S.Y., Kutish, G.F., Kolbasov, D., Rock, D.L. 2016. African swine fever virus serotype-specific proteins are significant protective antigens for African swine fever. *Journal of General Virology*, April 25. doi: 10.1099/jgv.0.000490.
- Joshi, L.R., Mohr, K.A., Clement, T., Hain, K.H., Myers, B., Yaros, Y., Nelson, E.A., Christopher-Hennings, J., Gava, D., Schaefer, R., Caron, L., Dee, S., **Diel, D.G.** 2016. Detection of the Emerging Picornavirus Senecavirus A in Pigs, Mice and Houseflies. *Journal of Clinical Microbiology*, June, 54(6):1536-45. doi: 10.1128/JCM.03390-15.
- Clement, T., Kutish, G.F., Nezworski, J., Scaria, J., Nelson, E., Christopher-Hennings, J., **Diel, D.G.**Complete genome sequence of a highly pathogenic avian influenza virus (H5N2) associated with an outbreak in commercial chickens, Iowa, USA, 2015. Manuscript accepted by GenomeA on May 13, 2015.

BOOK CHAPTERS

- Canal, C.V., **Diel**, **D.G.** 2012. Poxviridae. *In:* Flores, E.F. *Veterinary Virology*. 2 ed., Editora UFSM (Ed), Santa Maria, Brazil; Part II. Chapter 18, p.571.
- Delhon, G., **Diel, D. G.** 2012. Asfarviridae. *In:* Flores, E. F. *Veterinary Virology*. 2 ed., Editora UFSM (Ed), Santa Maria, Brazil; Part II. Chapter 19, p.605.
- Flores, E.F., Lovato, L.T., Silva, M.S., Dezengrini, R., **Diel, D.G.** 2007. Orthomyxoviridae. *In Veterinary Virology*, E. F. Flores, Editora UFSM (Ed), Santa Maria, Brazil; Part II, Chapter 28, p.721.

- i. Name: Dr Martin Gilbert M.R.C.V.S
- ii. **Position on this project:** Co-Investigator

Role: Contribute to data analysis and interpretation, including production of management guidelines.

- iii. **Current position**: Senior Research Associate, Department of Population Medicine and Diagnostic Sciences, Cornell University
- iv. Education:
 - 2016: PhD, Comparative Medicine. University of Glasgow. Thesis title "Understanding and managing canine distemper virus as a disease threat to Amur tigers"
 - 1999: Bachelor of Veterinary Medicine and Surgery (B.V.M.S.), University of Glasgow.
 - 1996: Bachelor of Science (Honours), Zoology, University of Glasgow.

v. **Previous positions:**

- 2014-16: Carnivore Health Specialist, Wildlife Conservation Society, Russia.
- 2011-14: MCEIRS Project (WCS-PI), Wildlife Conservation Society, Mongolia
- 2008-11: Associate Director Asia, Global Health Program, Wildlife Conservation Society
- 2004-07: Assistant Field Veterinarian, Field Veterinary Program, Wildlife Conservation Society, Cambodia
- 2000-04: Field Veterinarian, Asian Vulture Crisis Project, The Peregrine Fund, Pakistan.
- 1999-00: Field Veterinarian/Biologist, The Peregrine Fund, Madagascar.

Selected honours and awards:

2016: Nominated for the Joseph Black Medal and Alan Hird Prize, the highest distinction of excellence for PhD theses submitted to the University of Glasgow

v. Selected peer-reviewed publications:

- Zhu, H., B. Damdinjav, G. Gonzalez, V. L. Patrono, A. Parr, T. Hammond, E. Shiilegdamba, C. Leung, P. Malik, M. John F, J. Hughes, **M. Gilbert**, and P. R. Murcia. 2019. Absence of adaptive evolution is the main barrier against influenza emergence in horses in Asia despite frequent virus interspecies transmission from wild birds. PLoS Pathogens 15:e1007531.
- **Gilbert, M**. 2018. Chapter 54: Techniques for vaccinating wildlife. in R.E. Miller, N. Lamberski, and P. Calle, editors. Fowler's zoo and wild animal medicine, Volume 9: current therapy. Elsevier, St. Louis.
- Sulikhan, N. S., **M. Gilbert**, E. Y. Blidchenko, S. V. Naidenko, *et al.* 2018. Canine distemper virus in a wild Far Eastern leopard Panthera pardus orientalis. *J. Wildl. Dis.* 54(1):170-174.
- **Gilbert, M.**, B. Buuveibaatar, A. E. Fine, L. Jambal, and S. Strindberg. 2016. Declining breeding populations of white-naped cranes in Eastern Mongolia, a ten-year update. Bird. Cons. Int. 26:490–504.
- Ostrowski, S., and **M. Gilbert**. 2016. Diseases of free-ranging snow leopards and primary prey species. Pages 97–112 in T. McCarthy and D. Mallon, editors. Snow leopards of the World. First Edit. Elsevier, London, United Kingdom.
- **Gilbert, M.**, S. Soutrina, I. Seryodkin, N. Sulikhan, *et al.* 2015. Canine distemper virus as a threat to wild tigers in Russia and across their range. Int. Zool. 10:329–343.
- Logan, N., E. McMonagle, A. A. Drew, E. Takahashi, M. McDonald, M. D. Baron, **M. Gilbert**, et al. 2015. Efficient generation of vesicular stomatitis virus-pseudotypes bearing morbilliviral glycoproteins and their use in quantifying virus neutralising antibodies. Vaccine 34:814–822.

- **Gilbert, M.**, D. G. Miquelle, J. M. Goodrich, R. Reeve, *et al.* 2014. Estimating the potential impact of canine distemper virus on the Amur tiger population in Russia. PLoS ONE 9:e110811.
- **Gilbert, M.**, B. F. Koel, T. M. Bestebroer, N. S. Lewis, D. J. Smith, and R. A. M. Fouchier. 2014. Serological evidence for non-lethal exposures of Mongolian wild birds to highly pathogenic avian influenza H5N1 virus. PLoS ONE 9:e113569.
- **Gilbert, M.**, R. Tingay, L. Jambal, N. Sureda, *et al.* 2014. Distribution and status of the Pallas's Fish Eagle *Haliaeetus leucoryphus* in Mongolia: a cause for conservation concern? Bird. Cons. Int. 24:379–388.
- Margalida, A., G. Bogliani, C. G. R. Bowden, J. A. Donazar, F. Genero, **M. Gilbert**, *et al.* 2014. One Health approach to use of veterinary pharmaceuticals. SCIENCE 346:1296–8.
- Olson, S. H., **M. Gilbert**, M. C. Cheng, J. A. K. Mazet, and D. O. Joly. 2013. Prevalence and distribution of avian influenza virus A (H7N9) among wild birds. Emerg. Infect. Dis. 19:2031–2033.
- **Gilbert, M.**, L. Jambal, W. B. Karesh, A. Fine, *et al.* 2012. Highly pathogenic avian influenza virus among wild birds in Mongolia. PLoS ONE 7:e44097.
- **Gilbert, M.**, D. Bickford, L. Clark, A. Johnson, *et al.* 2012. Amphibian pathogens in Southeast Asian frog trade. EcoHealth 9:9(4):386-98.
- **Gilbert, M.**, C. Sokha, P. H. Joyner, R. L. Thomson, and C. Poole. 2012. Characterizing the trade of wild birds for merit release in Phnom Penh, Cambodia and associated risks to health and ecology. Biol. Cons. 153:10–16.
- **Gilbert, M.**, and J. Philippa. 2012. Avian influenza H5N1 virus: epidemiology in wild birds, zoo outbreaks, and zoo vaccination policy. Pages 343–348. in R. E. Miller and M. E. Fowler, editors. Fowler's zoo and wild animal medicine, Volume 7: current therapy. Elsevier Saunders, St. Louis.
- **Gilbert, M.**, R. T. Watson, S. Ahmed, M. Asim, and J. A. Johnson. 2007. Vulture restaurants and their role in reducing diclofenac exposure in Asian vultures. Bird. Cons. Int. 17:63–77.
- **Gilbert, M.**, R. T. Watson, M. Z. Virani, J. L. Oaks, *et al.* 2006. Rapid population declines and mortality clusters in three Oriental white-backed vulture *Gyps bengalensis* colonies in Pakistan due to diclofenac poisoning. Oryx 40:388–399.
- Oaks, J. L., **M. Gilbert**, M. Z. Virani, R. T. Watson, *et al.* 2004. Diclofenac residues as the cause of vulture population decline in Pakistan. Nature 427:630–633.

i. Name: Christina N. Gorsuch

ii. **Position on this project:** Co-Investigator

Role: Liaison with zoos to deliver captive trial; help with design and interpretation of this trial; translation of research findings into practical applications for captive population.

iii. Current position: Curator, Cincinnati Zoo and Botanical Garden

3400 Vine Street. Cincinnati, OH 45220 USA

Coordinator, AZA African Painted Dog Species Survival Program

iv. **Education**:

2000: B. A. Anthropology and English, Tulane University.

v. Previous positions:

2010-2015: Lead Keeper: Africa, Chicago Zoological Society's Brookfield Zoo

2002-2010: Keeper III to Lead Keeper: Mammals, Zoo Atlanta 2000-2002: Keeper I: Primates then Children's Zoo, Audubon Zoo

Selected advisory positions:

2014-present: Member of African Painted Dog SSP management group since 2014

• 2019: African Painted Dog SSP Coordinator

• 2016-2019: African Painted Dog SSP Vice-coordinator

2014: African Painted Dog Studbook editor then studbook keeper

2016-present: AZA Canid TAG Secretary

2015-present: AZA Reproductive Management Center Advisory Board

2018-present: AZA Asian Elephant SAFE Steering Committee

- i. Name: Dr Matthew Mutinda Ndunda
- ii. **Position on this project:** Co-investigator

Role: Contribute to trial design and interpretation to ensure data collection will inform conservation needs; contribute to production of management guidelines.

iii. **Current position**: Head, Mountain Region mobile veterinary clinic, Kenya Wildlife Service Collaborative Researcher, Mpala Research Centre, Kenya

iv. Education:

2012: MSc, International Animal Health, University of Edinburgh, UK. Thesis title "Molecular Epidemiology of Trypanosomes among selected Wildlife populations in Kenya"

2005: BVetMed (Hons) University of Nairobi, Kenya.

v. **Previous positions:**

2008-2010: Field Veterinary Officer, Kenya Wildlife Service

Major incidents addressed include:

2006 - Field trial of anthrax vaccination of Grevy's zebra

2008 - Serosurvey for Rift Valley Fever

2010 - Rinderpest surveillance for Meru National Park

2011 - Investigation into outbreak of elephant herpes virus

2012 - Management of rabies outbreak in African wild dogs

2012 - Investigated mass die-off of laughing doves

2012 - Avian influenza surveillance

2012 - Hirola translocation

2017 - Managed Canine Distemper outbreak in African wild dogs and domestic dogs

Advisory positions:

present: Technical committee, Sweetwaters Chimpanzee Sanctuary.

Selected honours and awards:

2011: Employee of the year, Lewa wildlife conservancy. For excellent performance as the veterinarian in charge of the Mountain Region Mobile Veterinary Unit.

v. Peer-reviewed publications:

- Mutinda, M., Crofoot, M.C., Kishbaugh, J.C., Hayek, L.A.C., Zimmerman, D., Tunseth, D.A. & Murray, S. (2019) Blood Biochemical Reference Intervals for Free-Ranging Olive Baboons (*Papio anubis*) in Kenya. *International Journal of Primatology*, 40, 187-196.
- Isbell, L.A., Bidner, L.R., Omondi, G., **Mutinda**, M. & Matsumoto-Oda, A. (2019) Capture, immobilization, and Global Positioning System collaring of olive baboons (*Papio anubis*) and vervets (*Chlorocebus pygerythrus*): Lessons learned and suggested best practices. *American Journal of Primatology*, 81.
- Guevara, L., Abdelgawad, A., Onzere, C., Greenwood, A.D., Davidson, Z., Bishop, R. & **Mutinda**, M. (2018) Seroprevalence of Equine Herpesviruses 1 and 9 (EHV-1 and EHV-9) in Wild Grevy's Zebra (*Equus grevyi*) in Kenya. *Journal of Wildlife Diseases*, 54, 848-851.
- Rajeev, M., **Mutinda**, M. & Ezenwa, V.O. (2017) Pathogen Exposure in Cattle at the Livestock-Wildlife Interface. *Ecohealth*, 14, 542-551.
- **Mutinda**, M., Devin A. Tunseth; Dawn Zimmerman; Margaret C. Crofoot; Samson Mutura Lee-Ann C. Hayek; Joshua Engel; Suzan Murray. **2017.** Biochemical reference intervals for free-ranging olive baboons (*Papio anubis*) in Kenya. BMC Veterinary Research.

- Sitt, T., Poole, E.J., Ndambuki, G., Mwaura, S., Njoroge, T., Omondi, G.P., **Mutinda**, M., Mathenge, J., Prettejohn, G., Morrison, W.I. & Toye, P. (2017) Exposure of vaccinated and naive cattle to natural challenge from buffalo-derived *Theileria parva*. *International Journal for Parasitology-Parasites and Wildlife*, 6, 219-219.
- Ghai, R.R., **Mutinda**, M. & Ezenwa, V.O. (2016) Limited sharing of tick-borne hemoparasites between sympatric wild and domestic ungulates. *Veterinary Parasitology*, 226, 167-173.
- Obanda, V., Michuki, G., Jowers, M.J., Rumberia, C., **Mutinda,** M., Lwande, O.W., Wangoru, K., Kasiiti-Orengo, J., Yongo, M. & Angelone-Alasaad, S. (2016) Complete genomic sequence of virulent pigeon paramyxovirus in laughing doves (*Streptopelia senegalensis*) in Kenya. *Journal of Wildlife Diseases*, 52, 599-608.
- Otiende, M.Y., Kivata, M.W., Jowers, M.J., Makumi, J.N., Runo, S., Obanda, V., Gakuya, F., **Mutinda**, M., Kariuki, L. & Alasaad, S. (2016) Three Novel Haplotypes of *Theileria bicornis* in Black and White Rhinoceros in Kenya. *Transboundary and Emerging Diseases*, 63, E144-E150.
- Otiende, M.Y., Kivata, M.W., Makumi, J.N., **Mutinda**, M.N., Okun, D., Kariuki, L., Obanda, V., Gakuya, F., Mijele, D., Soriguer, R.C. & Alasaad, S. (2015) Epidemiology of *Theileria bicornis* among black and white rhinoceros metapopulation in Kenya. *BMC veterinary research*. 11.
- Hawkins, E., Kock, R., McKeever, D., Gakuya, F., Musyoki, C., Chege, S.M., **Mutinda**, M., Kariuki, E., Davidson, Z., Low, B., Skilton, R.A., Njahira, M.N., Wamalwa, M. & Maina, E. (2015) Prevalence of *Theileria equi* and *Babesia caballi* as well as the identification of associated ticks in sympatric Grevy's zebra (*Equus grevyi*) and donkeys (*Equus africanus asinus*) in northern Kenya. *Journal of Wildlife Diseases*, 51, 137-147.
- Sitt, T., Poole, E.J., Ndambuki, G., Mwaura, S., Njoroge, T., Omondi, G.P., **Mutinda**, M., Mathenge, J., Prettejohn, G., Morrison, W.I. & Toye, P. (2015) Exposure of vaccinated and naive cattle to natural challenge from buffalo-derived *Theileria parva*. *International Journal for Parasitology-Parasites and Wildlife*, 4, 244-251.
- **Mutinda, M.**, Chenge, G., Gakuya, F., Otiende, M., Omondi, P., Kasiki, S., Soriguer, R.C. & Alasaad, S. (2014) Detusking Fence-Breaker Elephants as an Approach in Human-Elephant Conflict Mitigation. *PLOS One*, 9.
- Alasaad, S., Permunian, R., Gakuya, F., **Mutinda**, M., Soriguer, R.C. & Rossi, L. (2012) Sarcoptic-mange detector dogs used to identify infected animals during outbreaks in wildlife. *BMC veterinary research*, 8.
- **Mutinda, M**., Otiende, M., Gakuya, F., Kariuki, L., Obanda, V., Ndeere, D., Ndambiri, E., Kariuki, E., Lekolool, I., Soriguer, R.C., Rossi, L. & Alasaad, S. (2012) Putative filariosis outbreak in white and black rhinoceros at Meru National Park in Kenya. *Parasites & Vectors*, 5.
- Ndeereh, D., Okita-Ouma, B., Gaymer, J., **Mutinda, M**. & Gakuya, F. (2012) Unusual mortalities of the eastern black rhinoceros (*Diceros bicornis michaeli*) due to clostridial enterotoxaemia in Ol Jogi Pyramid Sanctuary, Kenya. *Pachyderm*, 45-51.

i. Name: Dr Michael Ngatho Njoroge

ii. Position on this project: Co-investigator

Role: Contribute to trial design and interpretation to ensure data collection will inform conservation needs; contribute to production of management guidelines.

iii. Current position:

2014-present Head, Southern Conservation Area Mobile Veterinary Unit, Kenya Wildlife Service

iv. **Education**:

2002: BVetMed (Hons) University of Nairobi, Kenya.

v. **Previous positions:**

2005-2011: Chief Veterinary Officer, Kenya Police Dog Unit, Kenya National Police Service Oversaw all elements of the health of police dogs and horses

2011-2014 Veterinary Officer, Kenya Wildlife Service

Clinical intervention with sick and injured wildlife

Wildlife rescue, translocation and relocation

Disease investigation, monitoring and surveillance within the ecosystem.

Actively participate in research studies within the Southern conservation area involved in protection of carnivores, elephants and other wildlife inclusive of their habitat.

Provision of short and long term solutions to human wildlife conflict issues especially those involving carnivores and elephants within the region.

Training of intern students, imparting of knowledge to the local community on wildlife conservation and management and also the importance of wildlife as a natural resource for their benefit and the country in general.

Report on conservation progress within the ecosystem to Kenya Wildlife Service policy makers, scientists and donors funding various operations.

- i. **Name:** Dr Louis van Schalkwyk
- ii. **Position on this project:** Co-Investigator

Role: Coordinate, and help analyse and interpret, the field trial in Kruger National Park, including immobilizations, vaccinations, sampling, health monitoring and contributions to data analysis and interpretation.

iii. **Current position**: State Veterinarian, Directorate Animal Health, Department of Agriculture, Forestry & Fisheries, Skukuza, Kruger National Park, South Africa.

iv. Education:

2015: PhD, University of Pretoria. Thesis: "A spatio-temporal probability model of cattle and African buffalo (Syncerus caffer) contact as a proxy for foot-and-mouth disease risk: a case study at the wildlife-livestock interface of the Kruger National Park, South Africa".

2004: MSc, University of Pretoria. Dissertation: "Bone density and Calcium and Phosphorus content of the giraffe (Giraffa camelopardalis) and African buffalo (Syncerus caffer) skeletons."

2002: BVSc, University of Pretoria.

v. Selected advisory positions:

South African Veterinary Council: Registered member (D02/4511) (2002-present)

South African Veterinary Foundation: Director (2012 – 2014)

South Africa Society for Veterinary Epidemiology & Preventive Medicine: Member (2004-present)

Bovine Tuberculosis Study Group: Regular participant/contributor (2001-present)

Anthrax Advisory Group: Convener/Chairman (2019-present)

Wild Dog Advisory Group: Regular participant/contributor (2016-present)

Selected honours and awards:

Wildlife and Environment Society of South Africa Individual Award (National), 2019

Managing Executive Special Award, Kruger National Park, 2019

Managing Executive Appreciation Award, Kruger National Park, 2018

Academic Honorary Colours, University of Pretoria, 2015

Academic Honorary Colours, University of Pretoria, 2004

Honorary Colours, Faculty of Veterinary Science, University of Pretoria, 2002

Service Award, Faculty of Veterinary Science, University of Pretoria, 2002

v. Selected peer-reviewed publications:

- C MARNEWECK, DG MARNEWECK, OL **VAN SCHALKWYK**, G BEVERLEY, HT DAVIES-MOSTERT, DM PARKER. 2019. Spatial partitioning by a subordinate carnivore is mediated by conspecific overlap. *Oecologia* 191:531–540.
- R HIGGITT, OL VAN SCHALKWYK, L DE KLERK-LORIST, P BUSS, P CALDWELL, L ROSSOUW, T MANAMELA, G HAUSLER, J HEWLETT, EP, MITCHELL, P VAN HELDEN, S PARSONS, M MILLER. 2019. Prevalence of *Mycobacterium bovis* Infection in African Wild Dogs, Kruger National Park, South Africa. Emerging Infectious Diseases 25(7):1425-1427.
- M CORTEY, L FERRETTI, E PÉREZ-MARTÍN, F ZHANG, L DE KLERK-LORIST, K SCOTT, G FREIMANIS, J SEAGO, P RIBECA, L **VAN SCHALKWYK**, N JULEFF, F MAREE, B CHARLESTON. 2019. Persistent infection of African buffalo (*Syncerus caffer*) with Foot-and-Mouth Disease Virus: limited viral evolution and no evidence of antibody neutralization escape. Journal of Virology 17:93(15)pii: e00563-19.
- R HIGGITT, OL **VAN SCHALKWYK**, L DEKLERK LORIST, P BUSS, P CALDWELL, L ROSSOUW, T MANAMELA, G HAUSLER, P VAN HELDEN, S PARSONS, M MILLER. 2019. An interferon

- gamma release assay for the detection of immune sensitization to *Mycobacterium bovis* in African wild dogs (*Lycaon pictus*). Journal of Wildlife Diseases 55(3).
- MA MILLER, P BUSS 2, EO ROOS, G HAUSLER, A DIPPENAAR, E MITCHELL, L VAN SCHALKWYK, S ROBBE-AUSTERMAN, W RAY WATERS, A SIKAR-GANG, KP LYASHCHENKO, SDC PARSONS, R WARREN, P VAN HELDEN. 2019. Fatal Tuberculosis in a Free-Ranging African Elephant and One Health Implications of Human Pathogens in Wildlife. Frontiers in Veterinary Science 6:18.
- MA MILLER, P BUSS, SDC PARSONS, E ROOS, J CHILESHE, WJ GOOSEN, L VAN SCHALKWYK, L DE KLERK-LORIST, M HOFMEYR, G HAUSLER, L ROSSOUW, T MANAMELA, EP MITCHELL, R WARREN, P VAN HELDEN. 2018. Conservation of White Rhinoceroses Threatened by Bovine Tuberculosis, South Africa, 2016–2017. Emerging Infectious Diseases 24(12):2373-2375.
- DD LAZARUS, OL **VAN SCHALKWYK**, REJ BURROUGHS, A MPEHLE, B REININGHAUS, O RIKHOTSO, L HEATH, FF MAREE, B BLIGNAUT, GT FOSGATE. 2018. Serological responses of cattle inoculated with inactivated trivalent foot-and-mouth disease vaccine at the wildlife-livestock interface of the Kruger National Park, South Africa. Preventive Veterinary Medicine 158:89-96.
- PJ STEENKAMP, H VAN HEERDEN, OL **VAN SCHALKWYK**. Ecological suitability modeling for anthrax in the Kruger National Park, South Africa. 2018. PLOS ONE 13(1): e0191704.
- CK GLIDDEN, B BEECHLER, PE BUSS, B CHARLESTON, L DE KLERK-LORIST, FF MAREE, T MULLER, E PÉREZ-MARTIN, KA SCOTT, OL **VAN SCHALKWYK**, A JOLLES. 2018. Detection of pathogen exposure in African buffalo using non-specific markers of inflammation. Frontiers in Immunology 8:1944.
- DD LAZARUS, GT FOSGATE, OL **VAN SCHALKWYK**, REJ BURROUGHS, L HEATH, FF MAREE, B BLIGNAUT, B REININGHAUS, A MPEHLE, O RIKHOTSO, GR THOMSON. 2017. Serological evidence of vaccination and perceptions concerning Foot-and-Mouth Disease control in cattle at the wildlife-livestock interface of the Kruger National Park, South Africa. Preventive Veterinary Medicine 147:17-25.
- OL VAN SCHALKWYK, DL KNOBEL, EM DE CLERCQ, C DE PUS, G HENDRICKX, P VAN DEN BOSSCHE. 2016. Heterogeneity in a communal cattle farming system along a zone endemic for foot and mouth disease in South Africa. Geospatial Health 11:338.
- FF MAREE, L DE KLERK-LORIST, S GUBBINS, F ZHANG, J SEAGO, E PÉREZ-MARTÍN, E REID, K SCOTT, OL **VAN SCHALKWYK**, RG BENGIS, B CHARLESTON, N JULEFF. 2016. Differential persistence of foot-and-mouth disease virus in African buffalo is related to virus virulence. Journal of Virology 90(10): 5132-5140.
- OL VAN SCHALKWYK, DL KNOBEL, EM DE CLERCQ, C DE PUS, G HENDRICKX, P VAN DEN BOSSCHE. 2016. Description of events where African buffaloes (*Syncerus caffer*) strayed from the endemic foot-and-mouth disease zone in South Africa, 1998–2008. Transboundary and Emerging Diseases 63(3): 333-347.
- E DION, L VAN SCHALKWYK, EF LAMBIN. 2011. The landscape epidemiology of foot-and-mouth disease in South Africa: A spatially explicit multi-agent simulation. Ecological Modelling 222: 2059-2072.
- G MITCHELL, OL **VAN SCHALKWYK**, JD SKINNER. 2005. The calcium and phosphorus content of giraffe (*Giraffa camelopardalis*) and buffalo (*Syncerus caffer*) skeletons. Journal of Zoology 267:55-61.
- R VERSTER, C BOTHA, V NAIDOO, OL **VAN SCHALKWYK**. 2004. Aldicarb poisoning of dogs and cats in Gauteng during 2003. Journal of the South African Veterinary Association, 75(4):177-181.
- OL VAN SCHALKWYK, JD SKINNER, G MITCHELL. 2004. A comparison of the bone density and morphology of giraffe (*Giraffa camelopardalis*) and buffalo (*Syncerus caffer*) skeletons. Journal of Zoology (London), 264(3):307-315.

O. Letters of support

Letters of support are provided from collaborators Prof Christl A. Donnelly and Tom Smallwood. Statistician Prof Donnelly was unable to act as coinvestigator due to rules at her institution which require her to seek salary support when acting as a coinvestigator. Modeller Tom Smallwood is a PhD student, too early in his career to act as a coinvestigator.



Imperial College London

MRC Centre for Global Infectious Disease Analysis Department of Infectious Disease Epidemiology

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12 November 2019

Christ Ann Donnells

DEPARTMENT OF STATISTICS

24-29 St Giles', Oxford OX1 3LB, UK

Tel: +44(0)1865 281 228 christl.donnelly@stats.ox.ac.uk https://www.stats.ox.ac.uk/all-people/professor-christl-donnelly/

Dear Selection Panel,

I am writing to convey my enthusiastic support for Prof Woodroffe's proposal: **Protecting endangered African wild dog populations from Canine Distemper Virus by vaccination**. I collaborated with Prof Woodroffe in the development of the study design and corresponding sample size calculation. I look forward to collaborating with the team on the analysis of the data from this important CDV vaccination study.

Sincerely

Christl A. Donnelly CBE FMedSci FRS

Professor of Applied Statistics

University of Oxford

Professor of Statistical Epidemiology Imperial College London



Imperial College London

Institute of Zoology Zoological Society of London

Outer Circle Regent's Park London, NW1 4RY, UK

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MRC Centre for Global Infectious Disease Analysis Department of Infectious Disease Epidemiology

School of Public Health Imperial College London St Mary's Campus Norfolk Place London, W2 1PG, UK

thomas.smallwood14@imperial.ac.uk https://www.imperial.ac.uk/people/thomas.smallwood14

13 November 2019

Dear Selection Panel.

I am writing to convey my enthusiastic support for Professor Woodroffe's proposal: **Protecting endangered African wild dog populations from Canine Distemper Virus by vaccination**. I collaborated with Professor Woodroffe on the African wild dog population modelling which contributed to the preliminary data outlined in the proposal. I look forward to continuing to collaborate with the team on the further development and application of the mathematical modelling to help develop guidelines for CDV management.

Yours sincerely,

Thomas R C Smallwood

PhD Candidate

Imperial College London

PhD Candidate

Institute of Zoology

Zoological Society of London

JANE HOPPER

Veterinary Dept, Aspinall Foundation, Port Lympne Reserve, Aldington Rd, Lympne, Kent, CT21 4PD

0792 1474677 <u>janeh@aspinallfoundation.org</u>

Full name: Jane Susannah Hopper

Other surnames: (married surname)

Nationality:

Professional registration: MRCVS 6310895

Education and professional development

- RCVS advanced practitioner in Zoo Animal Medicine, 2015
- RCVS Certificate in Zoological Medicine, 2007
- VetMB degree in Veterinary Medicine, Cambridge University, 2002
- MA, Cambridge University, 2002
- BA (I), Cambridge University, 1999

Employment history

- 2017- present senior veterinary partner of UK charity Wildlife Vets International
 - Wildlife Vets International provides specialist veterinary services/ funds projects carrying out conservation medicine in a variety of countries.
- 2008-2019 Director of Montgomery Veterinary Clinic, Kent
 - o Founding and starting the clinic jointly with my husband
 - o Managing 14 members of staff and ensuring the clinic provides a high standard of care to many species of animal.
- 2006- present Head of Veterinary Services for the Aspinall Foundation (comprising Howletts and Port Lympne Wild Animal Parks and TAF's overseas projects).
 - Leading and managing the Aspinall Foundation's international vet departments. This includes veterinary management of captive collections in the UK and conservation projects in Congo, Gabon, Java and Madagascar. Managing all aspects of the veterinary budget in 4 countries.
 - Recruiting, managing, coaching and training international teams of veterinary surgeons and support staff.
 - All aspects of husbandry, medicine and surgery of a wide range of exotic mammal species. The Aspinall Foundation holds the largest collection of zoological mammals in Europe.
 - Conservation project design, planning, implementation, development and management. Full responsibility for design, planning and management of the health side of the Foundation's projects, including surveillance, disease risk analysis, disease pathways and one health input.
 - o Frequent overseas travel. I have worked in Congo, Gabon, Java and various other developing countries regularly for the last 14 years. I have

- daily contact with teams on the ground, using mainly English but also French.
- o Representing the Foundation and presenting its work as required
- o Founding and chairing the Aspinall Foundation's ethics committee
- 2004- 2005 Locum veterinary officer at Johannesburg Zoo, South Africa
 - Two 3 month periods of locum work at a busy zoological park including periods of sole charge
- 2004- 2006. Regular work at Howletts and Port Lympne Wild Animal Parks (through Burnham House) providing veterinary services to both parks
- 2002-2006 Burnham House Veterinary Surgery, UK.
 - Mixed practice. As well as mixed practice work I was responsible for veterinary care for 2 small zoological collections (Wildwood and Wingham Wildlife Park)
- 2001 Student externship at Perth Zoo, Australia (3 months)
- 2000 Student externship for Mauritian Wildlife Foundation, Mauritius (3 months)
- 1999 Student externship at St Louis Zoo, USA (3 months)

Selected publications

- Hopper, J., Holding, E., Scurrell, S. and Featherstone H. Vernal-like conjunctivitis in a western lowland gorilla (*Gorilla gorilla gorilla*). Veterinary Ophthalmology 2017
- Hopper, J. Pre-release health considerations for gorillas. In Cooper, J. and Hull, G. (eds). Gorilla pathology and health. First edition. Elsevier; 2017. 197-203.
- Hopper, J. Common marmosets. In Kubiak. M. (ed). Handbook of Exotic Pet Medicine. First edition. Wiley Blackwell; 2021. 27-42
- Scurrell, E., Holding, E., Hopper, J., Denk, D., Fuchs-Baumgartiner A., Silbermayr, K., and Nell, B. Bilateral lenticular Encephalitozoon cuniculi infection in a snow leopard (*Panthera uncia*). Veterinary Ophthalmology. 2015 18(1): 143-7
- Bishop, G., Zuba, J., Pessier, A, Hopper, J, Kendall G, Rosychuk R, and Magdesian K.
 Medical management of recurrent eosinophilic granuloma in two black rhinoceros (*Diceros bicornis*). J Zoo Wildl Med. 2016 47(3): 855-861
- Cracknell, J., Lawrie, A., Yon, L., Hopper, J., Martinez Pereria Y. and Pizzi,
 R. Outcomes of conservatively managed coracoid fractures in wild birds in the United Kingdom. J Avian Med Surg. 2018 32(1): 19-24

- Ogden, R., Ghazali M., Hopper J., Culik, L. and King T. Genetic assessments for antelope reintroduction planning in four European breeding programmes. IZAR. 2018 6(3): 79-84
- Diks, K., Webster, L., McDowall, I., Muya, S., Hopper, J. and O'Donoghue,
 P. Validation studies in dinucleotide STRs for forensic identification of black rhinoceros Diceros bicornis. Forensic Sci Int Genet. 2017 e25-e28 doi: 10.1016/j.fsigen.2016.10.016
- Vega, R., Hopper, J., Kitchener, A., Catinaud, J., Roullet, D., Robsomanitrandrasana, E., Roos, C. & King, T. 2019. A preliminary report on the population genetics of captive ruffed lemurs (Abstract). *Primate Eye* 128: 32.
- Wedana, M., Masnur, I., Ibrahim, S., Semiawan, I.E., Ford, M., Hopper, J. & King, T. (2021) Reinforcement of an isolated Javan silvery gibbon population at Mt. Tilu, West Java, Indonesia. In: Soorae, P.S. (ed.) Global Conservation Translocation Perspectives: 2021. Case studies from around the globe. Gland, Switzerland: IUCN SSC Conservation Translocation Specialist Group, Environment Agency Abu Dhabi and Calgary Zoo, Canada. pp. 229-234.
- Kurniawan, I., Wedana, M., Masnur, I., Semiawan, I.E., Hopper, J. & King, T. (2021)
 Reinforcement of isolated Javan ebony langur populations in East Java,
 Indonesia. In: Soorae, P.S. (ed.) Global Conservation Translocation Perspectives:
 2021. Case studies from around the globe. Gland, Switzerland: IUCN SSC
 Conservation Translocation Specialist Group, Environment Agency Abu Dhabi
 and Calgary Zoo, Canada. pp241-245
- Wedana, M., Masnur, I., Ibrahim, S., Semiawan, I.E., May, A., Hopper, J. & King, T. (2021) Reinforcement of isolated populations of Javan grizzled langur and western Javan ebony langur at Mt. Tilu, West Java, Indonesia In: Soorae, P.S. (ed.) Global Conservation Translocation Perspectives: 2021. Case studies from around the globe. Gland, Switzerland: IUCN SSC Conservation Translocation Specialist Group, Environment Agency Abu Dhabi and Calgary Zoo, Canada. pp.235-140

Memberships

- British Veterinary Zoological Society (BVZS)- member.
- BVZS Council member and meetings committee member (2011-2017)
- American Association of Zoo Veterinarians (AAZV)- member
- European Association of Zoos and Aquaria (EAZA). Veterinary advisor for:

European Felid Taxon Advisory Group Clouded leopard EEP Amur and Sumatran Tiger EEPs (co-advisor) Bush dog International studbook Javan gibbon EEP Black rhinoceros EEP Greater bamboo lemur EEP

- Primate specialist group- member, and working group vet for section for small apes
- Great Ape Welfare Group Advisory panel 2021
- Pan African Sanctuary Alliance (PASA)- member

Scientific reviewer for

- Journal of zoo and wildlife medicine
- Journal of zoo and aquarium research
- Journal of medical primatology
- Veterinary Record Case Reports

Languages

- •
- •

PRIVATE AND CONFIDENTIAL ZOOLOGICAL SOCIETY OF LONDON - ETHICS COMMITTEE RESEARCH PROJECT ETHICAL REVIEW FORM (Animal Impacts)



The ZSL Ethics Committee considers proposals for research involving the use of animals by ZSL and external staff, and advises the ZSL Directors on ethical and welfare aspects of the work. The Committee consists of ZSL staff members, external members and an external Chair. It holds 2 main meetings a year and other interim meetings as required. Proposals are dealt with promptly both during and between meetings.

This form should be completed, signed by the Applicant and their Line Manager/Supervisor/Programme Manager, and then emailed to the Secretary to the Ethics Committee (bill.holt@zsl.org), at least ONE MONTH in advance of the proposed start date for the work. If appropriate, supporting documents may be appended to the application.

Please contact the Secretary if you require assistance or wish to discuss any points before submitting the form.

SECTION A – Project Details	
01. Project Title	02. ZPD Ref Code
Can vaccination protect African wild dogs from canine distemper? Addressing a conservation emergency.	IOZ69

03. Applicant Name and Home Institution

Rosie Woodroffe, IoZ

04. Project Abstract and Lay Summary

Please provide both a scientific and 'lay' summary (to describe the project in simpler terms).

The African wild dog is a globally endangered species, with fewer than 700 packs remaining in the wild. Canine Distemper Virus (CDV) was assumed to pose little risk to the species, because field studies in many parts of Africa had found healthy animals with antibodies to the virus, suggesting that wild dogs often survived the disease. Then suddenly, in the past three years, six separate fatal CDV outbreaks have been recorded, with the worst all but wiping out the largest population in the northern hemisphere. Previous ZSL research shows that CDV cannot easily be controlled by vaccinating domestic dogs, suggesting that wild dogs themselves might need to be vaccinated where CDV risks are most acute. Unfortunately, no safe and effective vaccination protocol has been devised for use on free-ranging wild dogs. This project aims to identify such a protocol, to inform urgent conservation efforts.

Our project has three components. First, a captive trial designed to test whether presumed protective antibody titres can be triggered on a single handling event, appropriate for field use. Second, a field trial in South Africa designed to evaluate whether the captive protocol is safe and likely effective for free-ranging wild dogs, and whether it can be implemented with a reasonable level of effort. Third, we shall use these new data to parameterise an existing dynamic model of CDV dynamics and control, to identify the management approaches most likely to reduce population extinction risks, allowing us to develop guidelines for managing the conservation impacts of this deadly disease.

05. Project Aims and Context

Please also include information about the following issues: Benefits to science/conservation/welfare/education; Why animal use is necessary (are there any other possible approaches?); The appropriateness of the species used; Justification for the numbers of animals to be used (include details of the experimental design, numbers of animals required to achieve significant results and factors that may affect this): Links to references to previous work or previous usage of techniques.

Hypothesis and Objectives

This project aims to test the hypothesis that extinction risks to African wild dog populations can be reduced by vaccination against Canine Distemper Virus. The project has three key objectives, thus:

(1) Working with captive African Wild Dogs, identify a protocol for vaccination against Canine Distemper Virus which is safe, effective, and likely to be practical for field use.

- (2) Working with free-ranging African Wild Dogs, assess the safety, efficacy, and practicality of the vaccination protocol
- Using an existing mathematical model, and findings from Objectives 1 and 2, quantify impacts on extinction risk to develop guidelines for CDV management in African wild dog populations.

The need to manage Canine Distemper risks to African wild dog populations

The African wild dog (*Lycaon pictus*) is an endangered species threatened by infectious disease, and Canine Distemper Virus (CDV) appears to be a growing threat. Habitat loss and deliberate killing have extirpated the species rabies being considered the greatest disease threat to the species. In contrast, CDV exposure was often nonfatal, with multiple field studies reporting seropositivity in apparently healthy animals¹⁻⁵. Although sporadic whole-pack deaths

were reported^{6,7}, the only major confirmed outbreak was in a captive breeding centre⁸. However, in 2016 CDV killed whole packs at three separate sites in South Africa^{9,10}, and the following year another pack succumbed in Tanzania's Serengeti ecosystem. In 2017 a major CDV epidemic caused the near-extinction of the wild dog population in the Ewaso ecosystem in Kenya, killing ≥20 packs¹¹. By 2019, three packs had re-formed from the remnants of the Ewaso population, but CDV killed one of them. Evidently, CDV is a serious and emerging threat to this endangered species.



Because CDV is a canine pathogen, there have been several attempts to reduce wildlife CDV risks by vaccinating domestic dogs^{11,12}. However, this approach may have limited effectiveness, since

- (i) Domestic dog populations may not act as reservoir hosts for CDV. Mass dog vaccination around the Serengeti reduced CDV incidence in dogs but not in wild lions¹², suggesting that the virus was persisting in wildlife. Likewise, molecular analyses suggest that CDV affecting tigers in the Russian far east came from wildlife, rather than domestic dogs¹³. Our previous research within the Ewaso ecosystem showed that CDV was not persisting in local domestic dogs¹⁴, and that wild dogs with greater opportunities for domestic dog contact were not more likely to have been exposed to CDV³.
- (ii) Even if domestic dogs did act as a CDV reservoir, controlling infection would be challenging because CDV, like other morbilliviruses (e.g. measles¹⁵, phocine distemper virus¹⁶), may persist only on very large geographic scales, and control may require vaccination coverage of $\geq 95\%^{17}$.
- (iii) While governments are committed to eradicating dog-mediated rabies by 2030¹⁸, CDV has no human health impacts, and hence no eradication strategy. For this reason, any local CDV vaccination of domestic dogs would need to be maintained by conservationists in perpetuity.

Since vaccination of domestic dogs appears to be an imperfect way to reduce CDV threats to African wild dogs, in some circumstances vaccination of wild dogs may need to be considered.

Choice of CDV vaccine

Three categories of vaccine are currently available: inactivated, modified-live, and recombinant.

Modified-live vaccines (MLVs) are highly effective in domestic dogs¹⁹, ¹⁷, and can prompt seroconversion in captive African wild dogs²⁰. Nevertheless MLVs have occasionally induced clinical distemper in a number of nondomestic carnivores^{21,22}, including African wild dogs²³⁻²⁵. Risks appear to be low, however²⁶, and MLVs are widely used on captive African wild dogs in Europe.

<u>Inactivated vaccines</u> have been used on African wild dogs in captivity to avoid all risk of vaccine-induced distemper²⁶. However, they have consistently failed to provoke serological responses^{20,27}, and failed to prevent CDV from killing 49 of 52 wild dogs in a captive facility in Tanzania⁸.

<u>Recombinant vaccines</u> likewise cannot induce distemper, because they do not contain a complete viral genome. Such vaccines have induced seroconversion in African wild dogs²⁸, and other sensitive species²⁹. However, a trial in captive tigers showed that recombinant vaccines produced weaker immune responses than MLVs³⁰. Moreover, use of the recombinant CDV vaccine on free-ranging wild dogs in an outbreak situation might be difficult, because the import of GMOs is forbidden in some African countries and requires time-consuming permitting in others³¹. Moreover, the vaccine has faced repeated supply problems^{32,33}.

As MLV appears to be immunogenic, low risk, and widely available in Africa, it is a strong candidate for use in protecting free-ranging populations of African wild dogs threatened by canine distemper. However, there is currently no established vaccination protocol suitable for field use.

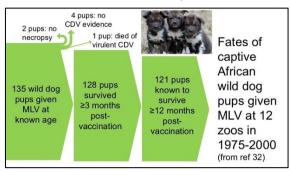
Choice of vaccination protocol

Like domestic dogs, most captive wild dogs are given their first CDV vaccinations as young puppies, although maternal antibodies may neutralise the vaccine³⁴. To ensure vaccine "take", doses are repeated at 2-4 week intervals until 16 weeks of age³⁴. However, because vaccination of free-ranging wild dogs would require darting, it would have to target older animals, as darting would injure young pups. If a domestic dog receives its first vaccinations at >20 weeks, after maternal antibodies have waned, a single MLV dose is protective³⁴. If the same were true in wild dogs,

MLV might be able to protect free-ranging wild dogs after a single handling event. However, this point is uncertain because wild dogs which seroconverted in published studies had previously been given MLV³⁵ or inactivated²⁰ CDV vaccine. If a single dose proved insufficient, immune responses might be strengthened by giving multiple doses simultaneously, as in rabies control^{36,37}. We anticipate that a double dose would be safe, because the dose for a 5month pup (2ml/15.9kg³⁸ or 0.13ml/kg) would be lower than that for a 2-month pup (1ml/6.1kg or 0.16ml/kg), and that for an adult of a small domestic dog breed (e.g. adult chihuahua, 1ml/3kg or 0.33ml/kg). The monovalent MLV contains no adjuvant³⁹ which some have tentatively linked to adverse vaccine reactions in small domestic dog breeds⁴⁰. It may thus be helpful to evaluate both single and double doses of MLV in African wild dogs.

Preliminary Data

We have previously evaluated the safety of modified live CDV vaccine in captive African wild dogs, by requesting zoos' vaccination records for the period 1975-2000, and comparing individual survival using studbook data^{41,42}. This



work²⁶ revealed no cases of confirmed vaccine-induced distemper among 135 pups given MLV for the first time at known age, suggesting a risk of 0% (exact binomial 95% confidence interval [CI] 0-2.7%). If one pup which died in 1983 of virulent CDV (likely not a vaccine strain) and two pups with no reported cause of death are conservatively assumed to have died of vaccine-induced distemper, the risk would be 2.2% (CI 0.5-6.4%).

We have also evaluated antibody responses to recombinant CDV vaccine in captive wild dog pups, showing that this vaccine is safe and immunogenic in captivity, if

litter 2

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litter 1

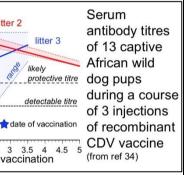
delivered by a parenteral route²⁸. All pups without detectable maternal antibodies at the start of vaccination showed strong, rising titres after a single dose, although those with maternal antibodies required multiple doses²⁸.

However, our evaluation of immune responses to recombinant CDV vaccine in free ranging wild dogs showed a much less promising immune response (van Schalkwyk, unpubl. data). Wild dogs in 20 packs given recombinant vaccine in Kruger National Park, South Africa, showed no

Responses to recombinant CDV vaccine in 38 free-ranging wild dogs (LvS unpubl. data) serum pseudotype neutralisation test assay no detectable weak response to response to vaccination vaccination high positive medium negative low

(antibody titre) immune response detectable by serum

7



neutralisation tests. A pseudotype assay on the same samples showed evidence of a weak response: only 11 of 38 individuals had high titres after a single vaccine dose, of which four had had high titres pre-vaccination (see left). These (unpublished) data raise concerns about the utility of recombinant CDV vaccine for freeranging wild dogs.

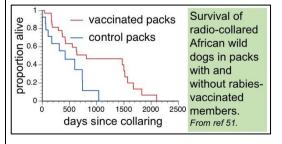
2 2.5

months post-vaccination

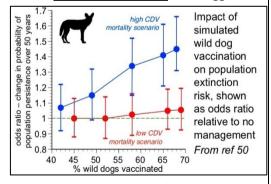
Nevertheless, our team's population modelling work suggests

that, if we could identify an effective vaccination

protocol, it would have conservation benefits. In a model (see right) simulating wild dog population dynamics (including within-and



between-pack dynamics⁴³), vaccination was associated with >40% reductions



in extinction risk if CDV could cause high mortality⁴⁴. Our team has previously used field trials to evaluate vaccine safety, including a trial at a site in Kenya⁴⁵ which showed that rabies vaccination was safe for use in African wild dogs (see left).

06. Brief description of the project methodology (Please keep to less than 200 words if possible)

Our project has three components. First, a captive trial designed to test whether presumed protective antibody titres can be triggered on a single handling event, appropriate for field use. This captive trial will be conducted in AZA-accredited zoos in North America and will entail measuring serum antibodies in African wild dog pups vaccinated according to three different protocols. Second, a field trial in South Africa designed to evaluate whether the captive protocol is safe and likely effective for free-ranging wild dogs, and whether it can be implemented with a reasonable level of effort. This field trial will entail comparing the survival of vaccinated and unvaccinated wild dogs in Kruger National Park, South Africa, and also comparing serum antibodies in vaccinated animals before and after vaccination. Third, we shall use these new data to parameterise an existing dynamic model of CDV dynamics and control, to identify the management approaches most likely to reduce population extinction risks, allowing us to develop guidelines for managing the conservation impacts of this deadly disease.

07. Licences and Permits

(a) Will any of the work need to be carried out under the Animals (Scientific Procedures) Act 1986?:

No, as it is conducted entirely overseas

(b) Do you already hold a Home Office Personal Licence?:

Yes, for a different project, so this research is conducted to the same standard

(c) Has the project been discussed with the relevant NVS and NACWO?:

This project has been discussed in detail with equivalent officers in the USA and South Africa, where the data will be collected

(d) Are other national or international licences or permits required (e.g. for capture and handling free-living animals, sample import or export)? If so, please provide details:

Yes. For the zoo work in the USA, the project will require ethical approval from participating zoos and may require other permits from state and/or federal agencies depending on which zoos take part. For the fieldwork, permits are needed for capture and handling, and for sample export (from South Africa) and import (to the USA). Research in South Africa will be conducted in collaboration with SANParks under permits from the Limpopo and Mpumalanga provincial authorities. Sample export permits from South Africa are issued by these same authorities. Import permits to the USA (for sample analysis at Cornell University) are issued by the US Fish & Wildlife Service. While African wild dogs are not listed on CITES, in the USA they are listed in the Endangered Species Act and are therefore subjected to the same permitting rules as CITES-listed species.

Both we and our collaborators already hold permits to capture and collar wild dogs in South Africa for other projects, so we are familiar with all the protocols.

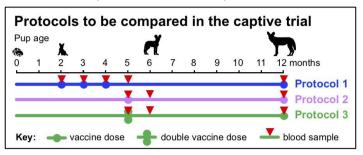
08. Details on the species, numbers and ages (e.g. juvenile, adult) of the animals involved

Please include a power calculation if possible to demonstrate that data will be statistically robust.

All data collection will be conducted on African wild dogs. The captive trial (Objective 1) will entail working with animals aged 2-12 months, whereas the field trial (Objective 2) will entail working with animals aged ≥12 months. The numbers of animals included in each element of the project have been carefully chosen to balance the numbers of animal used (which should be minimized) against the sample size required to address the study questions and, hence, to inform wild dog conservation efforts (which should be maximized). In performing our power calculations (described below), we have therefore taken account of the need to obtain estimates with adequate precision, while minimizing the number of animals involved. We have also accounted for the fact that, in the field study especially, some individuals may die from causes unrelated to the study (such as predation), requiring a slightly larger sample size to provide adequate precision.

<u>Objective 1:</u> Working with captive African Wild Dogs, identify a protocol for vaccination against Canine Distemper Virus which is safe, effective, and likely to be practical for field use.

Based on the evidence presented above, we propose evaluating MLV as a tool for protecting wild dogs against CDV. For simplicity, we shall use a monovalent CDV vaccine such as Neovac-D. In captivity, we plan to compare three protocols, designed to maximise information relevant to managing CDV risks in the field, while minimising both the health risks to captive animals, and disruption to zoo staff. The effect of each protocol will be measured using samples



collected at the time of vaccination, and approximately 30 days after the end of each vaccination course.

Protocol 1 is the standard zoo protocol (doses at 2, 3, and 4 months of age, with a booster at 12 months). It is not suitable for field use, but it provides a baseline against which other protocols can be compared.

Protocol 2 is the approach recommended for older domestic dog pups (a single dose at ≥5 months

with a booster at 12 months). In the field, vaccinated animals would be >10 months old, but we propose evaluating this protocol in captivity on pups aged 5 months, to avoid prolonging the period of CDV risk in animals which have cleared their maternal antibodies.

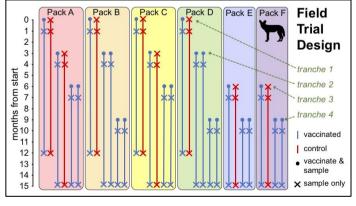
Protocol 3: is a modification of Protocol 2, using two simultaneous doses of vaccine at different sites.

The captive trial will be conducted at a minimum of four participating zoos, coordinated by the Association of Zoos and Aquaria (AZA), with all vaccination and blood sampling performed by the zoos' own veterinary staff. In Phase 1, two litters (approximately 18 pups, based on average captive litter size⁴¹) will be allocated to Protocol 1 while, in two other litters, equal numbers of pups will be randomly assigned to receive either Protocol 2 or Protocol 3 (approximately 9 pups per protocol). If no ill-effects are observed, the protocols will be repeated with another four litters in Phase 2.

Blood samples will be centrifuged on the day of collection, with serum stored at -20°C pending analysis. CDV antibody titres will be measured at the Animal Health Diagnostic Centre at Cornell University, using a serum neutralization test and the Onderstepoort virus strain. A published study suggests that the proportion of adult wild dogs with protective titres after a single MLV dose (8/8²⁰) falls between 100% and 63% (exact binomial confidence interval). Our study could improve the precision of this estimate to 90-100% (Protocol 1, 36 pups) and 81-100% (Protocols 2 & 3, 18 pups each). We shall compare individuals' titres before and after vaccination using Wilcoxon signed rank tests, and will use Mann-Whitney U-tests to compare post-vaccination titres between protocols; we prefer nonparametric statistics because titre data often include values (e.g., <8, >160) which cannot be analysed using parametric statistics. Test results from Phase 1 will determine the protocol to be used in the field trial.

Objective 2: Working with free-ranging African Wild Dogs, assess the safety, efficacy, and practicality of the vaccination protocol

The field trial will be conducted in Kruger National Park, South Africa. It will evaluate whether free-ranging wild dogs mount a strong immune response to MLV after a single handling event, and whether vaccinated individuals survive as well as unvaccinated pack-mates. Although the vaccine will have been tested in captivity, our field study will reflect guidance on designing "first in man" trials⁴⁶, initially vaccinating a small number of animals and increasing numbers if no ill-effects are found.



To measure vaccine safety, we plan to compare the survival of vaccinated and control animals, focusing on the first month of monitoring since all recorded cases of vaccine-induced distemper have occurred 10-22 days post-vaccination²³⁻²⁵. Animals will be recruited to the trial in four tranches. For tranche 1, two yearling animals will be darted in each of four packs, with one of each pair randomly selected to receive vaccine (either single or double dose, depending on captive trial findings) and a mortality-sensing satellite-linked GPS collar, while the other remains

unvaccinated and is fitted with a mortality-sensing VHF collar. Both animals will be blood sampled on initial collaring and again 1 month and 12 months later. We shall monitor mortality daily, and will attempt visual observations every 2-3 days in the first month post-vaccination. Any signs of ill health will prompt daily visual monitoring and immediate consultation with veterinarians. Any mortality signals will trigger immediate attempts to retrieve a carcass for necropsy, and screening for CDV using histologic examination, virus isolation, reverse transcriptase-PCR, and nucleotide sequencing at Cornell. If CDV is detected, vaccinations will be paused pending discussions within the team, and with SANParks, about how to proceed. If none of the vaccinated animals dies of CDV in the first three months of

monitoring, tranches 2 (six vaccinated, two control), 3 (six vaccinated, two control), and 4 (eight vaccinated) will be recruited at three-month intervals, as illustrated above. Using continuity correction and α =0.05, this study design should provide 80% power to detect mortality increases among vaccinated animals of 35% in the first month of monitoring, and 8% in the full 312 dog-month monitoring period⁴⁷. This **power calculation** is two-sided. The seroprevalence in the reference group (unvaccinated controls) is conservatively estimated as the upper exact binomial confidence limit for the most recent measure of seroprevalence (3/38 seropositive without vaccination, exact binomial CI 1.7-21.4%, therefore conservatively assume baseline seroprevalence of 21.4%). The seroprevalence in the vaccinated group is conservatively estimated as the lower exact binomial confidence limit for the only estimate of seroprevalence post-vaccination (8/8 seropositive post-vaccination, exact binomial CI 63.1-100%, therefore conservatively assume a post-vaccination seroprevalence of 63.1%). This calculation gives a conservatively-estimated expected difference of 42% between the vaccinated and unvaccinated groups. A sample size of 24 vaccinates and eight controls provides 85% power to detect such a difference.

To measure likely vaccine effectiveness, we plan to compare CDV antibody titres (measured at Cornell using serum neutralisation tests) in vaccinated animals one month post-vaccination with their own pre-vaccination titres, and with simultaneous titres of unvaccinated control animals, using nonparametric statistics as for the captive trial. Our proposed sample size (24 vaccinates and eight controls) should provide 85% power to detect the difference between conservatively-estimated baseline CDV seroprevalence and the expected proportion of seropositive animals post-vaccination⁴⁷. This **power calculation** includes a continuity correction, and is one-sided because the field trial is designed to evaluate whether vaccination causes excess mortality, not whether it reduces mortality. We shall use similar methods to compare vaccine titres 6-12 months post-vaccination, providing some information on likely duration of protection.

To measure the practicality of vaccine delivery, we shall record the effort (in person-hours, vehicle mileage, and other costs) required to deliver each vaccination and each visual observation.

<u>Objective 3</u>: Combine the findings from Objectives 1 and 2 with ongoing mathematical modelling to develop and agree guidelines for CDV management in African wild dog populations

Our captive and field trials will provide data on the proportion of wild dogs likely to be protected from CDV by MLV, and the duration of such benefits. We shall parameterise our existing population model⁴⁴ with these new data to estimate the likely consequences for population persistence of different vaccine coverage scenarios. Our population model has the capacity to represent vaccine coverage as a function of pack encounter probability per unit effort⁴⁴; hence, we can use our data on the effort and costs associated with delivering each vaccination to parameterise our model. We can then compare the likely conservation gains associated with specific investments in effort. We shall use the outcomes of this empirical and modelling work, together with our own and others' previous evidence, to develop guidelines on CDV management in African wild dog populations.

09. Where is the work to be carried out? (Please list all sites)

(a) ZSL sites:

Project management and modelling will be conducted at IoZ. All data collection will be conducted overseas in collaborative partnerships.

(b) Collaborating Institutions (Please provide the name(s) and contact details of your collaborator(s), and state whether ethical approval has been granted by any collaborating institutions with reference number if possible)

US Zoos

The specific zoos participating in the captive trial have not yet been selected. Zoos will be invited to participate by the Association of Zoos and Aquaria (AZA) Canid and Hyaenid Technical Advisory Group (TAG) through its African Wild Dog Species Survival Plan (SSP). Our contacts are Karen Bauman (TAG Chair, KBauman@stlzoo.org) and Christina Gorsuch (SSP Lead, Christina.Gorsuch@cincinnatizoo.org). Each participating zoo will need to complete its own ethical review of the project.

Fieldwork

Field data collection will be conducted in Kruger National Park, in collaboration with SANParks. Our contact there is SANParks veterinarian Dr Louis van Schalkwyk (<a href="https://liveo.org/liveo

Sample screening

All blood samples will be screened for CDV antibodies at Cornell University, which has the international standard laboratory for CDV-screening of wildlife. Collaborators at Cornell are Dr Diego Diel (Director, Virology Laboratory,

Department of Population Medicine and Diagnostic Sciences, dgdiel@cornell.edu) and Dr Martin Gilbert (wildlife epidemiologist, Department of Population Medicine and Diagnostic Sciences, m.gilbert@cornell.edu). Cornell will require evidence that the samples have been collected and exported/imported under appropriate permits and with ethical clearance by contributing teams.

Preparation of management guidelines

The management guidelines will be developed in collaboration with all project team members, including Kenya Wildlife Service where we are working closely with wildlife veterinarians Dr Matthew Mutinda (mmutinda@kws.go.ke) and Dr Michael Njoroge (njorogengatho@gmail.com).

10. Details of how and where the animals will be housed

Are suitable facilities available for the procedures which will be carried out?

All animals in the captive study will be housed in AZA-accredited zoos under high standards of animal care.

All animals in the field study will be free-ranging and will be released to the wild within 2 hours of capture

11. Provide details of the procedures involved with reference to the following (including durations) where relevant (provide links and references to previous work or previous usage of techniques)

(a) Source of animals/capture techniques:

Animals enrolled in the captive trial (**Objective 1**) will be captive-born pups in AZA-accredited zoos in North America. Pups will be vaccinated and blood sampled by manual handling while they are small enough, and using behavioural training when they are older. Vaccination and blood sampling will be conducted by the staff at the participating zoos, who will decide on the most appropriate methods at each age.

Animals involved in the field trial (**Objective 2**) will be wild-born in Kruger National Park, South Africa, and will remain in the wild for the duration of the study. Free-ranging wild dogs will be captured by darting from a vehicle. Darting is conducted using a CO₂-powered rifle at distances of ≤20m, targeting the large muscle mass in the hindquarter of a stationary standing or sitting animal. No darts are fired where there is a risk of hitting a non-target animal. Darted wild dogs typically move 10-30m, then settle down again before becoming recumbent. Other pack members typically do not respond at all, or move with the darted animal; this behaviour regularly allows two animals to be darted on a single occasion. Pack members that are not immobilized usually remain within a few hundred metres (often less) while handling is conducted and are rapidly re-united with the immobilized animal once handling is complete.

(b) Handling:

In the captive trial (**Objective 1**), animal handling will be conducted by experienced veterinary or keeper staff, following the protocols of the individual (AZA-accredited) participating zoos.

In the field trial (**Objective 2**), all study animals will be fitted with VHF radio-collars (weighing ≤350g), GPS collars (usually Vectronic Aerospace, weighing ≤350g), or bespoke collars incorporating GPS, accelerometer, temperature, and light sensors (≤350g, http://www.wildbyte-technologies.com/products). Analyses conducted on wild dogs in several ecosystems have revealed no evidence that such collars cause stress or increase mortality. All tracking collars fitted will include mortality sensors, which transmit a distinctive signal when the collar does not move for several hours. This almost invariably indicates that the animal has died.

Full biometric measures are taken on each capture. These comprise weight (measured using a spring balance), head-body length, tail length, ear height, neck diameter, chest girth, hindfoot length, and the length of the carpals, metacarpals, radius, humerus and scapula. All animals are photographed to permit recognition of their individual coat patterns. Tooth wear (an approximate measure of age) is also recorded. Reproductive status is noted (pregnancy and lactation status for females, testis length and width for males). Any injuries sustained during capture are also recorded if they occur.

(c) Anaesthesia:

The captive trial (**Objective 1**) will not involve any anaesthesia, unless study animals are anaesthetised for other purposes and blood sampled or vaccinated at the same time.

For the field trial (Objective 2), all immobilizations take place between dawn and early afternoon, to avoid the risk of predation on disoriented animals that could occur at nightfall. All immobilized animals are kept in the shade, with regular monitoring of body temperature, respiration, and heart rate; animals are cooled with water if necessary. For our long-term wild dog study in Kenya, wild dogs are immobilized with a combination of medetomidine (Domitor, Pfizer Animal Health; approximately 26µg/kg) and ketamine (approximately 2.6 mg/kg) which immobilizes animals within 5 minutes, lasting 45-75 minutes. For this study in Kruger National Park, however, the overseeing SANParks veterinarians may select alternative immobilising drugs, using their clinical judgement. Moisturising eye ointment is administered immediately on immobilization to prevent drying of the corneas, and all animals are blindfolded throughout handling. Ketamine is kept on-hand in case of a need for a top-up (though this is very seldom needed).

(d) Surgical procedures:

This project does not entail any surgery.

(e) Sampling (samples, quantities, frequencies, sites):

In the captive trial (**Objective 1**), blood samples will be collected at the time of each vaccination, and 30 days after the final vaccination in a course (3-5 times in 12 months, depending on the protocol). Blood collection sites will be chosen by the collaborating veterinarians. Blood volumes to be collected on each capture will be minimised, and will not exceed 2% of blood volume on any one occasion, or 5% of blood volume in any 30-day period (e.g., 5ml blood collected from a 6.1kg pup³⁸ aged 2 months would represent 1% blood volume).

In the field trial (**Objective 2**), blood samples will be collected from the jugular vein of anaesthetised wild dogs using vacutainers. In addition, the following will be collected from each animal where possible: ectoparasites; saliva swabs (to check for rabies virus and CDV); faecal sample. Blood volumes will not exceed 2% of blood volume on any one occasion, or 5% of blood volume in any 30-day period (e.g., 20ml blood collected from a 25kg adult would represent 1% blood volume).

(f) Analgesia:

No analgesia is anticipated for this project, since the procedures used are likely to impose only very transitory, minor pain.

(g) Recovery:

In the captive trial (Objective 1), we do not anticipate chemical immobilization, and so no recovery period is needed.

In the field trial (**Objective 2**), once heart and respiratory rates start to rise, or 60-75 minutes after administration of the initial dose (whichever is the sooner), medetomidine anaesthesia is reversed with an intramuscular or intravenous injection of atipamezole (Antisedan, Pfizer Animal Health; 130µg/kg). This usually leads to the animal standing within 10 minutes of administration, with full coordination attained within a further 10 minutes. Animals are monitored closely throughout this period.

(h) Release:

In the captive trial (**Objective 1**), study animals will be returned to their enclosures (if they ever leave them) immediately after handling.

In the field trial (**Objective 2**), all study animals will be immobilized, handled, and reversed at the capture site, to minimise stress and ensure the most rapid return to the wild. Other pack members usually remain nearby during handling, and immobilized wild dogs are often seen to be reunited with their pack within a few minutes of reversal. No aggression towards recovering animals has ever been witnessed. All immobilized wild dogs have remained with their packs for at least several weeks after handling.

(i) Subsequent care and monitoring:

In the captive trial (**Objective 1**), study animals will be carefully monitored by zoo staff to watch for any signs of ill health.

In the field trial (**Objective 2**), all animals will be monitored both visually (to assess health and behaviour), and by listening to radio signals (to assess survivorship).

On each occasion when study animals are sighted, observers will record (i) any evidence of neurological signs that might indicate CDV or rabies infection; (ii) any other evidence of ill health.

Whenever radio-signals are detected, the animal's approximate location will be noted, along with whether the signal is normal or indicating mortality. Any mortality signal will be followed up immediately to recover the animal's carcass and to conduct a detailed necropsy.

12. What adverse welfare effects are anticipated and what steps are to be taken to ameliorate/avoid these?

Consider each stage of the project, from obtaining the animals, transport, husbandry, procedures (see Question 11) etc., to euthanasia or re-homing.

(a) Capture techniques

In the captive trial (**Objective 1**), removing or handling animals for vaccination and/or blood sampling could lead to stress for other group members, as well as stress or injury the animals being handled. These risks will be ameliorated by ensuring that all training and handling are conducted by experienced staff following protocols of the participating zoos.

In the field trial (**Objective 2**), animals could become injured by a misplaced dart. This is avoided by exercising extreme caution when darting, firing only at short range, and when the position of the target animal, and other nearby animals, is such that a dart which goes high or low, or is moved laterally by the wind, is likely to miss entirely rather than hit another animal or a body part which could be harmed. Darting accuracy is maintained by avoiding darting on windy days, regular practice, and frequent checking of gun sights. Over the past 20 years, no injuries have occurred on this project in >150 wild dog dartings.

There is a small risk of groups of animals being broken up by darting. However, this has never been recorded in the 20 years of the Samburu-Laikipia Wild Dog Project. Precautions are taken to hide darted animals from their group-mates (e.g., behind a vehicle or a bush) to avoid possible stress to animals not being darted.

(b) handling

In the captive trial (**Objective 1**), animals could be injured by restraint using poorly designed equipment or inexperienced staff. These risks will be minimised by sharing experiences between participating zoos, and also with staff at London Zoo where wild dogs are routinely trained to accept vaccination and blood sampling (though London Zoo cannot participate as it has no opportunities to raise pups at present).

In the field trial (**Objective 2**), animals might be harmed by handling procedures if over-large radio-collars were fitted. The collars used are similar to those used on other wild dog projects and constitute 1.2-1.5% of body weight. No collar injuries have been observed in 20 years of monitoring.

(c) anaesthesia

Animals in the field trial (**Objective 2**) might be harmed during anaesthesia by a major drug overdose. This is avoided by using immobilizing drugs with a wide safety margin, using doses which have been refined through field experience to be the lowest needed to achieve immobilization, and reviewing drug doses on an ongoing basis. Reversal agents are kept on hand through immobilization. Animals' pulse and respiratory rates, and SpO₂ where possible, are monitored through immobilization, with early reversal or administration of respiratory stimulant (Dopram V) possible should this appear necessary.

Animals might also over-heat during immobilization. This is avoided by keeping animals in the shade, and monitoring body temperature throughout anaesthesia. Animals are cooled with water (either onto the skin or by wrapping in wet towels) when temperature appears elevated.

(d) surgical procedures

NA

(e) sampling

Animals might be harmed by sampling if too large a quantity of blood were collected. The volumes to be collected reflect the 1% suggested by guidelines as the maximum that can be removed in the course of repeated sampling.

(f) analgesia

NA

(g) recovery

Animals in the field trial (**Objective 2**) might over-heat during recovery if they move out of the shade while still somewhat disoriented. This is avoided by attempting to minimise the period of disorientation through careful choice of drug doses and administration times. The doses of immobilizing drugs have been refined to use the minimum dose of ketamine (which is not reversible) needed to achieve recumbency. Careful monitoring of pulse, respiration rate, eye position, muscle tone, blink response etc is used to assess depth of anaesthesia and to delay administration of atipamezole (the reversal agent for medetomidine) as long as possible. This means that, on removal of the effects of medetomidine at reversal, animals are left with a very low residual dose of ketamine, minimising the length of the recovery period. Animals are monitored closely during recovery to allow intervention should ill effects be detected. However, no such problems have been encountered in over 150 past immobilizations.

(h) release

At release in the field, animals are often disoriented for a period of one or two hours, and might be harmed by larger carnivores (e.g. lions and hyaenas), or by people. Minimising the length of the recovery period (see above) reduces these risks. In addition, all immobilizations occur in daylight, ideally in the morning but in all cases several hours before dusk, so that recovery periods do not coincide with the (nocturnal) activity period of lions and hyaenas. In areas of the park with high tourist volumes, project staff can remain close to study animals resting post-recovery, to dissuade vehicles from approaching them.

(i) subsequent monitoring

All animals in the captive trial (**Objective 1**) will be captive born in zoos open to the public; hence the animals will be accustomed to visual observation.

In the wild (**Objective 2**), all monitoring of collared animals is conducted so as to minimise disturbance. Observations are conducted entirely from vehicles, which wild dogs do not fear if carefully driven. The absence of negative stimuli associated with monitoring wild dogs is illustrated by the fact that packs usually allow vehicles to approach to 10-15m without apparent concern. Most routine observations are conducted at 30-40m.

NOTE: Ethics approval comes with a requirement to inform the committee if any adverse effects arise during the course of the project.

13. In case of euthanasia, what method(s) will be used?

Please provide references about the methods and their suitability for the species concerned.

No euthanasia of study animals is anticipated as part of the project protocol. Zoo veterinarians would make clinical decisions about euthanasia of animals in the captive trial (Objective 1) and SANParks veterinarians would make decisions about euthanasia of animals in the field trial (Objective 2). In the unlikely event of euthanasia being required, free-ranging animals would most likely be darted and euthanised by intravenous injection (https://www.avma.org/KB/Policies/Documents/euthanasia.pdf). If darting was impossible, euthanasia by gunshot would be considered (Longair JA, Finley GG, Laniel MA, et al. Guidelines for euthanasia of domestic animals by firearms. Can Vet J. 1991;32(12):724–726).

14. Co-Investigators and Technicians (and affiliations)

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Dr Louis van Schalkwyk Directorate Animal Health, Kruger National Park, South Africa Email: LvS@vodamail.co.za				
15. What type of proposal is this?				
 ✓ A new application ✓ An amendment to an existing approved project ✓ Referred to the Ethics Committee by the Department Director 				
16. Preferred start date of work	17. Estimated completion date			
1 Jan 2021	31 Dec 2023			

SECTION B – Experience & Training

18. Provide details of your previous relevant experience in care of the species and with the proposed procedures

RW has experience of over 180 wild dog immobilizations, as well as a total of several hundred lions, hyaenas, leopards, jackals, foxes and mongooses, and over a thousand European badgers.

Collaborating SANParks veterinarian Dr Louis van Schalkwyk is experienced in field practice, including having run a previous field trial of recombinant CDV vaccine in Kruger National Park.

The immobilisation and collaring procedures described here have been refined over the decades of field research, and staff are fully familiar with all the methods involved.

19. Do you have formal relevant qualifications or can you demonstrate competency in other ways?

All immobilizations, vaccinations, and sample collection will be conducted by, or under the guidance of, qualified and registered veterinarians in the host countries.

20. Is any new formal training required?

no

SECTION C – Project Completion

21. Describe arrangements for animals at the end of the study (return to stock, reuse, re-home, other)

All animals in the captive trial (**Objective 1**) will remain in their enclosures, except in the unlikely event that they are moved between collections under the population management of the African wild dog SSP.

All animals in the field trial (**Objective 2**) will be released immediately after handling. If and when the project comes to an end, attempts will be made to remove any collars.

<u>Please note that the committee will expect to receive a brief report (500 words max.) when the approved</u>

<u>procedures have been completed.</u> Information required will include a summary of the most important results; how many subjects were required for the study; how many were actually recruited; did any of the subjects drop out – if yes, did this affect the results?

22. Biosecurity aspects and disposal of materials

Explain arrangements for the disposal of materials, unwanted samples and consumables within ZSL premises and/or at field sites (Note that arrangements must comply with ZSL requirements). Applicants should take all possible precautions to reduce the risk of spreading pathogens between field sites and individuals.

Materials from this project are unlikely to pass through ZSL premises.

Samples from the captive trial (**Objective 1**) will be collected at the participating zoos and sent directly to the laboratory at Cornell for analysis.

Samples from the field trial (**Objective 2**) will be stored initially in South Africa prior to export to the USA for analysis at Cornell.

Samples will be brought to ZSL premises only if required for analysis or biobanking.

Contaminated materials will be destroyed by incineration either in the USA or in South Africa.

SECTION D – Project Approval			
23. Name & Signature of Applicant	Date		
Name: Rosie Woodroffe	12 May 2020		
Signature: 24. Name & Signature of ZSL Line Manager, Supervisor or Programme Manager	Date		
Name: Trent Garner Signature:	12/5/2020		
25. Name & Signature of Chair, Ethics Committee	Date		
Name: Richard Kock Signature:	22/05/2020		
26. Name & Signature of Department Director	Date		
Name: Chris Carbone Signature:	26/05/20		

PLEASE NOTE: For all projects that receive ethical approval by the Committee, the Applicant will be required to submit an *Ethical Review Feedback Form* after the commencement of the project. The form will be a short report confirming where there were issues of ethical concern and how these evolved or were addressed, and in particular the success or otherwise of novel techniques that were developed within the project, or established techniques applied in novel ways

or moved across to other species. For projects with human impacts, the Committee would like to hear about any issues or concerns that arose during the work and advice for improved methods involving human participants.

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MODIFIED LIVE DISTEMPER VACCINES CARRY LOW MORTALITY RISK FOR CAPTIVE AFRICAN WILD DOGS, LYCAON PICTUS

Rosie Woodroffe, BA, DPhil

Abstract: Recently, canine distemper virus (CDV) has been linked to population declines in the endangered African wild dog (Lycaon pictus). As CDV appears able to persist in wildlife, threats to free-ranging wild dogs cannot be eliminated by vaccinating domestic dogs. Conservation managers may therefore consider CDV vaccination of wild dogs in highly threatened populations. For use in field conservation, the ideal CDV vaccine would be safe, immunogenic, and readily available in Africa. The CDV vaccine type most commonly used for domestic dogs (modified live vaccine) is available in Africa, and apparently immunogenic in wild dogs, but has been linked to fatal vaccine-induced distemper in captive wild dogs. However, alternatives are either ineffective (inactivated vaccine) or difficult to obtain in Africa (recombinant vaccine). Data from a questionnaire survey of zoo vaccination practices were therefore combined with studbook tracing to assess the safety of modified live CDV vaccine in captive African wild dogs. Among 135 wild dog pups given modified live CDV vaccine for the first time, there was a single, unconfirmed, case of potential vaccine-induced distemper. Pups given modified live vaccine survived better than those given inactivated vaccine or no vaccine. Although studbook tracing revealed higher overall pup survival at zoos which responded to the questionnaire than at zoos which did not, tracing of all pups born during a 20-yr period that lived long enough to be vaccinated (n = 698 pups in 155 litters) revealed no mortality events consistent with vaccine-induced distemper. Modified live CDV vaccine thus appears to carry low mortality risks for African wild dog pups in captivity, and may warrant trials in free-ranging populations.

INTRODUCTION

The threat to wildlife populations from infectious disease is increasingly recognized,⁴⁰ but this recognition seldom prompts intervention in the wild.^{52,63} In mammals, the pathogens that threaten wildlife populations are often shared with domestic animals, yet vaccines that are safe and effective in domestic animals may be ineffective or even harmful in wildlife.^{8,54,63} Hence, concerns about vaccine safety have impeded the development of field vaccination programs for several wildlife species.^{6,30}

The African wild dog, *Lycaon pictus*, is an endangered species that is threatened by infectious disease. Rabies has been considered the greatest disease threat, because it was confirmed in the Serengeti wild dog population just before that population went extinct, and has also thwarted reintroduction attempts in Namibia and South Africa. 18,23,27,49,65 Canine distemper virus (CDV) was previously thought to be a less serious threat than rabies, because seropositive wild dogs were reported from many healthy populations, suggesting widespread nonlethal exposure. 2,5,13,31,41 Moreover, prior to 2016, the only major CDV outbreak confirmed in wild dogs had occurred in captivity,

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where packs were held in close proximity; the only two confirmed outbreaks in wild populations were restricted to single packs.^{1,20,57} However, in 2016– 2017 CDV killed ≥20 packs in Kenya's Ewaso ecosystem, 3 packs in South Africa, and 1 pack in Tanzania.^{15,21,32,38} There is evidence, therefore, that CDV can represent a serious threat to free-ranging wild dog populations. This pattern of highly variable mortality, with widespread nonlethal exposure and occasional mass mortality, is also a feature of CDV epidemiology in some other carnivore species.^{10,37,47,56}

Strategic plans for wild dog conservation call for mitigation of disease threats.24-26 For rabies, mass vaccination of domestic dogs is likely to reduce these threats, as wild dogs acquire rabies mainly from domestic dogs and, fortuitously, ongoing strategies to end human rabies deaths entail domestic dog vaccination on the very large scales needed to protect wild dogs. 9,42,45,64,66,67 In contrast, domestic dog vaccination may contribute little to protecting wild dog populations from CDV. The virus appears not to persist in domestic dog populations at the local scale, with several studies suggesting persistence in wildlife. 10,19,42 Wild dog exposure to CDV is not associated with domestic dog contact, and mass CDV vaccination of domestic dogs in the Serengeti ecosystem failed to prevent new wildlife infections.41,61,66 Where there is a recognized need to protect free-ranging wild dogs from CDV, conservation managers may therefore need to consider vaccination of wild dogs themselves.

For use in the wild, the ideal CDV vaccine would be safe, immunogenic, and readily available in African wild dog range states. Unfortunately, it is not clear that any of the three types of CDV vaccine (modified live, inactivated, and recombinant) meet all three criteria. Modified live CDV vaccine is widely used in domestic dogs, including in Africa; however, it has been reported to cause fatal vaccine-induced distemper in African wild dogs, as well as some other carnivore species.8,16,22,35,58 Inactivated vaccine carries no such risk of vaccine-induced distemper; however, it has not prompted strong immune responses and failed to prevent mass mortality at a wild dog captive breeding facility in Tanzania.36,57,62 Recombinant CDV vaccine has been shown to trigger immune responses in captive wild dogs, but supply issues can limit access to this vaccine type, and restrictions on the import of genetically modified organisms to many African countries could hinder its use in wild populations.^{7,11,29}

For North American zoos, husbandry guidelines recommend the use of recombinant CDV vaccines, to avoid any risk of vaccine-induced distemper.⁴ In Europe, however, recombinant CDV vaccines are not available and modified live vaccine is widely used. Limited studies suggest that modified live CDV vaccine is immunogenic in captive wild dogs.^{51,59} A recent study of captive tigers (*Panthera tigris*) found that modified live CDV vaccines were markedly more immunogenic than recombinant vaccines.⁴⁸

The immunogenicity and widespread availability of modified live CDV vaccines mean that this vaccine type would be the best tool for field conservation, if the risks of vaccine-induced distemper were acceptably low. Although several cases of vaccine-induced distemper have been reported in African wild dogs, cases when modified live distemper vaccine caused no ill effects are seldom reported, and so the risk of vaccineinduced distemper is uncertain. Moreover, risks may have changed over time in response to changing conditions. A widely used vaccine strain (the Rockborn strain, adapted to canine kidney cell culture) that was controversially linked to vaccine-induced distemper was withdrawn during the 1990s, potentially reducing the risk in subsequent years.33 Additionally, there is some evidence that the immunosuppressive effect of parvovirus infection may increase risks of vaccine-induced distemper, and so the extent of parvovirus control may influence the safety of modified live CDV vaccine.²⁸ Finally, the use of multivalent preparations including modified live vaccines against both distemper and parvovirus has been tentatively linked to the risk of vaccine-induced distemper.⁵³

This study therefore evaluated the mortality risks associated with administering modified live CDV vaccine to African wild dogs in captivity, by combining studbook data with data from a questionnaire survey of zoos, conducted in the years before recombinant CDV vaccines became available.

MATERIALS AND METHODS

Published reports

Published accounts were used to characterize the mortality patterns associated with vaccineinduced distemper, including timing of clinical signs and death relative to vaccination, and mortality rate within affected litters. 16,35,58

Questionnaire survey

Questionnaire data were gathered in 2000, by sending paper forms to veterinary staff responsible for the care of wild dogs held in captivity. Questionnaires were sent to 55 collections worldwide, listed on the International Species Information System (ISIS) as holding wild dogs within their collections at the time, and to two unlisted private collections known to hold wild dogs. The questionnaire asked for data on current and past policy on vaccinating wild dogs (with reasons for any changes), and whether any wild dogs had ever become sick or died following the administration of vaccines. In addition, data were requested on the vaccination histories (vaccination dates, vaccine brand names and, where possible, batch numbers) of any wild dogs held. Data were not requested on incidents of wild-type distemper. To maximize responses, the questionnaire was worded to reassure participants that information from individual collections would be held in confidence. Questionnaires were sent a second time to collections that had not responded 4 mo after the initial mailing.

Where zoos provided individual vaccination records, an independent estimate of mortality postvaccination was obtained by using studbooks (which cover the European and North American captive populations) to trace the survival of vaccinated animals. 46,60 As questionnaires were sent worldwide, not just to the regions covered by the studbooks, the fates of some animals mentioned in the questionnaire responses could

Table 1.	Characteristics of presumed vaccine-induced distemper in African wild dogs as reported in the literature.
All pub	blished cases involved pups being given their first dose of modified live distemper vaccine, in a polyvalent
formula	ation including canine parvovirus (CPV) vaccine. NR indicates information which was not reported.

Litter	Individual	Year			Days to death	Reference	
A	A 1	<1989	Candur SHLP, Behringwerke,	57	18	22	16
A	A2	<1989	Marburg 35041	57	24	30	16
A	A3	<1989	Strain: Rockborn	57	25	31	16
A	A4	<1989	CPV: inactivated	57	26	32	16
В	B1	1981	Paramune 5, Dellen Laboratories,	150	10	12	35
В	B2	1981	Omaha, NE 68134, USA	150	10	13	35
В	В3	1981	Strain: unknown	150	10	15	35
В	B4	1981	CPV: inactivated	150	10	17	35
В	B5	1981		150	10	19	35
В	B 6	1981		150	10	20	35
В	B 7	1981		150	10	survived	35
C	C1	<1987	Quantum 4, Pitman-Moore,	56	13	15	58
C	C2	<1987	Washington Crossing, NJ 08560,	56	13	15	58
C	C3	<1987	USA	56	13	15	58
C	C4	<1987	Strain: Onderstepoort	56	13	16	58
C	C5	<1987	CPV: live	56	13	16	58
C	C6	<1987		56	13	15	58
D	D1	<1987	Quantum 6, Pitman-Moore,	77	NR	13	58
D	D2	<1987	Washington Crossing, NJ 08560,	77	NR	13	58
D	D3	<1987	USA	77	NR	13	58
D	D4	<1987	Strain: Onderstepoort CPV: live	77	NR	14	58
mean \pm SD		SD		91.5 ± 43.1	14.2 ± 5.6	17.8 ± 6.2	

not be traced in this way. A Cox proportional hazards model (including litter identity as a random effect, fitted using package coxme in the statistical package R) was used to compare the survival of pups which were, and were not, given modified live CDV vaccine.44,55 The Cox proportional hazards model is a type of survival analysis, which allows assessment of associations between independent variables (e.g. vaccination) and the time taken for an event to occur (e.g. death).¹² A hazard ratio less than one indicates a lower probability of death (i.e. higher survival). The inclusion of a random effect (here, litter identity) accounts for the statistical nonindependence of pups from the same litter, as they are genetically related, exposed to the same environmental conditions, and likely to receive vaccine from the same batch.

Studbook analyses

Questionnaire surveys will give inaccurate results if respondents represent a biased sample of the population. To assess whether the zoos which returned their questionnaires were representative, studbook data were used to estimate pup survival

across the European and North American captive populations during the period covered by most questionnaire responses (1980–2000). Pup survival was compared between zoos which did, and did not, respond to the questionnaire survey using a Cox proportional hazards model including litter identity as a random effect, fitted using package *coxme* in the statistical package *R*.^{44,55}

RESULTS

Published reports

All of the published reports of vaccine-induced distemper involved pups given their first vaccinations (Table 1). All pups had been given modified live CDV vaccine in a polyvalent formulation that also included parvovirus vaccine; however, some formulations included modified live parvovirus vaccine and others contained inactivated parvovirus vaccine (Table 1).

Clinical signs were observed 14.2 days postvaccination on average (SD 5.6 days), with death occurring 17.8 days (SD 6.2 days) postvaccination (Table 1). Across the four affected litters, 20 of 21 pups died (Table 1; 95.2% mortality, exact bino-

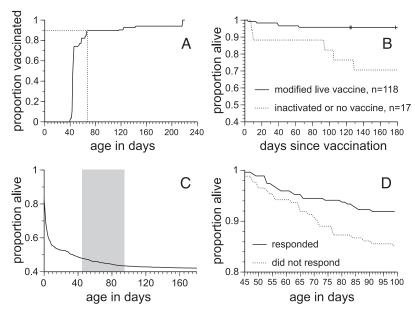


Figure 1. Vaccination of captive African wild dogs against canine distemper virus (CDV). Panel A shows age at first vaccination for 135 pups given modified live CDV vaccine; the dotted line indicates the age by which 90% of pups had been vaccinated. Panel B compares the survival of pups first given modified live CDV vaccine at known age in North America and Europe, with those given either no vaccine or an inactivated vaccine. Panel C shows the survival, from birth to 180 days, of 1,459 pups born in North America and Europe in 1980–2000, with shading showing the age (45–99 days) when the risk of vaccine-induced distemper was estimated to be highest. Panel D compares the survival, from 45 to 99 days, of pups born at zoos which did, and did not, respond to the questionnaire about vaccination practices.

mial 95% confidence interval (CI) 76.2–99.9%), with deaths occurring across a period of 2–11 days within each litter (Table 1).

Questionnaire responses

Questionnaires were returned by 36 collections (a 63% response rate), of which 26 reported having vaccinated wild dogs against CDV. Of 13 zoos that gave details of vaccines used, 12 had used modified live CDV vaccines, in either monovalent (Fervac [United Vaccines, Fitchburg, WI 53593, USA], Galaxy-D [Schering Plough, Omaha, NE 68138, USA], Fromm-D [Solvay, Mendota Heights, MN 55120, USA]), or multivalent (Candur [Behringwerke, Marburg 35041 Germany], Canvac [Dyntec, Terezin 41155, Czech Republic], Duramune [Elanco, Fort Dodge, IA 50501, USA], Kavak [Fort Dodge, Overland Park, KS 66210, USA], Nobivac [Intervet, Millsboro, DE 19966, USA], Protech [Arthur Webster Pty, Sydney, Australia], Vanguard [Pfizer, Ramsgate CT13 9ND, UK]) preparations. Two captive facilities had used both inactivated and modified live CDV vaccines, and one had used inactivated vaccine exclusively (CDV-ISCOM, Rotterdam 3720, Netherlands).

One hundred thirty-five wild dogs were reported to have received modified live CDV vaccine for the first time at known age, although the vaccine brand was only reported for 37 of these animals. These first vaccinations were given at a mean age of 61 days (SD 43 days, range 40–217 days), with 90% of animals having received their first vaccination by the age of 67 days (Fig. 1A).

Of 26 zoos which reported having vaccinated wild dogs against CDV, 25 reported no observations of ill effects postvaccination. One zoo reported a case of a single wild dog pup that died, aged 82 days, 10 days after its second dose of modified-live CDV vaccine. Neither the brand nor type of vaccine (monovalent/polyvalent) was reported. The postmortem report provided a diagnosis of canine distemper based on clinical symptoms, necropsy, and histopathology, and CDV was confirmed by a fluorescent antibody test. However, failure to isolate the virus in cell culture led the virologist consulted to suggest that this was likely to have been a wild-type virus, because "...vaccine strains of CDV are highly celladapted and usually will propagate in cell cultures..." (unpublished necropsy report). This animal had seven littermates; one died aged 43 days, on the day of first vaccination (and so could not have died of vaccine-induced distemper), and another died aged 57 days, following bite-wounding. Five other littermates survived to adulthood. This incident therefore entailed lower postvaccination mortality than the published cases shown in Table 1 (28.6% vs 95.2% mortality; Fisher exact P = 0.001). Nevertheless, if the two animals that died postvaccination are assumed to have contracted vaccine-induced distemper, it would suggest a maximum of two deaths among 135 vaccinated animals, giving a vaccine-associated mortality estimate of 1.5% (CI 0.2-5.3%). If this incident did not represent vaccine-induced distemper, the estimate would be 0% (CI 0-2.7%).

Studbook tracing revealed that 118 pups given a modified live CDV vaccine in Europe or North America (aged 44 days on average) experienced higher survival over the subsequent 180 days than 17 pups (aged 44 days) given either no CDV vaccine (n = 14) or inactivated CDV vaccine (n = 3); Cox proportional hazards model including litter identity as a random effect, effect of modified live vaccine: hazard ratio = 0.142, P = 0.012; Fig. 1B). The survival of pups reported to have been given modified live distemper vaccine in a monovalent formulation (n = 15) could not be compared with that of pups given polyvalent formulations containing parvovirus vaccine which was either inactivated (n = 6) or modified live (n = 15), because none of these 36 animals died in the 180 days following vaccination.

Studbook analyses

Studbooks recorded mortality at all ages: of 1,459 pups born in the years 1980–2000, 40% died in the first 5 days of life, and 42% survived more than 6 mo (Fig. 1C).

The minimum age for vaccine-induced distemper (45 days) was estimated by adding the minimum reported age at first vaccination (40 d; Fig. 1A) to a conservative estimate of the time from vaccination to death, based on published studies (5 days, calculated as the mean [17.8 days] minus 2 SD [12.4 days]; Table 1). The likely maximum age for vaccine-induced distemper (99 days) was calculated by adding the age by which 90% of pups had received their first vaccination (67 days; Fig. 1A) to the maximum reported time from vaccination to death (32 days; Table 1). Among zoos that were sent questionnaires, pup survival at ages 45–99 days was consistently higher at zoos that responded to the questionnaire

than at those that did not respond (Cox proportional hazards model including litter identity as a random effect, effect of zoo response: hazard ratio = 0.322, P = 0.045, Fig. 1D).

As survival was consistently higher at zoos that responded to the questionnaire, an alternative estimate of potentially vaccine-induced mortality was derived, independent of the questionnaire results. Litters were identified as potential cases of vaccine-induced distemper if more than one pup died at age 45–99 days, because published accounts reported mortality of over 75% in affected litters (Table 1). To help interpret mortality patterns, data were also collated on deaths aged 20–44 days and 100–175 days in these litters (Table 2).

Among 155 litters with members surviving to 45 days (totaling 698 pups), 18 litters met this criterion, with 53 pups dying aged 45-99 days (Table 2). If all of these litters had received modified live CDV vaccine, and all of these deaths had reflected vaccine-induced distemper, the vaccine-associated mortality would be 7.6% (CI 5.7-9.8%). However, these 18 incidents represented marked lower mortality within affected litters than the published cases (42.4% vs 95.2%, Fisher exact P < 0.001). The only case of 100% mortality aged 45-99 days involved a litter from a zoo that had reported that it did not routinely vaccinate wild dog pups against CDV (Table 2). Moreover, in 10 of the 18 litters, the reported deaths were part of a series that extended before or after the period when pups were 45–99 days old (Table 2). One additional litter (which experienced no mortality aged 45-99 days, and is therefore not shown in Table 2) experienced 100% mortality (of three pups) aged 100-175 days, but this litter was confirmed to have received inactivated CDV vaccine, not modified live vaccine. With no litters showing the brief episodes of high mortality reported in the published cases, at the age when vaccine-induced distemper would be expected, the incidence of apparent vaccine-associated mortality in pups aged ≥45 days appears to be 0/698 (0%, CI 0–0.53%). However, it is not known how many of the 698 pups had in fact received modified live CDV vaccine.

DISCUSSION

The results presented here suggest that modified live distemper vaccine carries a low mortality risk for captive African wild dogs. Zoos reported only one potential case of vaccine-induced distemper among 135 pups given modified live distemper vaccine, and this case was not con-

Table 2. Captive African wild dog litters identified through studbook tracing to have experienced ≥2 deaths aged 45–99 days (when the risk of vaccine-induced distemper was estimated to be highest). Deaths before and after this age are also shown; na (not applicable) indicates that no deaths were recorded within the stated time window. Canine distemper virus (CDV) vaccination status is reported as unknown if zoos were not sent questionnaires, or did not return them, as yes/no if zoos provided vaccination records for these individuals, and probably/probably not if zoos only indicated their typical vaccination practices.

	Litter	Pups alive at 45 days	Mortality 45–99 days	Days of age at death			CDV
Zoo				20–44	45–99	100-175	vaccinated?
A	E284	5	40%	na	65, 65	na	Unknown
Α	E354	4	50%	na	79, 87	105, 114	Unknown
В	E286	8	25%	na	77, 84	na	Unknown
В	E287	10	30%	29	56, 56, 72	na	Unknown
В	E288	5	80%	39	48, 65, 65, 77	na	Unknown
В	E289	8	50%	35	45, 54, 88, 91	na	Unknown
\mathbf{C}	N51	8	25%	na	56, 56	naª	Unknown
D	E166	8	63%	na	79, 81, 82, 82, 92	na	Unknown
E	E197	9	22%	na	50, 50	na	Unknown
F	E257	3	67%	35	53, 70	na	Unknown
F	E260	10	20%	36	65, 68	127, 155	Unknown
G	E92	8	100%	39	49, 53, 53, 56, 58, 63, 66, 66	na	Probably not
G	E95	8	25%	29, 41	53, 55	na	No
H	E233	6	50%	na	85, 86, 91	na	Probably not
I	E390	9	33%	na	62, 74, 80	na	Probably
J	N6	7	28%	43	57, 82	na	Yes—live
K	N46	6	50%	na	50, 54, 55	na	Unknown
L	E266	3	67%	33, 40	62, 71	na	Unknown
	Total	125	42.4%				

^a Three littermates transferred and lost to monitoring at 101 days of age.

firmed, differing in several ways from published accounts. Although the zoos that responded to the questionnaire represented a biased sample of wild dog litters with relatively low mortality, an independent evaluation of studbook records revealed no patterns of mortality similar to those described in published accounts of vaccine-induced distemper. Indeed, studbook tracing revealed that wild dog pups that received a modified live distemper vaccine experienced significantly lower mortality than those that did not.

The difference in pup mortality recorded at zoos that did, and did not, respond to the questionnaire represents a form of nonresponse bias, a well-known source of bias in postal surveys. Nonresponse bias was evaluated because zoo staff might have been reluctant to report cases of vaccine-induced distemper, and such reluctance is one potential explanation for the higher pup mortality observed at nonresponding zoos (Fig. 1D). However, this explanation is not consistent with analyses of studbook data, which did not reveal mortality patterns similar to published cases of vaccine-induced distemper, in either responding or nonresponding zoos. An

alternative explanation is that nonresponding zoos may not have routinely vaccinated wild dog pups against CDV, a practice that was also associated with lower pup survival (Fig. 1B). Reassuringly, the questionnaire survey and the studbook analysis both suggested consistently low risks of vaccine-induced distemper.

Wild dog pups given either no CDV vaccine or inactivated vaccine experienced lower survival than those given modified-lived CDV vaccine (Fig. 1B). Potentially, this pattern might reflect mortality from wild-type CDV, which has been reported from some captive facilities, including one where wild dogs had been given inactivated CDV vaccine.^{3,39,57} However, this pattern might also have reflected the protection against other canine pathogens afforded by the multivalent vaccines that most zoos used. Additionally, routine pup vaccination could indicate greater attention to veterinary care, improving survival. Irrespective of the mechanism behind it, this finding showed that modified live CDV vaccine was not associated with elevated pup mortality.

This study revealed a single case of confirmed distemper in a vaccinated wild dog pup, suspected

by the virologist to reflect infection with wild-type virus rather than a vaccine strain. This observation is consistent with a study of domestic dogs, in which molecular analyses revealed that 10 puppies with suspected vaccine-induced distemper were instead infected with wild-type CDV.³⁴ This finding may also cast doubt on the published cases of vaccine-induced distemper, none of which could rule out the possibility of wild-type CDV infection.^{16,35,58}

It is surprising that four cases of presumed vaccine-induced distemper were published during the 1980s (Table 1), when similar mortality events were not recorded in studbooks during 1980-2000 (Table 2). Three of the published cases involved litters born outside the captive populations monitored by the studbooks, and the fourth case (reported from an unspecified zoo on an unspecified date) could not be identified within studbooks. The literature on CDV vaccines' reversion to virulence suggests several possible explanations for these potential cases of vaccine-induced distemper. Vaccine strain variation is one explanation; however, the four published cases of presumed vaccine-induced distemper involved at least two different vaccine strains, including the Onderstepoort strain, which is widely considered safe (Table 1).14,33 Likewise, immunosuppression caused by coinfection with parvovirus (or even modified live parvovirus vaccine) has been proposed as a cause of vaccine-induced distemper, 17,28 but only two of the four reported cases involved modified live parvovirus vaccine (Table 1). Alternatively, host genetic susceptibility has been linked to vaccine-induced distemper in domestic dogs, and it is possible that the careful genetic management of captive populations (which developed from the 1980s onwards, and is the purpose of maintaining studbooks) might have reduced the risk of vaccine-induced distemper. 17,43

Evidence concerning the safety of vaccines in captive animals is, of course, only one component of the information needed to evaluate vaccination as a conservation tool for free-ranging animals. Evidence would also be needed of vaccine safety and effectiveness in the wild, where animals are more likely to be nutritionally stressed, as well as immunologically challenged by other pathogens. The apparently low mortality risk associated with modified live CDV vaccines in captive African wild dogs, combined with the apparently high immunogenicity of such vaccines, and their widespread availability in Africa, suggest that conservation managers may wish to consider a field trial to evaluate them further.

Acknowledgments: The author wishes to thank the staff from the various captive facilities who provided (often detailed) data to this study.

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Re: [EXTERNAL] RE: Inquiry Regarding CS7222595 - 3-200-37e: Import of biological specimens (ESA) for scientific research

Ketram, Natchanon N < natchanon_ketram@fws.gov>

Fri 6/7/2024 3:16 PM

To:Jen Powers < jhb19@cornell.edu>

Good afternoon Jen,

Okay I have had a chance to review the old application. I don't have any other follow-up questions on your application. That said, for your awareness, the Federal Register publication for your application was valid only for one year from date of publication of the notice, 11/21/22. So I will need to put your reissuance request back into the queue for publication.

Thank you,

Natchanon Ketram
Permit Biologist
Branch of Permits
Division of Management Authority
International Affairs Program
U.S. Fish and Wildlife Service
Falls Church, VA, USA

From: Jen Powers <jhb19@cornell.edu> Sent: Thursday, June 6, 2024 4:25 PM

To: Ketram, Natchanon N < natchanon ketram@fws.gov>

Subject: RE: [EXTERNAL] RE: Inquiry Regarding CS7222595 - 3-200-37e: Import of biological specimens (ESA) for

scientific research

Yes, no changes except on the original application I listed 224 specimens and on this new one I listed 261. During the original application process the number of animals increased due to new births. The permit was issued with 261. The client thought they were going to get the samples shipped in time and then did not. When I inquired about renewal they did not respond and I missed the deadline.

Thank You, Jen

Jennifer H. Powers
Manager, Virology Laboratory
Animal Health Diagnostic Center
New York State Veterinary Diagnostic Laboratory
Cornell University
jhb19@cornell.edu

Phone: 607-253-3900 Phone: 607-253-4458

From: Ketram, Natchanon N < natchanon ketram@fws.gov>

Sent: Thursday, June 6, 2024 4:22 PM **To:** Jen Powers <jhb19@cornell.edu>

Subject: Re: [EXTERNAL] RE: Inquiry Regarding CS7222595 - 3-200-37e: Import of biological specimens (ESA) for scientific research

Good afternoon Jen,

Oh I did not know that this is a renewal of an issued permit. I apologize but I did not see on your submission that this is a renewal of a previously issued permit. You may have included that request in the new submission that identifies this as a reissuance request but I must have miss it. Please ignore my questions for now. I am going to look into the old files and circle back with you if I have follow-up questions.

Besides that, I do have a quick question for you. Is this a reissuance request with no changes?

Thank you,

Natchanon Ketram
Permit Biologist
Branch of Permits
Division of Management Authority
International Affairs Program
U.S. Fish and Wildlife Service
Falls Church, VA, USA

From: Jen Powers < jhb19@cornell.edu> Sent: Thursday, June 6, 2024 4:14 PM

To: Ketram, Natchanon N < natchanon_ketram@fws.gov>

Subject: [EXTERNAL] RE: Inquiry Regarding CS7222595 - 3-200-37e: Import of biological specimens (ESA) for

scientific research

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Hi Natchanon,

I can reach out to the PI and get these answers for you. However, I did want to make sure you noted that this is a renewal of a previous permit. We missed the renewal deadline, so I was told to submit a new application and note the previous permit number of MAPER0042576. I believe most if not all of these questions were answered on for the original permit application. Are you able to obtain these answers there or should I reach out to the PI?

Thank you for the help with this>

Thank You, Jen

Jennifer H. Powers

Manager, Virology Laboratory

Animal Health Diagnostic Center

New York State Veterinary Diagnostic Laboratory

Cornell University

jhb19@cornell.edu Phone: 607-253-3900 Phone: 607-253-4458

From: Ketram, Natchanon N < natchanon ketram@fws.gov >

Sent: Thursday, June 6, 2024 3:43 PM **To:** Jen Powers < jhb19@cornell.edu>

Subject: Inquiry Regarding CS7222595 - 3-200-37e: Import of biological specimens (ESA) for scientific research

Good afternoon,

The USFWS has questions regarding your application for a permit. I will provide these questions below:

- 1. According to the answer you provided in the Section E of the form, the samples are currently with Port Lympne Zoo. However, the exporter from the UK is the Zoological Society of London. Will the Zoological Society of London have ownership of the samples prior to shipment or will they only be the caretaker of the samples?
- 2. For confirmation, are all 261 samples currently with Port Lympne Zoo?
- 3. You provided the Species 360 reports for some of the animals the specimens were extracted from but not all specimens you identified had a species report included. Can you confirm if all the animals that the samples were derived from were captive born and bred?
- 4. Attached to the application is an excel table that identified pups the samples were extracted from. The specimen reports for the other individuals were from deceased ones. Were the samples from the pups extracted from live individuals?
 - a. If the samples were extracted from live individuals, please answer 8d and 8e of the 3-200-37e form. I know that these questions are technically asking about wild animals. However, for the purpose of assessing samples acquired from live animals, the same set of considerations is applied.
 - b. Additionally, please answer question 10c if the samples were extracted from live individuals.

In accordance with 50 CFR 13.11(e), if the requested information is not received by this office by **July 21, 2024**, your application will be abandoned and administratively closed. Once a file is closed you will need to submit a new application and all required fees for the Service to consider your proposed activity. Please refer to permit application number CS7222595 in your correspondence.

Thank you,

Natchanon Ketram
Permit Biologist
Branch of Permits
Division of Management Authority
International Affairs Program
U.S. Fish and Wildlife Service
Falls Church, VA, USA

Re: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Lamberson, Amanda M <amanda_lamberson@fws.gov>

Thu 5/5/2022 10:30 AM

To: Jen Powers <jhb19@cornell.edu>

Good morning Jen,

Thank you for the additional information. I will review the information and if I have any further questions, I will let you know.

Kind regards,

Amanda Lamberson Biologist U.S. Fish and Wildlife Service Division of Management Authority Branch of Permits, MS: IA 5275 Leesburg Pike

Falls Church, VA 22041

From: Jen Powers <jhb19@cornell.edu> Sent: Tuesday, May 3, 2022 9:47 AM

To: Lamberson, Amanda M <amanda_lamberson@fws.gov>

Subject: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

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Hello Amanda.

Sorry for the delay in following up on this. Attached is the requested document. Hopefully it is not too late. I will also upload in epermits.

Thank You, Jen

Jennifer H. Powers
Supervisor, Virology Laboratory
Animal Health Diagnostic Center
New York State Veterinary Diagnostic Laboratory
Cornell University
jhb19@cornell.edu

Phone: 607-253-3900 Phone: 607-253-4458

From: Lamberson, Amanda M <amanda_lamberson@fws.gov>

Sent: Monday, March 7, 2022 12:27 PM **To:** Jen Powers <jhb19@cornell.edu>

Subject: Addi onal Informa on Required - FWS Applica on CS0082219

Hello Jennifer,

Thank you for your application for a federal import permit. I apologize for the delay. I have reviewed the application and found some additional information is required. Based on the proposal submitted in response to question 10a., it is not fully clear what purpose the 224 serum samples serve and why these specific samples are necessary to the proposed project. In a separate document, please provide an explanation of the purpose these samples, how they fit within the objectives of the proposed project, and methods of any activities to be conducted with these samples. If you have any questions, please let me know. Thank you.

In accordance with 50 CFR 13.II(e), if the requested informa on is not received by this office within 45 calendar days of the date of this email, your applica on will be abandoned and administra vely closed. Once a file is closed, you will need to submit a new applica on, and all required fees, for the Service to consider your proposed ac vity.

Kind regards,

Amanda Lamberson Biologist U.S. Fish and Wildlife Service Division of Management Authority Branch of Permits, MS: IA 5275 Leesburg Pike Falls Church, VA 22041

Justification for inclusion of samples from the UK

Our study involves developing a safe and effective protocol for vaccinating African wild dogs (*Lycaon pictus*) against canine distemper virus (CDV). The intended first step of this work was to conduct a vaccine trial in captivity. This work needed to be performed using pups, because we needed to measure antibody titres before and after the first vaccination; as distemper vaccination is routine in most zoos, older animals would almost certainly have received vaccine previously, and would therefore be expected to show stronger and more rapid antibody responses not representative of those that might be seen in the wild.

As described in our research proposal (included as Annex 10a in our submission), our original plan was to conduct our captive trial in North American zoos. Wild dog reproduction is highly seasonal, and so this trial was scheduled to take place in early 2021, involving pups born in the fall of 2020 (see Timeline in Annex 10a). The plan was to compare several different vaccination protocols (involving one doses, two simultaneous doses, and three sequential doses), to identify the most appropriate for use in a field setting. However, two factors caused us to modify this original plan.

- (1) Unfortunately, the North American zoo population experienced a complete breeding failure in 2020. An unusually large number of African wild dog pups had been born in 2019, and so only four zoos were given permission to attempt breeding in 2020, under the rules of the Association of Zoos and Aquaria's (AZA) Species Survival Plan (SSP). Unexpectedly, all four litters failed. With no pups available, the project could not proceed as originally planned.
- (2) We were made aware of a paper¹, not listed on the Science Citation Index and therefore not previously known to our team, which provided preliminary evidence of the safety and likely efficacy of the vaccine we were aiming to trial. This paper suggested that a single dose of vaccine was likely to prompt a strong immune response¹, meaning that our planned comparison of one versus multiple doses was unnecessary.

We nevertheless wished to generate additional data on the likely efficacy of modified live vaccine against canine distemper virus, to reassure managers of free-ranging populations that the vaccine was appropriate for trialling in the wild.

In seeking alternative litters in Europe, we discovered that, by chance, veterinarians at Port Lympne zoo in the UK had been administering modified live distemper vaccine to African wild dogs (including pups) for 20 years, and banking serum when blood was collected opportunistically for other purposes (e.g., in the course of periodic health checks, or when animals were moved between enclosures). The result was a remarkable serum bank which would allow us to not only measure responses to initial vaccination (among more pups than would have been possible in our original design), but also to explore the duration of serum antibodies post-vaccination (with some animals resampled up to five years since their first vaccine dose) and the impacts of booster vaccination (with some animals sampled before and after up to 4-5 successive doses of vaccine).

In conclusion, analysing the samples from Port Lympne will allow us to fulfil our original objectives, and go beyond them. These samples were collected opportunistically for zoo management purposes .They will be screened at Cornell for antibodies to canine distemper virus, to assess immune responses to vaccination. The results will inform a field trial in Kruger National Park, South Africa.

¹Wahldén, L *et al.* Evaluation of immunogenicity of a commercially available live attenuated vaccine for dogs containing canine distemper virus and canine parvovirus in African wild dog (*Lycaon pictus pictus*). *Hosts and Viruses* **5**, 26-34 (2018).

Re: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Lamberson, Amanda M <amanda_lamberson@fws.gov>

Thu 6/9/2022 1:24 PM

To: Jen Powers <jhb19@cornell.edu>

Hello Jen,

Thank you for your email. I am currently working on my assessment of the application and how it meets each of the 50 CFR 17.22 issuance criteria under the ESA. I have some additional clarifying questions I will need to ask but I will complete my assessment first before sending those. Once I've ensured I do not need any further information, the application will be submitted to the Federal Register for a 30-day period for public comment as is required for requests for endangered species. This can take a few weeks before it is published. After it is published and the 30-day period lapses we will proceed with making a final decision. Unfortunately, I am not able to give you a more specific timeline of completion.

While I am completing my assessment I do need to request the complete species 360 reports for each of the African wild dogs who were not born at Port Lympne Safari Park. The species 360 report should be at least a full page, if not multiple pages showing each time a wild dog was transferred between facilities. Otherwise if the full reports are not available we will need statements from each of the original breeders (other than Port Lympne Safari Park). If I can clarify on any of the above information, please let me know. Thank you.

In accordance with 50 CFR 13.ll(e), if the requested information is not received by this office within 45 calendar days of the date of this email, your application will be abandoned and administratively closed. Once a file is closed, you will need to submit a new application, and all required fees, for the Service to consider your proposed activity.

Kind regards,

Amanda Lamberson
Biologist
U.S. Fish and Wildlife Service
Division of Management Authority
Branch of Permits, MS: IA
5275 Leesburg Pike
Falls Church, VA 22041

From: Jen Powers <jhb19@cornell.edu> Sent: Wednesday, June 8, 2022 3:26 PM

To: Lamberson, Amanda M <amanda_lamberson@fws.gov>

Subject: RE: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Hi Amanda,

I just sent a message in epermits regarding this permit, but thought I would follow up directly as well. Do you have an idea of when we can expect this permit to be approved?

Thank You, Jen

Jennifer H. Powers Supervisor, Virology Laboratory Animal Health Diagnostic Center New York State Veterinary Diagnostic Laboratory

Cornell University jhb19@cornell.edu Phone: 607-253-3900

Phone: 607-253-3900 Phone: 607-253-4458

From: Lamberson, Amanda M <amanda_lamberson@fws.gov>

Sent: Thursday, May 5, 2022 10:31 AM **To:** Jen Powers <jhb19@cornell.edu>

Subject: Re: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Good morning Jen,

Thank you for the additional information. I will review the information and if I have any further questions, I will let you know.

Kind regards,

Amanda Lamberson Biologist U.S. Fish and Wildlife Service Division of Management Authority Branch of Permits, MS: IA 5275 Leesburg Pike Falls Church, VA 22041

From: Jen Powers <jhb19@cornell.edu> Sent: Tuesday, May 3, 2022 9:47 AM

To: Lamberson, Amanda M <<u>amanda_lamberson@fws.gov</u>>

Subject: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

This email has been received from outside of DOI - Use caution before clicking on links, opening attachments, or responding.

Hello Amanda,

Sorry for the delay in following up on this. Attached is the requested document. Hopefully it is not too late. I will also upload in epermits.

Thank You, Jen

Jennifer H. Powers
Supervisor, Virology Laboratory
Animal Health Diagnostic Center
New York State Veterinary Diagnostic Laboratory
Cornell University
jhb19@cornell.edu

Phone: 607-253-3900 Phone: 607-253-4458 From: Lamberson, Amanda M amanda lamberson@fws.gov>

Sent: Monday, March 7, 2022 12:27 PM **To:** Jen Powers < jhb19@cornell.edu>

Subject: Additional Information Required - FWS Application CS0082219

Hello Jennifer,

Thank you for your application for a federal import permit. I apologize for the delay. I have reviewed the application and found some additional information is required. Based on the proposal submitted in response to question 10a., it is not fully clear what purpose the 224 serum samples serve and why these specific samples are necessary to the proposed project. In a separate document, please provide an explanation of the purpose these samples, how they fit within the objectives of the proposed project, and methods of any activities to be conducted with these samples. If you have any questions, please let me know. Thank you.

In accordance with 50 CFR 13.ll(e), if the requested information is not received by this office within 45 calendar days of the date of this email, your application will be abandoned and administratively closed. Once a file is closed, you will need to submit a new application, and all required fees, for the Service to consider your proposed activity.

Kind regards,

Amanda Lamberson Biologist U.S. Fish and Wildlife Service Division of Management Authority Branch of Permits, MS: IA 5275 Leesburg Pike Falls Church, VA 22041 Re: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Lamberson, Amanda M <amanda_lamberson@fws.gov>

Fri 7/22/2022 3:33 PM

To: Jen Powers < jhb19@cornell.edu>

Hi Jen,

Thank you for your reply and for the additional information. I apologize for the delay in my response. We will need the 360 reports before we can proceed with submitting the application to the Federal Register. Thank you.

Kind regards,

Amanda Lamberson

From: Jen Powers <jhb19@cornell.edu>
Sent: Wednesday, July 13, 2022 10:57 AM

To: Lamberson, Amanda M <amanda_lamberson@fws.gov>

Subject: RE: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Hi Amanda,

Below are answers to your questions. The PI is still working on getting the 360 reports together and it looks like they will be providing an updated list of animals because of new animals being born. I will be in touch with more information once I receive it from the PI. For now does this email suffice to keep the process moving or should I upload this information to the FWS permit system as well?

1. Will the modified live vaccine that was used for the foreign captive-bred African wild dogs be the same type used in the field portion of the study?

The UK-based captive wild dogs were vaccinated with a number of different vaccine brands. However, the more recent samples come from animals vaccinated with the same strain as will be used in the field trial (the Onderstepoort strain, which is contained in the Nobivac available in both the UK, where the captive animals were vaccinated, and in South Africa, where the field trial will take place).

2. Will the methods for analyzing antibody titers differ from the original proposed protocol? If so, please describe the changes in methods.

No, the methods will be the same

3. On page 3, the table specifies that samples were collected up till May 2020, however the description in 10 b states samples were collected up till 2021. Please provide clarification.

Not for FWS - Yikes – OK I have looked into this and the problem is that this process was taking so long that more dogs have been born! I have just requested the id numbers for the 2021 litter, we will need to add it to the list.

4. Per 50 CFR 17.22(a)(2)(iv), the following issuance criteria must be met: Whether the purpose for which the permit is required would be likely to reduce the threat of extinction facing the species of wildlife sought to be covered by the permit. While it is understood that collection of data can be valuable to informing better management practices, please provide further details as to how this proposed research will be likely to reduce the threat of extinction to African wild dogs in the wild.

This project is specifically designed to support the conservation of free-ranging African wild dogs. The African wild dog is a globally endangered species, with fewer than 700 packs remaining in the wild. In the past five years, six separate fatal outbreaks of Canine Distemper Virus (CDV) have been recorded across Africa, with the worst all but wiping out the largest population in the northern hemisphere. Conservation managers throughout Africa urgently need guidelines on effective ways to manage this disease. Previous research shows that CDV cannot easily be controlled by vaccinating domes c dogs. However, simulation modelling suggests that, where CDV risks are most acute, vaccinating wild dogs themselves could greatly reduce extinction risks. Unfortunately, recent attempts to implement CDV vaccination in Kruger National Park, using a recombinant vaccine, provoked minimal immune responses. This project involves trialling a different (modified live) vaccine, widely used on domestic dogs and likely to be both safe and

effective in wild dogs. Trialling of this vaccine was recently described as "urgent and necessary" by participants in a workshop on protecting wild dogs on Kenya from the threat of infectious disease. We propose screening samples from captive-born wild dogs as a prelude to such a field trial, and to generate more precise es mates of likely vaccine effectiveness. These results will help conservation managers to evaluate the likely benefits of vaccination, as well as the potential risks, in deciding whether, where, and when to deploy vaccination as a management tool. To assist such decision-making, we will use the results of the captive and field data collection to parameterise a simulation model linking wild dog management to extinction risk, to evaluate different vaccination strategies and identify the most cost-efficient. More precise estimates of vaccine effectiveness (drawing on these captive data as well as data from free-ranging animals) will allow this model to be parameterised more precisely and will give more robust outcomes.

Thank You, Jen

Jennifer H. Powers
Supervisor, Virology Laboratory
Animal Health Diagnostic Center
New York State Veterinary Diagnostic Laboratory
Cornell University
jhb19@cornell.edu

Phone: 607-253-3900 Phone: 607-253-4458

From: Lamberson, Amanda M <amanda_lamberson@fws.gov>

Sent: Thursday, June 23, 2022 3:48 PM **To:** Jen Powers <jhb19@cornell.edu>

Subject: Re: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Good afternoon Jen,

I have a few additional questions regarding your application.

- 1. Will the modified live vaccine that was used for the foreign captive-bred African wild dogs be the same type used in the field portion of the study?
- 2. Will the methods for analyzing antibody titers differ from the original proposed protocol? If so, please describe the changes in methods.
- 3. On page 3, the table specifies that samples were collected up till May 2020, however the description in 10 b states samples were collected up till 2021. Please provide clarification.
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Just as a reminder, I will need the complete species 360 reports as well. Thank you and please let me know if you have any questions.

Kind regards,

Amanda Lamberson Biologist U.S. Fish and Wildlife Service Division of Management Authority Branch of Permits, MS: IA 5275 Leesburg Pike Falls Church, VA 22041

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From: Jen Powers <jhb19@cornell.edu> Sent: Friday, June 10, 2022 8:30 AM

To: Lamberson, Amanda M < amanda lamberson@fws.gov >

Subject: RE: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Hi Amanda,

Thank you for the update.

I just want to confirm that I completed everything correctly and you have the right information. We are only looking to ship serum from these animals for canine distemper antibody testing. All of this is required for that?

Thank you, Jen

From: Lamberson, Amanda M < amanda lamberson@fws.gov >

Sent: Thursday, June 9, 2022 1:24 PM **To:** Jen Powers < jhb19@cornell.edu>

Subject: Re: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Hello Jen,

Thank you for your email. I am currently working on my assessment of the application and how it meets each of the 50 CFR 17.22 issuance criteria under the ESA. I have some additional clarifying questions I will need to ask but I will complete my assessment first before sending those. Once I've ensured I do not need any further information, the application will be submitted to the Federal Register for a 30-day period for public comment as is required for requests for endangered species. This can take a few weeks before it is published. After it is published and the 30-day period lapses we will proceed with making a final decision. Unfortunately, I am not able to give you a more specific timeline of completion.

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To: Lamberson, Amanda M < amanda lamberson@fws.gov >

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jhb19@cornell.edu

Phone: 607-253-3900 Phone: 607-253-4458

From: Lamberson, Amanda M amanda lamberson@fws.gov>

Sent: Thursday, May 5, 2022 10:31 AM **To:** Jen Powers < jhb19@cornell.edu>

Subject: Re: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Good morning Jen,

Thank you for the additional information. I will review the information and if I have any further questions, I will let you know.

Kind regards,

Amanda Lamberson Biologist U.S. Fish and Wildlife Service Division of Management Authority Branch of Permits, MS: IA 5275 Leesburg Pike Falls Church, VA 22041

From: Jen Powers <jhb19@cornell.edu> Sent: Tuesday, May 3, 2022 9:47 AM

To: Lamberson, Amanda M < amanda lamberson@fws.gov >

Subject: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

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New York State Veterinary Diagnostic Laboratory
Cornell University
jhb19@cornell.edu

Phone: 607-253-3900 Phone: 607-253-4458

From: Lamberson, Amanda M < amanda lamberson@fws.gov >

Sent: Monday, March 7, 2022 12:27 PM To: Jen Powers < jhb19@cornell.edu>

Subject: Additional Information Required - FWS Application CS0082219

Hello Jennifer,

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P21994	Threespot	M	9080	Born at PL Af Exp	958 000 010 946 536	04.11.20	Lenny	Saddle
P21995	Strike	F	9081	Born at PL Af Exp	958 000 010 947 173	04.11.20	Lenny	Saddle
P21996	Widget	M	9082	Born at PL Af Exp	958 000 010 947 798	04.11.20	Lenny	Saddle
P21997	Shroom	M	9083	Born at PL Af Exp	958 000 010 946 803	04.11.20	Lenny	Saddle
P21998	Zand	M	9084	Born at PL Af Exp	958 000 010 946 112	04.11.20	Lenny	Saddle
P21999	Diamond	M	9085	Born at PL Af Exp	958 000 010 947 202	04.11.20	Lenny	Saddle
P22070	Flecks	F	9086	Born at PL Af Exp	958 000 010 946 809	04.11.20	Lenny	Saddle
P22071	Kwenna	F	9087	Born at PL Af Exp	958 000 010 946 309	04.11.20	Lenny	Saddle
P22072	Stomp	M	9088	Born at PL Af Exp	958 000 010 947 390	04.11.20	Lenny	Saddle
P22143 AHD Pup	1 (2022 litter) Wicks	M		Born at PL Af Exp - vet hosp #RH broken leg	958 000 010 961 315	03.01.22	Lenny	Saddle
P22144 AHD Pup	2 (2022 litter)	F		Born at PL Af Exp	958 000 010 961 713	03.01.22	Lenny	Saddle
P22145 AHD Pup	3 (2022 litter) Spyro	F		Born at PL Af Exp	958 000 010 960 208	03.01.22	Lenny	Saddle
P22146 AHD Pup	4 (2022 litter)	F		Born at PL Af Exp	958 000 010 961 379	03.01.22	Lenny	Saddle
P22147 AHD Pup	5 (2022 litter) Skunk	M		Born at PL Af Exp	958 000 010 960 573	03.01.22	Lenny	Saddle
P22148 AHD Pup	6 (2022 litter)	M		Born at PL Af Exp	958 000 010 960 526	03.01.22	Lenny	Saddle
P22149 AHD Pup	7 (2022 litter)	M		Born at PL Af Exp	958 000 010 961 129	03.01.22	Lenny	Saddle
P22150 AHD Pup	8 (2022 litter)	M		Born at PL Af Exp	958 000 010 961 763	03.01.22	Lenny	Saddle
P22151 AHD Pup	9 (2022 litter)	M		Born at PL Af Exp	958 000 010 961 018	03.01.22	Lenny	Saddle
P22152 AHD Pup	10 (2022 litter)	M		Born at PL Af Exp	958 000 010 961 277	03.01.22	Lenny	Saddle
P22153 AHD Pup	11 (2022 litter)	M		Born at PL Af Exp	958 000 010 960 405	03.01.22	Lenny	Saddle
P22154 AHD Pup	12 (2022 litter)	M		Born at PL Af Exp	958 000 010 960 912	03.01.22	Lenny	Saddle
P22155 AHD Pup	13 (2022 litter) Bunda	М		Born at PL Af Exp - anal hematoma	958 000 010 960 375	03.01.22	Lenny	Saddle

Local ID: LYMPNE / P20047

Species360 MIG12-17979419

GAN

Lycaon pictus African hunting dog

Studbooks EAZA, WAZA,

PAAZA, AZA, ZAA

Order Carnivora **Family** Canidae **CITES**

Endangered (EN) **IUCN**

Start Date Jan 01, 1800 **End Date** Aug 08, 2022

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Out Oct 17, 2005

Basic Animal Information Local Data Differences

Sex - Contraception Male -Status Dead Scientific Name Lycaon pictus pictus Jan 08, 1998 - 7Y,9M,9D at the Common Name African hunting dog Birthdate - Age **Preferred ID** LYMPNE / P20047

time of death

Safaripark Beekse Bergen **Origin** Rearing Parent Birth Type Captive Birth/Hatch **Hybrid Status** Not Hybrid

MIG12-12389065 (HILVARENB / Dam Sire MIG12-29593418 (HILVARENB /

> M90210) M94111)

Current Collection Main Institution Animal Collection Collection Trip Clutch / Litter **Enclosure**

Visit History

Date in Acquisition - Vendor/Local ID Disposition - Recipient/Local ID Phy Own Reported By Phy Own Date Out Jan 08, 1998 Birth/Hatch HILVARENB / M98004 Loan Out To LYMPNE/P20047 Out Oct 04, 2000 LYMPNE / P20047 Oct 05, 2000 Loan In From Sender: In Death Out Oct 17, 2005 HILVARENB/M98004 Vendor: HILVARENB/M98004

HILVARENB / M98004 Death (Ownership Only)

Identifiers

Reported By Effective Date <u>Identifier</u> Comments Location Status 5 4 1 **Type** Local ID LYMPNE Oct 05, 2000 P20047 Active **HILVARENB** Mar 01, 1998 Regional EAZA/5332 Active Legacy SLocation: EAZA Studbook Number Legacy Comment: LYMPNE Jan 26, 1998 Transponder 00-013C-C10F In-Use HILVARENB Jan 26, 1998 Transponder 00-013C-C10E In-Use Legacy SLocation: Legacy Comment: 00-013C-C10E CHIP LYMPNE Jan 08, 1998 EAZA/5332 Legacy SLocation: EAZA Regional Active Studbook Number Legacy Comment: House Name HILVARENB Jan 08, 1998 TSENGA BB 72 Active LYMPNE Jan 08, 1998 House Name TSENGA BB 72 Active Jan 08, 1998 Local ID M98004 HILVARENB Active WAZA Jan 08, 1998 Intl Stdbk# 5332 Active Studbook: Lycaon pictus Regional **EAZA** Jan 08, 1998 EAZA/5332 Active Studbook: Lycaon pictus Studbook Number

Sex Information

Reported By Comments Date <u>Sex</u> LYMPNE Oct 05, 2000 Male HILVARENB Jan 08, 1998 Male

Parent Info

Reported By In ZIMS Parent Info Type / Probability Birth Date Comments HILVARENB MIG12-29593418 [HILVARENB / Dam/100% Dec 07, 1994 Yes M94111] MIG12-12389065 [HILVARENB / HILVARENB Yes Sire/100% May 05, 1990 M90210] LYMPNE Yes MIG12-29593418 [HILVARENB / Dam/100% Dec 07, 1994 M94111] LYMPNE MIG12-12389065 [HILVARENB / Yes Sire/100% May 05, 1990 M90210]

Ancestry Information (calculated by Species 360 from shared data)

% Pedigree Known % Pedigree Certain **Taxonomic Inconsistencies** No. Identified Ancestors No

Death Information

Specimen Report: MIG12-17979419 | Local ID: LYMPNE / P20047

Printed: Aug 08, 2022 03:09 Species360 ZIMS version 2.25.5



Reported By
Manner of Death
Death DateHILVARENB
UndeterminedNecropsy Topology
Necropsy Etiological
Relevant Death Information
UndeterminedUnknownDeath DateOct 17, 2005Relevant Death Information
Carcass DispositionUndetermined

Primary Body System Affected

Body Part Institution Recipient ID Date Sent Date Received Genetic Results

-

Reported By
Manner of DeathLYMPNENecropsy Topology
Necropsy EtiologicalUndeterminedDeath DateOct 17, 2005Relevant Death Information
Carcass DispositionUndeterminedDeath In TransitNoCarcass DispositionUndetermined

Primary Body System Affected

Body Part Institution Recipient ID Date Sent Date Received Genetic Results

Species360 MIG12-27324767

GAN

Lycaon pictus

African hunting dog

Studbooks EAZA, WAZA,

PAAZA, AZA, ZAA

Order Carnivora

IUCN Endangered (EN)

Start Date Jan 01, 1800

CITES

Family

End Date Aug 08, 2022

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Basic Animal Information Local Data Differences

Birthdate - Age Jan 08, 1998 - 11Y,11M,17D at Preferred ID

the time of death

Status

Dead LYMPNE / P20048

Canidae

Local ID: LYMPNE / P20048

Scientific Name Lycaon pictus pictus **Common Name** African hunting dog

<u>Origin</u> Safaripark Beekse Bergen Rearing Parent **Birth Type** Captive Birth/Hatch **Hybrid Status** Not Hybrid

MIG12-12389065 (HILVARENB / Dam MIG12-29593418 (HILVARENB /

M94111)

M90210) **Current Collection** Main Institution Animal Collection Collection Trip

Clutch / Litter

Sex - Contraception Male -

Enclosure

Visit History

<u>Sire</u>

Date in	Acquisition - Vendor/Local ID	<u>Phy</u>	<u>Own</u>	Reported By	Disposition - Recipient/Local ID	Phy Owr	Date Out
Jan 08, 1998	Birth/Hatch	In	In	HILVARENB / M98003	Loan Out To LYMPNE/P20048	Out -	Oct 04, 2000
Oct 05, 2000	Loan In From Sender: HILVARENB/M98003 Vendor: HILVARENB/M98003	In	-	LYMPNE / P20048	Loan Transfer To BEKESBRNE/H20236	Out -	Dec 23, 2002
Dec 23, 2002	Loan In From Sender: LYMPNE/P20048 Vendor: LYMPNE/P20048	In	-	BEKESBRNE / H20236	Loan Return To Owner LYMPNE/P20048	Out -	Apr 01, 2006
Apr 01, 2006	Loan In From Vendor: BEKESBRNE/H20236	In	-	LYMPNE / P20048	Death	Out -	Dec 25, 2009
		-	-	HILVARENB / M98003	Death (Ownership Only)	- Out	Dec 25, 2009

Identifiers

Reported By	Effective Date	<u>Type</u>	<u>Identifier</u>	Location	<u>Status</u>	Comments
BEKESBRNE	Dec 31, 2002	House Name	Mzungu		Active	
BEKESBRNE	Dec 31, 2002	Transponder	00-013C-B790		In-Use	
BEKESBRNE	Dec 23, 2002	Local ID	H20236		Active	
LYMPNE	Oct 05, 2000	Local ID	P20048		Active	
LYMPNE	Jan 26, 2000	Transponder	00-013C-B790		In-Use	
LYMPNE	Jan 08, 2000	House Name	MZUNGU BB 71		Active	
HILVARENB	Mar 01, 1998	Regional Studbook Number	EAZA/5331		Active	Legacy SLocation: EAZA Legacy Comment:
HILVARENB	Jan 26, 1998	Transponder	00-013C-B790		In-Use	Legacy SLocation: Legacy Comment: 00-013C-B790 CHIP
HILVARENB	Jan 08, 1998	House Name	MZUNGU BB 71		Active	
LYMPNE	Jan 08, 1998	Regional Studbook Number	EAZA/5331		Active	Legacy SLocation: EAZA Legacy Comment:
HILVARENB	Jan 08, 1998	Local ID	M98003		Active	
EAZA	Jan 08, 1998	Regional Studbook Number	EAZA/5331		Active	Studbook: Lycaon pictus
WAZA	Jan 08, 1998	Intl Stdbk#	5331		Active	Studbook: Lycaon pictus
BEKESBRNE	Jan 08, 1998	Regional Studbook Number	EAZA/5331		Active	Legacy SLocation: EAZA Legacy Comment:

Sex Information

Reported By	<u>Date</u>	<u>Sex</u>	<u>Comments</u>
BEKESBRNE	Jan 02, 2003	Male	
LYMPNE	Oct 05, 2000	Male	
HILVARENB	Jan 08, 1998	Male	

Parent Info

Specimen Report: MIG12-27324767 | Local ID: LYMPNE / P20048

Printed: Aug 08, 2022 02:55 Species360 ZIMS version 2.25.5

Reported By	In ZIMS	Parent Info		Type / Probability		<u>Comments</u>
BEKESBRNE	Yes	MIG12-29593418 [HILVAF M94111]	RENB /	Dam/100%	Dec 07, 1994	
BEKESBRNE	Yes	MIG12-12389065 [HILVAF M90210]	RENB /	Sire/100%	May 05, 1990	
HILVARENB	Yes	MIG12-29593418 [HILVAF M94111]	RENB /	Dam/100%	Dec 07, 1994	
HILVARENB	Yes	MIG12-12389065 [HILVAF M90210]	RENB /	Sire/100%	May 05, 1990	
LYMPNE	Yes	MIG12-29593418 [HILVAF M94111]	RENB /	Dam/100%	Dec 07, 1994	
LYMPNE	Yes	MIG12-12389065 [HILVAF M90210]	RENB /	Sire/100%	May 05, 1990	
Ancestry Inforn	nation (calcı	ulated by Species360 fro	om share	ed data)		
% Pedigree Know	<u>vn</u>	% Pedigree Certain		Taxonomic Inc	onsistencies	No. Identified Ancestors
0.00%		0.00%		No		5
Death Informati	<u>ion</u>					
Reported By	HILV	ARENB	<u>Ne</u>	cropsy Topology	Undetermined	
Manner of Death	<u>.</u> Unde	etermined	<u>Ne</u>	cropsy Etiological	Unknown	
Death Date	Dec 2	25, 2009	Re	levant Death Informati	on Undetermined	
Death In Transit	No		<u>Ca</u>	rcass Disposition	Undetermined	
Primary Body Sy	stem Affecte	<u>d</u>				
Body Part	Institution	Recipient ID	Date Se	ent Date Receive	ed Genetic	Results
-	-	-				
Reported By	LYMI	DNE	No	cropsy Topology	Undetermined	
Manner of Death		ral/Non-euthanasia			Unknown	
Death Date	•	25, 2009	· ·	<u>cropsy Etiological</u> levant Death Informati		Disease or Condition (Geriatric)
Death In Transit		23, 2003		rcass Disposition	Undetermined	Disease of Condition (Genatic)
Primary Body Sy		<u>d</u>	<u>0a</u>	icass Disposition	Ondetermined	
	Institution	_	Data Ca	unt Data Basalis	ad Camatia	Decute
Body Part	เทรินเนนเปก	Recipient ID	Date Se	ent Date Receive	ed Genetic I	Results

Species360 MIG12-29031991

GAN

Lycaon pictus

African hunting dog

Studbooks EAZA, WAZA,

PAAZA, AZA, ZAA

Order Carnivora
IUCN Endangered (EN)

Family Canidae

CITES

Start Date Jan 01, 1800 **End Date** Aug 08, 2022

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No Local Data Differences Found

Basic Animal Information

<u>Sex - Contraception</u> Male - <u>Status</u> Dead

Oct 17, 2004 - 11Y,2M,21D at the **Preferred ID** time of death

LYMPNE / P20169

Local ID: LYMPNE / P20169

unie oi deaui

Miejski Ogrod Zoologiczny

Rearing Parent

Warsaw
Captive Birth/Hatch

Hybrid Status Not Hybrid

Sire MIG12-17704088 (WARSAW /

<u>Dam</u> 5814177 (WARSAW / S5635)

S4802)

Current Collection Main

Main Institution Animal Collection Collection Trip

Clutch / Litter Enclosure

Visit History

Birthdate - Age

Origin

Date in	Acquisition - Vendor/Local ID	Phy	Owr	Reported By	Disposition - Recipient/Local ID	Phy Own Date Out
Oct 17, 2004	Birth/Hatch	In	In	WARSAW / S6231	Donation To LONDON RP/4540	Out Out Mar 13, 2006
Mar 14, 2006	Donation From WARSAW/S6231	In	In	LONDON RP / 4540	Donation To LYMPNE/P20169	Out Out Nov 02, 2010
Nov 02, 2010	Donation From LONDON RP/4540	In	In	LYMPNE / P20169	Death	Out Out Jan 07, 2016

Identifiers

Reported By	Effective Date	<u>Type</u>	<u>Identifier</u>	Location	<u>Status</u>	Comments
LYMPNE	Nov 02, 2010	Transponder	0006354322		Inactive	
LYMPNE	Nov 02, 2010	House Name	Tatu		Active	
LYMPNE	Nov 02, 2010	Local ID	P20169		Active	
LONDON RP	Jun 20, 2007	House Name	TATU		Active	Legacy SLocation: Legacy Comment: Swahili: "Three"
LONDON RP	May 26, 2006	Regional Studbook Number	EAZA/5671		Active	Legacy SLocation: EAZA Legacy Comment:
WARSAW	Mar 21, 2006	Regional Studbook Number	EAZA/5671		Active	Legacy SLocation: EAZA Legacy Comment:
LONDON RP	Mar 14, 2006	Local ID	4540		Active	
WARSAW	Nov 10, 2004	Transponder	00-0635-4322		Inactive	Legacy SLocation: LEFT SHOULDER Legacy Comment:
LONDON RP	Nov 10, 2004	Transponder	00-0635-4322		Inactive	Legacy SLocation: LEFT SHOULDER Legacy Comment: Left thorax
ZAA	Oct 17, 2004	Regional Studbook Number	ZAA/5671		Active	Studbook: Lycaon pictus
AZA	Oct 17, 2004	Regional Studbook Number	AZA/5671		Active	Studbook: Lycaon pictus
WAZA	Oct 17, 2004	Intl Stdbk#	5671		Active	Studbook: Lycaon pictus
WARSAW	Oct 17, 2004	Local ID	S6231		Active	
EAZA	Oct 17, 2004	Regional Studbook Number	EAZA/5671		Active	Studbook: Lycaon pictus

Sex Information

Reported By	<u>Date</u>	<u>Sex</u>	Comments
LYMPNE	Nov 02, 2010	Male	
LONDON RP	Mar 14, 2006	Male	
WARSAW	Oct 17, 2004	Male	

Parent Info

Reported By	In ZIMS	Parent Info	Type / Probability	Birth Date	Comments
LONDON RP	Yes	5814177 [WARSAW / S5635]	Dam/100%	Nov 29, 2000	
LONDON RP	Yes	MIG12-17704088 [WARSAW / S4802]	Sire/100%	Apr 15, 1996	
LYMPNE	Yes	5814177 [WARSAW / S5635]	Dam/100%	Nov 29, 2000	
LYMPNE	Yes	MIG12-17704088 [WARSAW / S4802]	Sire/100%	Apr 15, 1996	
WARSAW	Yes	5814177 [WARSAW / S5635]	Dam/100%	Nov 29, 2000	
WARSAW	Yes	MIG12-17704088 [WARSAW / S4802]	Sire/100%	Anr 15 1996	

Ancestry Information (calculated by Species 360 from shared data)

 % Pedigree Known
 % Pedigree Certain
 Taxonomic Inconsistencies
 No. Identified Ancestors

 0.00%
 Yes-Subspecies level
 15

Death Information

Specimen Report: MIG12-29031991 | Local ID: LYMPNE / P20169

Printed: Aug 08, 2022 02:57 Species360 ZIMS version 2.25.5 Reported ByLYMPNEManner of DeathEuthanasiaDeath DateJan 07, 2016Death In TransitNo

Primary Body System Affected

Necropsy Topology Necropsy Etiological Relevant Death Information Carcass Disposition

Family

CITES

Species360 MIG12-29430290

GAN

Lycaon pictus

Studbooks

Local ID: LYMPNE / P20170

Canidae

LYMPNE / P20170

5814177 (WARSAW / S5635)

Dead

Parent

Not Hybrid

African hunting dog EAZA, WAZA,

PAAZA, AZA, ZAA

Order Carnivora

IUCN Endangered (EN)

Start Date Jan 01, 1800 **End Date** Aug 08, 2022

Status

Rearing

<u>Dam</u>

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No Local Data Differences Found

Basic Animal Information

Sex - Contraception Male -Birthdate - Age

Oct 17, 2004 - 12Y,9M,1D at the Preferred ID

time of death

Miejski Ogrod Zoologiczny

Warsaw

Captive Birth/Hatch

MIG12-17704088 (WARSAW / <u>Sire</u> S4802)

Current Collection

Main Institution Animal Collection Collection Trip

Enclosure

Hybrid Status

Visit History

Clutch / Litter

Origin

Birth Type

Phy Own Reported By Date in Acquisition - Vendor/Local ID Disposition - Recipient/Local ID Phy Own Date Out Oct 17, 2004 Birth/Hatch WARSAW / S6225 Donation To LONDON RP/4537 Out Out Mar 13, 2006 Mar 14, 2006 Donation From WARSAW/S6225 In In LONDON RP / 4537 Donation To LYMPNE/P20170 Out Out Nov 02, 2010 Nov 02, 2010 Donation From LONDON RP/4537 LYMPNE / P20170 In Death Out Out Jul 18, 2017 In

Identifiers

Reported By	Effective Date	<u>Type</u>	<u>Identifier</u>	Location	<u>Status</u>	Comments
LYMPNE	Nov 02, 2010	Transponder	000634FA68		In-Use	
LYMPNE	Nov 02, 2010	House Name	Wili		Active	
LYMPNE	Nov 02, 2010	Local ID	P20170		Active	
LONDON RP	Jun 11, 2007	House Name	WILI		Active	Legacy SLocation: Legacy Comment: Swahili: "Two"
LONDON RP	May 26, 2006	Regional Studbook Number	EAZA/5666		Active	Legacy SLocation: EAZA Legacy Comment:
WARSAW	Mar 21, 2006	Regional Studbook Number	EAZA/5666		Active	Legacy SLocation: EAZA Legacy Comment:
LONDON RP	Mar 14, 2006	Local ID	4537		Active	
EAZA	Oct 17, 2004	Regional Studbook Number	EAZA/5666		Active	Studbook: Lycaon pictus
WAZA	Oct 17, 2004	Intl Stdbk#	5666		Active	Studbook: Lycaon pictus
WARSAW	Oct 17, 2004	Local ID	S6225		Active	
LONDON RP	Oct 11, 2004	Transponder	00-0634-FA68		In-Use	Legacy SLocation: LEFT SHOULDER Legacy Comment: left abdomen
WARSAW	Oct 11, 2004	Transponder	00-0634-FA68		In-Use	Legacy SLocation: LEFT SHOULDER Legacy Comment:

Sex Information

<u> </u>	<u></u>		
Reported By	<u>Date</u>	<u>Sex</u>	<u>Comments</u>
LYMPNE	Nov 02, 2010	Male	
LONDON RP	Mar 14, 2006	Male	
WARSAW	Oct 17 2004	Male	

Parent Info

Reported By	In ZIMS	Parent Info	Type / Probability	Birth Date	Comments
LONDON RP	Yes	5814177 [WARSAW / S5635]	Dam/100%	Nov 29, 2000	
LONDON RP	Yes	MIG12-17704088 [WARSAW / S4802]	Sire/100%	Apr 15, 1996	
LYMPNE	Yes	5814177 [WARSAW / S5635]	Dam/100%	Nov 29, 2000	
LYMPNE	Yes	MIG12-17704088 [WARSAW / S4802]	Sire/100%	Apr 15, 1996	
WARSAW	Yes	5814177 [WARSAW / S5635]	Dam/100%	Nov 29, 2000	
WARSAW	Yes	MIG12-17704088 [WARSAW / S4802]	Sire/100%	Apr 15, 1996	

Ancestry Information (calculated by Species 360 from shared data)

% Pedigree Certain No. Identified Ancestors % Pedigree Known Taxonomic Inconsistencies 0.00% 0.00% Yes-Subspecies level 15

Death Information

Specimen Report: MIG12-29430290 | Local ID: LYMPNE / P20170

Printed: Aug 08, 2022 02:58 Species360 ZIMS version 2.25.5 Reported By LYMPNE

<u>Manner of Death</u> Euthanasia, medical

Death DateJul 18, 2017Death In TransitNo

Primary Body System Affected

Necropsy Topology Necropsy Etiological Relevant Death Information

Carcass Disposition

Specimen Report: MIG12-29430290 | Local ID: LYMPNE / P20170

Species360 23529436

GAN

Lycaon pictus pictus

African hunting dog

Studbooks EAZA, WAZA,

PAAZA, AZA, ZAA

Order Carnivora **Family** Canidae

Local ID: LYMPNE / P20520

IUCN Endangered (EN)

CITES Start Date Jan 01, 1800 **End Date**

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No Local Data Differences Found

Basic Animal Information

Status Sex - Contraception Female -Dead

time of death

Sep 15, 2002 - 5Y,1M,20D at the Preferred ID LYMPNE / P20520

<u>Origin</u>

STB Kanta - Friguia Zoo Rearing Parent Captive Birth/Hatch **Hybrid Status** Not Hybrid MIG12-29642501 (FRIGUIA /

<u>Sire</u> F00042) MIG12-29642500 (FRIGUIA / F00043)

Main Institution Animal Collection Collection Trip

Current Collection Clutch / Litter

Birthdate - Age

Birth Type

Enclosure

Visit History

Date in	Acquisition - Vendor/Local ID	<u>Phy</u>	<u> Ow</u>	n Reported By	Disposition - Recipient/Local ID	Phy Own Date Out
Sep 15, 2002	Birth/Hatch	In	In	FRIGUIA / F02065	Donation To LYMPNE/P20520	Out Out Apr 25, 2005
Apr 25, 2005	Donation From FRIGUIA/F02065	In	In	LYMPNE / P20520	Death	Out Out Nov 04, 2007

Identifiers

Reported By	Effective Date	<u>Type</u>	<u>Identifier</u>	Location	<u>Status</u>	Comments
LYMPNE	May 24, 2005	House Name	Tunis		Active	
LYMPNE	Apr 25, 2005	Local ID	P20520		Active	
LYMPNE	Jan 01, 2003	Regional Studbook Number	EAZA/T5853		Active	Legacy SLocation: EAZA Legacy Comment:
FRIGUIA	Sep 15, 2002	House Name	Tunis		Active	
ZAA	Sep 15, 2002	Regional Studbook Number	ZAA/6834		Active	Studbook: Lycaon pictus
EAZA	Sep 15, 2002	Regional Studbook Number	EAZA/6834		Active	Studbook: Lycaon pictus
AZA	Sep 15, 2002	Regional Studbook Number	AZA/6834		Active	Studbook: Lycaon pictus
LYMPNE	Sep 15, 2002	Transponder	250229600007110)	In-Use	
WAZA	Sep 15, 2002	Intl Stdbk#	6834		Active	Studbook: Lycaon pictus
FRIGUIA	Sep 15, 2002	Transponder	250229600007110)	In-Use	
FRIGUIA		Local ID	F02065		Active	

Sex Information

Reported By	<u>Date</u>	<u>Sex</u>	<u>Comments</u>	
LYMPNE	Apr 25, 2005	Female		
FRIGUIA	Sen 15, 2002	Female		

Parent Info

Reported By	In ZIMS	Parent Info	Type / Probability	Birth Date	<u>Comments</u>
FRIGUIA	Yes	MIG12-29642500 [FRIGUIA / F00043]	Dam/100%	Aug 29, 2000	
FRIGUIA	Yes	MIG12-29642501 [FRIGUIA / F00042]	Sire/100%	Aug 29, 2000	
LYMPNE	Yes	MIG12-29642500 [FRIGUIA / F00043]	Dam/100%	Aug 29, 2000	
LYMPNE	Yes	MIG12-29642501 [FRIGUIA / F00042]	Sire/100%	Aug 29, 2000	

Ancestry Information (calculated by Species 360 from shared data)

% Pedigree Known % Pedigree Certain No. Identified Ancestors Taxonomic Inconsistencies 0.00% 0.00%

Death Information

I YMPNF Reported By **Necropsy Topology** Respiratory **Manner of Death** Natural/Non-euthanasia **Necropsy Etiological** Bacterial Nov 04, 2007 **Relevant Death Information Death Date** Infectious disease **Death In Transit** No **Carcass Disposition** Undetermined

Primary Body System Affected

Institution **Date Sent Body Part** Recipient ID **Date Received Genetic Results**

Specimen Report: 23529436 | Local ID: LYMPNE / P20520

Printed: Aug 08, 2022 02:56 Species360 ZIMS version 2.25.5

CITES

Species360 MIG12-16946612

GAN

Order

Lycaon pictus pictus

African hunting dog

Local ID: LYMPNE / P20911

Studbooks EAZA, WAZA,

PAAZA, AZA, ZAA

Canidae **Family**

Carnivora **IUCN** Endangered (EN)

Start Date Jan 01, 1800 **End Date** Aug 08, 2022

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No Local Data Differences Found

Basic Animal Information

Sex - Contraception Male -**Status** Dead

Oct 27, 2005 - 9Y,11M,26D at the Preferred ID

time of death

West Midland Safari & Leisure

Rearing

Parent

LYMPNE / P20911

Park Ltd **Birth Type**

Captive Birth/Hatch

Hybrid Status

<u>Sire</u> DOG4)

MIG12-29374654 (BEWDLEY /

MIG12-28090217 (BEWDLEY / <u>Dam</u>

DOG9)

Not Hybrid

Main Institution Animal Collection Collection Trip

Current Collection

Enclosure

Visit History

Clutch / Litter

Birthdate - Age

Origin

Date in	Acquisition - Vendor/Local ID	Phy	<u>Owr</u>	Reported By	Disposition - Recipient/Local ID	Phy Own	Date Out
Oct 27, 2005	Birth/Hatch	In	In	BEWDLEY / DOG21	Loan Out To LYMPNE/UNK	Out -	Mar 12, 2009
Mar 12, 2009	Donation From BEWDLEY/DOG21	In	In	LYMPNE / P20911	Donation To BEKESBRNE/H21297	Out Out	Nov 19, 2013
Nov 19, 2013	Donation From LYMPNE/P20911	In	In	BEKESBRNE / H21297	Donation To LYMPNE/P21552	Out Out	Jul 23, 2015
Jul 23, 2015	Donation From BEKESBRNE/H21297	In	In	LYMPNE / P20911	Death	Out Out	Oct 23, 2015

Identifiers

Reported By	Effective Date	<u>Type</u>	<u>Identifier</u>	Location	<u>Status</u>	Comments
BEKESBRNE	Nov 19, 2013	Local ID	H21297		Active	
LYMPNE	Mar 30, 2009	House Name	Vango		Active	
LYMPNE	Mar 12, 2009	Regional Studbook Number	EAZA/T6055		Active	Legacy SLocation: EAZA Legacy Comment:
LYMPNE	Mar 12, 2009	Local ID	P20911		Active	
LYMPNE	Mar 12, 2009	Transponder	956000000461385	5	Inactive	
BEWDLEY	Feb 18, 2008	Regional Studbook Number	EAZA/T6055		Active	Legacy SLocation: EAZA Legacy Comment:
BEWDLEY	Aug 24, 2006	House Name	Scanner		Active	
BEWDLEY	Aug 24, 2006	Transponder	956000000461385	5	Inactive	
BEWDLEY	Oct 27, 2005	Local ID	DOG21		Active	
WAZA	Oct 27, 2005	Intl Stdbk#	7262		Active	Studbook: Lycaon pictus
EAZA	Oct 27, 2005	Regional	EAZA/7262		Active	Studbook: Lycaon pictus

Sex Information

Reported By	<u>Date</u>	<u>Sex</u>	<u>Comments</u>
BEKESBRNE	Nov 19, 2013	Male	
LYMPNE	Mar 12, 2009	Male	
BEWDLEY	Oct 27, 2005	Male	

Parent Info

Reported By	In ZIMS	Parent Info	Type / Probability	Birth Date	<u>Comments</u>
BEWDLEY	Yes	MIG12-28090217 [BEWDLEY / DOG9]	Dam/100%	Apr 01, 2002	
BEWDLEY	Yes	MIG12-29374654 [BEWDLEY / DOG4]	Sire/100%	Mar 01, 2002	
LYMPNE	Yes	MIG12-28090217 [BEWDLEY / DOG9]	Dam/100%	Apr 01, 2002	
LYMPNE	Yes	MIG12-29374654 [BEWDLEY / DOG4]	Sire/100%	Mar 01 2002	

Ancestry Information (calculated by Species360 from shared data)

% Pedigree Known % Pedigree Certain No. Identified Ancestors Taxonomic Inconsistencies

Death Information

Reported By LYMPNE Necropsy Topology Euthanasia **Necropsy Etiological Manner of Death Death Date** Oct 23, 2015 **Relevant Death Information Death In Transit Carcass Disposition**

Primary Body System Affected

Specimen Report: MIG12-16946612 | Local ID: LYMPNE / P20911

Printed: Aug 08, 2022 02:59 Species360 ZIMS version 2.25.5 Port Lympne Wild Animal Park

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CITES

Species360 MIG12-29700434

GAN

Lycaon pictus

African hunting dog

Studbooks EAZA, WAZA,

PAAZA, AZA, ZAA

Order Carnivora **Family**

IUCN Endangered (EN)

Start Date Jan 01, 1800 **End Date** Aug 08, 2022

Status

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No Local Data Differences Found

Basic Animal Information

Sex - Contraception Female - Medical method-

surgical/Active [COLCHESTR]

Jun 05, 2006 - 10Y,5M,6D at the Preferred ID

LYMPNE / P21301

time of death **Origin**

Les Terres de Nataé Captive Birth/Hatch MIG12-29110762

(PONTSCORF / 100303)

Parent Rearing **Hybrid Status** Not Hybrid <u>Dam</u>

MIG12-29111617 (PONTSCORF /

Local ID: LYMPNE / P21301

Canidae

100306)

Dead

Current Collection Clutch / Litter

Birthdate - Age

Birth Type

<u>Sire</u>

Main Institution Animal Collection Collection Trip

Enclosure

Visit History

Date in	Acquisition - Vendor/Local ID	Phy	<u>Own</u>	Reported By	Disposition - Recipient/Local ID	Phy Own	Date Out
Jun 05, 2006	Birth/Hatch	In	In	PONTSCORF / 100390	Donation To DUISBURG/5385	Out Out	Jun 14, 2007
Jun 14, 2007	Loan In From Vendor: PONTSCORF/100390	In	-	DUISBURG / 5385	Loan Transfer To COLCHESTR/CLL707	Out -	Nov 26, 2009
Nov 26, 2009	Loan In From Sender: DUISBURG/5385 Vendor: PONTSCORF/100390	In	-	COLCHESTR / CLL707	Loan Transfer To LYMPNE/P21301	Out -	May 13, 2013
May 13, 2013	Loan Transfer From Sender:	In	-	LYMPNE / P21301	Death	Out -	Nov 11, 2016

Identifiers

Reported By	Effective Date	<u>Type</u>	<u>Identifier</u>	Location	<u>Status</u>	Comments
LYMPNE	May 13, 2013	Local ID	P21301		Active	
COLCHESTR	May 28, 2010	Regional Studbook Number	EAZA/T5955		Active	Legacy SLocation: EAZA Legacy Comment:
COLCHESTR	Dec 01, 2009	House Name	Zuri		Active	
COLCHESTR	Nov 26, 2009	Old Accession Number	5385		Active	Legacy SLocation: Legacy Comment: Duisburg ID
COLCHESTR	Nov 26, 2009	Local ID	CLL707		Active	
DUISBURG	Jun 14, 2007	Local ID	5385		Active	
DUISBURG	Sep 01, 2006	Transponder	250229600032779		In-Use	Legacy SLocation: left shoulder Legacy Comment:
PONTSCORF	Jun 05, 2006	Local ID	100390		Active	
EAZA	Jun 05, 2006	Regional Studbook Number	EAZA/7369		Active	Studbook: Lycaon pictus
WAZA	Jun 05, 2006	Intl Stdbk#	7369		Active	Studbook: Lycaon pictus

Sex Information

Reported By	<u>Date</u>	<u>Sex</u>	<u>Comments</u>
LYMPNE	May 13, 2013	Female	
COLCHESTR	Nov 26, 2009	Female	
DUISBURG	Jun 14, 2007	Female	
PONTSCORF	Jun 05, 2006	Female	

Parent Info

Reported By	In ZIMS	Parent Info	Type / Probability	Birth Date	Comments
COLCHESTR	Yes	MIG12-29111617 [PONTSCORF / 100306]	Dam/100%	Nov 09, 2002	
COLCHESTR	Yes	MIG12-29110762 [PONTSCORF / 100303]	Sire/100%	Nov 29, 2000	
DUISBURG	Yes	MIG12-29111617 [PONTSCORF / 100306]	Dam/100%	Nov 09, 2002	
DUISBURG	Yes	MIG12-29110762 [PONTSCORF / 100303]	Sire/100%	Nov 29, 2000	
PONTSCORF	Yes	MIG12-29111617 [PONTSCORF / 100306]	Dam/100%	Nov 09, 2002	
PONTSCORF	Yes	MIG12-29110762 [PONTSCORF /	Sire/100%	Nov 29, 2000	

Ancestry Information (calculated by Species 360 from shared data)

Specimen Report: MIG12-29700434 | Local ID: LYMPNE / P21301

Printed: Aug 08, 2022 03:03 Species360 ZIMS version 2.25.5 Port Lympne Wild Animal Park

Page: 1 of 2

% Pedigree Known 37.50% % Pedigree Certain Taxonomic Inconsistencies No. Identified Ancestors Yes-Subspecies level

37.50%

Death Information

Reported By

LYMPNE

Natural/Non-euthanasia **Manner of Death**

Death Date Nov 11, 2016

Death In Transit

Necropsy Topology Necropsy Etiological

Relevant Death Information Carcass Disposition

Primary Body System Affected

Species360 MIG12-29923020

GAN

African hunting dog

Lycaon pictus pictus **Studbooks** EAZA, WAZA,

PAAZA, AZA, ZAA

Order Carnivora **Family** Canidae **CITES**

IUCN Endangered (EN)

Aug 08, 2022 **Start Date** Jan 01, 1800 **End Date**

Rearing

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No Local Data Differences Found

Basic Animal Information

Sex - Contraception Female -Status Dead

Nov 12, 2011 - 4Y,11M,29D at the Preferred ID time of death

Parent

Not Hybrid

LYMPNE / P21348

Birth Type Captive Birth/Hatch MIG12-29923018 (EBELTOFT / <u>Sire</u>

Hybrid Status

24056245 (EBELTOFT / LYC009)

Local ID: LYMPNE / P21348

LYC005)

Current Collection Main Institution Animal Collection Collection Trip Clutch / Litter **Enclosure**

Ree Park - Ebeltoft Safari

Visit History

Birthdate - Age

Origin

Date in Phy Own Reported By Disposition - Recipient/Local ID Phy Own Date Out Acquisition - Vendor/Local ID Nov 12, 2011 Birth/Hatch EBELTOFT / LYC015 Donation To LYMPNE/P21348 Out Out Nov 08, 2013 In Nov 08, 2013 Donation From EBELTOFT/LYC015 In In LYMPNE / P21348 Death Out Out Nov 10, 2016

Identifiers

Reported By **Effective Date Type Identifier** Location **Status** Comments LYMPNE Local ID Nov 08, 2013 P21348 Active Active **EBELTOFT** Jun 01, 2012 House Name Nadifa **EBELTOFT** Apr 12, 2012 Regional EAZA/T1144 Active Studbook Number Feb 03, 2012 Transponder 208246000008515 In-Use **EBELTOFT** WAZA Nov 12, 2011 Intl Stdbk# 5784 Active Studbook: Lycaon pictus **EBELTOFT** Nov 12, 2011 Local ID LYC015 Active FA7A Nov 12, 2011 Regional EAZA/5784 Active Studbook: Lycaon pictus Studbook Number

Sex Information

Reported By Comments <u>Date</u> <u>Sex</u> Nov 08, 2013 LYMPNE Female **EBELTOFT** Nov 12, 2011 Female

Parent Info

Reported By In ZIMS Parent Info Type / Probability Birth Date **Comments** 24056245 [EBELTOFT / LYC009] **EBELTOFT** Dam/100% Dec 04, 2006 Yes MIG12-29923018 [EBELTOFT / Sire/100% **EBELTOFT** Yes Jun 01, 2002 LYC005]

Ancestry Information (calculated by Species360 from shared data)

% Pedigree Certain No. Identified Ancestors % Pedigree Known Taxonomic Inconsistencies 0.00% Yes-Subspecies level

Death Information

LYMPNE Reported By Necropsy Topology **Manner of Death** Euthanasia **Necropsy Etiological** Nov 10, 2016 **Relevant Death Information Death Date Death In Transit Carcass Disposition**

Primary Body System Affected

Local ID: LYMPNE / P21349

Parent

Not Hybrid

Species360 27577683

GAN

Lycaon pictus

Studbooks EAZA, WAZA,

African hunting dog

PAAZA, AZA, ZAA

Canidae Order Carnivora **Family CITES**

IUCN Endangered (EN)

Start Date Jan 01, 1800 **End Date** Aug 08, 2022

Hybrid Status

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No Local Data Differences Found

Basic Animal Information

Sex - Contraception Male -**Status** Dead Birthdate - Age ~From Oct 17, 2009 To Oct 19, LYMPNE / P21349 Preferred ID

2009 - 4Y,8M,5D +/-1D at the

time of death

Dublin Zoo - Zoological Society of Rearing

Ireland

Captive Birth/Hatch

MULTIPLE 18411770 (DUBLIN / A7M006) <u>Sire</u> <u>Dam</u>

Current Collection Main Institution Animal Collection Collection Trip

Clutch / Litter **Enclosure**

Visit History

<u>Origin</u>

Birth Type

<u>Date in</u>	Acquisition - Vendor/Local ID	<u>Phy</u>	<u>Own</u>	Reported By	<u>Disposition - Recipient/Local ID</u>	Phy Own	<u>Date Out</u>
Oct 18, 2009	Birth/Hatch	In	In	DUBLIN / A9M046	Donation To BEKESBRNE/H21202	Out Out	Jan 26, 2012
Jan 27, 2012	Donation From DUBLIN/A9M046	In	In	BEKESBRNE / H21202	Donation To LYMPNE/P21349	Out Out	Nov 22, 2013
Nov 22, 2013	Donation From BEKESBRNE/H21202	In	In	LYMPNE / P21349	Death	Out Out	Jun 23, 2014

Identifiers

Reported By	Effective Date	<u>Type</u>	<u>Identifier</u>	Location	<u>Status</u>	Comments
LYMPNE	Nov 22, 2013	Local ID	P21349		Active	
BEKESBRNE	Jan 27, 2012	Transponder	956000001133489	9	Inactive	
BEKESBRNE	Jan 27, 2012	Regional Studbook Number	EAZA/T9704		Active	Legacy SLocation: EAZA Legacy Comment:
BEKESBRNE	Jan 27, 2012	House Name	Ruaha		Active	
BEKESBRNE	Jan 27, 2012	Local ID	H21202		Active	
DUBLIN	May 10, 2011	Regional Studbook Number	T9704		Active	
DUBLIN	Feb 23, 2010	House Name	Ruaha		Active	Legacy SLocation: Legacy Comment: Reserve in Africa
DUBLIN	Jan 07, 2010	Transponder	956000001133489	9	Inactive	
WAZA	Oct 18, 2009	Intl Stdbk#	7660		Active	Studbook: Lycaon pictus
EAZA	Oct 18, 2009	Regional Studbook Number	EAZA/7660		Active	Studbook: Lycaon pictus
DUBLIN	Oct 18, 2009	Local ID	A9M046		Active	

Sex Information

	-		
Reported By	<u>Date</u>	<u>Sex</u>	<u>Comments</u>
LYMPNE	Nov 22, 2013	Male	
BEKESBRNE	Jan 27, 2012	Male	
DUBLIN	Oct 18, 2009	Male	

Parent Info

Reported By	<u>In ZIMS</u>	Parent Info	Type / Probability	Birth Date	<u>Comments</u>
BEKESBRNE	Yes	18411770 [DUBLIN / A7M006]	Dam/100%	Nov 04, 2002	
BEKESBRNE	Yes	8807994 [DUBLIN / A4M036]	Sire/100%	Nov 24, 2001	
DUBLIN	Yes	18411770 [DUBLIN / A7M006]	Dam/100%	Nov 04, 2002	
DUBLIN	Yes	23378707 [DUBLIN / A4M038]	Sire/50%	Nov 02, 2002	
DUBLIN	Yes	8807994 [DUBLIN / A4M036]	Sire/50%	Nov 24, 2001	

Ancestry Information (calculated by Species360 from shared data)

No. Identified Ancestors % Pedigree Known % Pedigree Certain **Taxonomic Inconsistencies** 56.25% 56.25% Yes-Subspecies level

Death Information

Specimen Report: 27577683 | Local ID: LYMPNE / P21349

Printed: Aug 08, 2022 03:04 Species360 ZIMS version 2.25.5 Reported By LYMPNE

Manner of Death Natural/Non-euthanasia

Death Date Jun 23, 2014

Death In Transit No Primary Body System Affected Necropsy Topology Necropsy Etiological Relevant Death Information

Carcass Disposition

Specimen Report: 27577683 | Local ID: LYMPNE / P21349

Printed: Aug 08, 2022 03:04 Species360 ZIMS version 2.25.5

Family CITES

African hunting dog

Species360 27577687

GAN

Lycaon pictus

Studbooks EAZA, WAZA, PAAZA, AZA, ZAA

Order Carnivora

IUCN Endangered (EN)

Start Date Jan 01, 1800 **End Date** Aug 08, 2022 Copyright, Species360, 2022. All rights reserved.

No Local Data Differences Found

Basic Animal Information Sex - Contraception Male -

Oct 18, 2009 - 11Y,1M,1D +/-1D Preferred ID

Status

LYMPNE / P21350

Dead

Local ID: LYMPNE / P21350

Canidae

at the time of death

Dublin Zoo - Zoological Society of Rearing

Parent

Ireland **Birth Type**

Captive Birth/Hatch

Hybrid Status Not Hybrid 18411770 (DUBLIN / A7M006)

<u>Sire</u> **MULTIPLE** <u>Dam</u>

Current Collection Main Institution Animal Collection Collection Trip Clutch / Litter

Enclosure

Visit History

Birthdate - Age

Origin

Date in	Acquisition - Vendor/Local ID	<u>Phy</u>	<u>Own</u>	Reported By	Disposition - Recipient/Local ID	<u>Phy</u>	<u>Own</u>	Date Out
Oct 18, 2009	Birth/Hatch	In	In	DUBLIN / A9M050	Donation To BEKESBRNE/H21204	Out	Out	Jan 26, 2012
Jan 27, 2012	Donation From DUBLIN/A9M050	In	In	BEKESBRNE / H21204	Donation To LYMPNE/P21350	Out	Out	Nov 22, 2013
Nov 22, 2013	Donation From BEKESBRNE/H21204	In	In	LYMPNE / P21350	Loan Out To AALBORG/UNDETERM+	Out	-	Jul 14, 2017
Jul 14, 2017	Loan In From Sender: LYMPNE/P21350 Vendor: LYMPNE/P21350	In	-	AALBORG / LYC77	Death	Out	-	Nov 19, 2020
		_	_	LYMPNE / P21350	Death (Ownership Only)	_	Out	Nov 19 2020

Identifiers

Reported By	Effective Date	<u>Type</u>	<u>Identifier</u>	Location	<u>Status</u>	Comments
AALBORG	Jul 14, 2017	Local ID	LYC77		Active	
LYMPNE	Jan 24, 2017	House Name	Chobe		Active	
LYMPNE	Nov 22, 2013	Local ID	P21350		Active	
BEKESBRNE	Jan 27, 2012	Local ID	H21204		Active	
BEKESBRNE	Jan 27, 2012	Regional Studbook Number	EAZA/T9708		Active	Legacy SLocation: EAZA Legacy Comment:
BEKESBRNE	Jan 27, 2012	Transponder	956000001009124		In-Use	
BEKESBRNE	Jan 27, 2012	House Name	Chobe		Active	
DUBLIN	May 10, 2011	Regional Studbook Number	T9708		Active	
DUBLIN	Feb 23, 2010	House Name	Chobe		Active	Legacy SLocation: Legacy Comment: Reserve in Africa
DUBLIN	Jan 07, 2010	Transponder	956000001009124		In-Use	
DUBLIN	Oct 18, 2009	Local ID	A9M050		Active	
EAZA	Oct 18, 2009	Regional Studbook Number	EAZA/7664		Active	Studbook: Lycaon pictus
WAZA	Oct 18, 2009	Intl Stdbk#	7664		Active	Studbook: Lycaon pictus

Sex Information

Reported By	<u>Date</u>	<u>Sex</u>	Comments
AALBORG	Jul 14, 2017	Male	
LYMPNE	Nov 22, 2013	Male	
BEKESBRNE	Jan 27, 2012	Male	
DUBLIN	Oct 18, 2009	Male	

Parent Info

Reported By	In ZIMS	Parent Info	Type / Probability	Birth Date	<u>Comments</u>
BEKESBRNE	Yes	18411770 [DUBLIN / A7M006]	Dam/100%	Nov 04, 2002	
BEKESBRNE	Yes	8807994 [DUBLIN / A4M036]	Sire/100%	Nov 24, 2001	
DUBLIN	Yes	18411770 [DUBLIN / A7M006]	Dam/100%	Nov 04, 2002	
DUBLIN	Yes	23378707 [DUBLIN / A4M038]	Sire/50%	Nov 02, 2002	
DUBLIN	Yes	8807994 [DUBLIN / A4M036]	Sire/50%	Nov 24, 2001	

Ancestry Information (calculated by Species360 from shared data)

% Pedigree Known % Pedigree Certain **Taxonomic Inconsistencies** No. Identified Ancestors 56.25% 56.25% Yes-Subspecies level 47

Death Information

Specimen Report: 27577687 | Local ID: LYMPNE / P21350

Printed: Aug 08, 2022 02:48 Species360 ZIMS version 2.25.5 Reported ByAALBORGManner of DeathEuthanasia, medicalDeath DateNov 19, 2020

Death In Transit No Primary Body System Affected Necropsy Topology
Necropsy Etiological
Relevant Death Information

<u>Carcass Disposition</u> Incinerated

Reported By LYMPNE

Manner of DeathEuthanasia, medicalDeath DateNov 19, 2020Death In TransitNo

Primary Body System Affected

Necropsy Topology
Necropsy Etiological
Relevant Death Information

<u>Carcass Disposition</u> Incinerated

Specimen Report: 27577687 | Local ID: LYMPNE / P21350

Printed: Aug 08, 2022 02:48 Species360 ZIMS version 2.25.5

Species360 27577685

GAN

Order

Lycaon pictus

African hunting dog

Studbooks EAZA, WAZA,

Oct 18, 2009 - 8Y,9M,21D +/-1D Preferred ID

PAAZA, AZA, ZAA Carnivora

IUCN Endangered (EN) **Family** Canidae

CITES

Start Date Jan 01, 1800 **End Date** Aug 08, 2022

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No Local Data Differences Found

Basic Animal Information

Sex - Contraception Male -Dead **Status**

at the time of death

LYMPNE / P21477

Parent

Local ID: LYMPNE / P21477

Dublin Zoo - Zoological Society of Rearing

Ireland

Captive Birth/Hatch **Hybrid Status** Not Hybrid

<u>Sire</u> **MULTIPLE** 18411770 (DUBLIN / A7M006) <u>Dam</u>

Current Collection Main Institution Animal Collection Collection Trip Clutch / Litter **Enclosure**

Visit History

Birthdate - Age

<u>Origin</u>

Birth Type

Date in	Acquisition - Vendor/Local ID	<u>Phy</u>	<u>Own</u>	Reported By	Disposition - Recipient/Local ID	Phy Owr	n Date Out
Oct 18, 2009	Birth/Hatch	In	In	DUBLIN / A9M048	Donation To BEKESBRNE/H21203	Out Out	Jan 26, 2012
Jan 27, 2012	Donation From DUBLIN/A9M048	In	In	BEKESBRNE / H21203	Loan Out To DORTMUND/051456	Out -	Jul 25, 2013
Jul 25, 2013	Loan In From Sender: BEKESBRNE/H21203 Vendor: BEKESBRNE/H21203	In	-	DORTMUND / 051456	Loan Transfer To LYMPNE/P21477	Out -	Dec 18, 2014
		-	-	BEKESBRNE / H21203	Loan Out To (Change in Reported Holder) LYMPNE/P21477		Dec 18, 2014
Dec 18, 2014	Loan Transfer From Sender: DORTMUND/051456 Vendor: BEKESBRNE/H21203	In	-	LYMPNE / P21477	Physical Transfer To LONDON RP/G01803	Out -	Jul 22, 2015
		-	-	BEKESBRNE / H21203	Donation To (Ownership Only) LONDON RP/G01803	- Out	Jul 22, 2015
Jul 22, 2015	Donation From Sender: LYMPNE/P21477 Vendor: BEKESBRNE/H21203	In	In	LONDON RP / G01803	3 Death	Out Out	Aug 08, 2018

Identifiers

Reported By	Effective Date	<u>Type</u>	<u>Identifier</u>	Location	<u>Status</u>	Comments
LONDON RP	Aug 08, 2018	Death Number	ZM775/18		Active	
LONDON RP	Jul 22, 2015	Local ID	G01803		Active	
LONDON RP	Jun 22, 2015	House Name	KRUGER		Active	
LYMPNE	Dec 18, 2014	Local ID	P21477		Active	
DORTMUND	Jul 25, 2013	Local ID	051456		Active	
BEKESBRNE	Jan 27, 2012	Local ID	H21203		Active	
BEKESBRNE	Jan 27, 2012	Regional Studbook Number	EAZA/T9706		Active	Legacy SLocation: EAZA Legacy Comment:
BEKESBRNE	Jan 27, 2012	House Name	Kruger		Active	
BEKESBRNE	Jan 27, 2012	Transponder	956000001013696	•	In-Use	
DUBLIN	May 10, 2011	Regional Studbook Number	T9706		Active	
DUBLIN	Feb 23, 2010	House Name	Kruger		Active	Legacy SLocation: Legacy Comment: Reserve in Africa
DUBLIN	Jan 07, 2010	Transponder	956000001013696	;	In-Use	
DUBLIN	Oct 18, 2009	Local ID	A9M048		Active	
EAZA	Oct 18, 2009	Regional Studbook Number	EAZA/7662		Active	Studbook: Lycaon pictus
WAZA	Oct 18, 2009	Intl Stdbk#	7662		Active	Studbook: Lycaon pictus
LONDON RP	Jul 22, 2015	Transponder	956000001013696		In-Use	

Sex Information

Reported By	<u>Date</u>	<u>Sex</u>	<u>Comments</u>
LONDON RP	Jul 22, 2015	Male	
LYMPNE	Dec 18, 2014	Male	
DORTMUND	Jul 25, 2013	Male	
BEKESBRNE	Jan 27, 2012	Male	
DUBLIN	Oct 18, 2009	Male	

Parent Info

Specimen Report: 27577685 | Local ID: LYMPNE / P21477

Printed: Aug 08, 2022 03:06 Species360 ZIMS version 2.25.5 Port Lympne Wild Animal Park

Page: 1 of 2

Reported By	In ZIMS	Parent Info	Type / Probability	Birth Date	Comments
BEKESBRNE	Yes	18411770 [DUBLIN / A7M006]	Dam/100%	Nov 04, 2002	
BEKESBRNE	Yes	8807994 [DUBLIN / A4M036]	Sire/100%	Nov 24, 2001	
DUBLIN	Yes	18411770 [DUBLIN / A7M006]	Dam/100%	Nov 04, 2002	
DUBLIN	Yes	23378707 [DUBLIN / A4M038]	Sire/50%	Nov 02, 2002	
DUBLIN	Yes	8807994 [DUBLIN / A4M036]	Sire/50%	Nov 24, 2001	
Ancestry Inforr	nation (calcu	lated by Species360 from shared of	data)		
% Pedigree Know	<u>vn</u>	% Pedigree Certain	Taxonomic Inc	onsistencies	No. Identified Ancestors
56.25%		56.25%	Yes-Subspecies	s level	47

Specimen Report: 27577685 | Local ID: LYMPNE / P21477

Species360 27577682

GAN

Lycaon pictus

Local ID: LYMPNE / P21478

Studbooks EAZA, WAZA, African hunting dog

PAAZA, AZA, ZAA Carnivora

Order **IUCN** Endangered (EN) **Family CITES**

Jan 01, 1800 **End Date** Aug 08, 2022

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No Local Data Differences Found

Basic Animal Information

Sex - Contraception Male -Oct 18, 2009 - 10Y,8M,7D +/-1D Preferred ID

<u>Status</u> Dead

at the time of death

Start Date

LYMPNE / P21478

Canidae

Dublin Zoo - Zoological Society of Rearing Ireland

Parent

Not Hybrid

Birth Type

Captive Birth/Hatch **MULTIPLE**

Hybrid Status

<u>Dam</u>

18411770 (DUBLIN / A7M006)

Current Collection

Birthdate - Age

<u>Origin</u>

<u>Sire</u>

Main Institution Animal Collection Collection Trip

Enclosure

Visit History

Clutch / Litter

Data in	Acquisition - Vendor/Local ID	Dhy	O	Papartad By	Dianosition Posinient/Legal ID	Dhy Own	Doto Out
<u>Date in</u>	Acquisition - Vendon/Local ID	FIII	OWI	Reported By			Date Out
Oct 18, 2009	Birth/Hatch	ln	In	DUBLIN / A9M045	Donation To BEKESBRNE/H21201	Out Out	Jan 26, 2012
Jan 27, 2012	Donation From DUBLIN/A9M045	In	In	BEKESBRNE / H21201	Loan Out To DORTMUND/051457	Out -	Jul 25, 2013
Jul 25, 2013	Loan In From Sender: BEKESBRNE/H21201 Vendor: BEKESBRNE/H21201	In	-	DORTMUND / 051457	Loan Transfer To LYMPNE/P21478	Out -	Dec 18, 2014
		-	-	BEKESBRNE / H21201	Loan Out To (Change in Reported Holder) LYMPNE/P21478		Dec 18, 2014
Dec 18, 2014	Loan Transfer From Sender: DORTMUND/051457 Vendor: BEKESBRNE/H21201	In	-	LYMPNE / P21478	Physical Transfer To LONDON RP/G01804	Out -	Jul 22, 2015
		-	-	BEKESBRNE / H21201	Donation To (Ownership Only) LONDON RP/G01804	- Out	Jul 22, 2015
Jul 22, 2015	Donation From Sender: LYMPNE/P21478 Vendor: BEKESBRNE/H21201	In	In	LONDON RP / G01804	Death	Out Out	Jun 25, 2020

Identifiers

Reported By	Effective Date	<u>Type</u>	<u>Identifier</u>	Location	<u>Status</u>	Comments
LONDON RP	Jun 30, 2020	Death Number	ZM515/20		Active	
LONDON RP	Jul 22, 2015	Local ID	G01804		Active	
LONDON RP	Jul 22, 2015	House Name	SELOUS		Active	
LYMPNE	Dec 18, 2014	Local ID	P21478		Active	
DORTMUND	Jul 25, 2013	Local ID	051457		Active	
BEKESBRNE	Jan 27, 2012	Transponder	956000002053881		In-Use	
BEKESBRNE	Jan 27, 2012	House Name	Selous		Active	
BEKESBRNE	Jan 27, 2012	Local ID	H21201		Active	
BEKESBRNE	Jan 27, 2012	Regional Studbook Number	EAZA/T9703		Active	Legacy SLocation: EAZA Legacy Comment:
DUBLIN	May 10, 2011	Regional Studbook Number	T9703		Active	
DUBLIN	Feb 23, 2010	House Name	Selous		Active	Legacy SLocation: Legacy Comment: Reserve in Africa
DUBLIN	Jan 07, 2010	Transponder	956000001011906	3	In-Use	
WAZA	Oct 18, 2009	Intl Stdbk#	7659		Active	Studbook: Lycaon pictus
DUBLIN	Oct 18, 2009	Local ID	A9M045		Active	
EAZA	Oct 18, 2009	Regional Studbook Number	EAZA/7659		Active	Studbook: Lycaon pictus
DUBLIN	Jan 26, 2012	Transponder	956000002053881		In-Use	(SM) Current transponder not detected when anaesthetised for move to Howlett's so this transponder inserted.
LONDON RP	Jul 22, 2015	Transponder	956000002053881		In-Use	

Sex Information

Reported By	<u>Date</u>	<u>Sex</u>	Comments
LONDON RP	Jul 22, 2015	Male	
LYMPNE	Dec 18, 2014	Male	
DORTMUND	Jul 25, 2013	Male	
BEKESBRNE	Jan 27, 2012	Male	

Specimen Report: 27577682 | Local ID: LYMPNE / P21478

Printed: Aug 08, 2022 03:05 Species360 ZIMS version 2.25.5

Reported By	<u>Date</u>	<u>Sex</u>		<u>Comments</u>	
DUBLIN	Oct 18, 20	009 Male			
Parent Info					
Reported By	In ZIMS	Parent Info	Type / Probability	Birth Date	Comments
BEKESBRNE	Yes	18411770 [DUBLIN / A7M006]	Dam/100%	Nov 04, 2002	
BEKESBRNE	Yes	8807994 [DUBLIN / A4M036]	Sire/100%	Nov 24, 2001	
DUBLIN	Yes	18411770 [DUBLIN / A7M006]	Dam/100%	Nov 04, 2002	
DUBLIN	Yes	8807994 [DUBLIN / A4M036]	Sire/50%	Nov 24, 2001	
DUBLIN	Yes	23378707 [DUBLIN / A4M038]	Sire/50%	Nov 02, 2002	
Ancestry Inform	nation (calcu	lated by Species360 from shared	data)		
% Pedigree Know	<u>vn</u>	% Pedigree Certain	Taxonomic Inc	consistencies	No. Identified Ancestors
56.25%		56.25%	Yes-Subspecie	s level	47

Species360 MIG12-29371056

GAN

Lycaon pictus

EAZA, WAZA,

African hunting dog

Studbooks Carnivora

PAAZA, AZA, ZAA

Order **IUCN** Endangered (EN) **Family**

Canidae

Local ID: LYMPNE / P21479

CITES

Start Date Jan 01, 1800 **End Date** Aug 08, 2022

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No Local Data Differences Found

Basic Animal Information

Sex - Contraception Female -Oct 27, 2010 - 6Y,1M,12D at the Preferred ID

Status

Dead

time of death

LYMPNE / P21479

<u>Origin</u> Zoo Duisburg gGmbH

Captive Birth/Hatch

Rearing **Hybrid Status** Parent Not Hybrid MIG12-18594391 (DUISBURG /

<u>Sire</u> 5999)

MIG12-29370812 (DUISBURG /

5389)

Current Collection Clutch / Litter

Birthdate - Age

Birth Type

Main Institution Animal Collection Collection Trip

Enclosure

Visit History

<u>Date in</u>	Acquisition - Vendor/Local ID	<u>Phy</u>	<u>Own</u>	Reported By	<u> Disposition - Recipient/Local ID</u>	Phy Own	Date Out
Oct 27, 2010	Birth/Hatch	In	In	DUISBURG / 6169	Trade To DORTMUND/UNK	Out Out	Mar 28, 2013
Mar 28, 2013	Trade From DUISBURG/6169	In	In	DORTMUND / 051455	Donation To LYMPNE/P21479	Out Out	Dec 18, 2014
Dec 18, 2014	Donation From DORTMUND/051455	In	In	LYMPNE / P21479	Donation To LONDON RP/G01805	Out Out	Jul 22, 2015
Jul 22, 2015	Donation From LYMPNE/P21479	In	In	LONDON RP / G01805	5 Death	Out Out	Dec 09, 2016

Identifiers

Reported By	Effective Date	<u>Type</u>	<u>Identifier</u>	Location	<u>Status</u>	Comments
LONDON RP	Dec 09, 2016	Death Number	ZM1786/16		Active	
LONDON RP	Jul 22, 2015	Local ID	G01805		Active	
LYMPNE	Dec 18, 2014	Local ID	P21479		Active	
DORTMUND	Mar 28, 2013	House Name	Branca		Active	
DORTMUND	Mar 28, 2013	Local ID	051455		Active	
DUISBURG	Feb 09, 2011	Transponder	276096909069309)	In-Use	Legacy SLocation: left shoulder Legacy Comment:
DUISBURG	Oct 27, 2010	Local ID	6169		Active	
WAZA	Oct 27, 2010	Intl Stdbk#	7743		Active	Studbook: Lycaon pictus
EAZA	Oct 27, 2010	Regional Studbook Number	EAZA/7743		Active	Studbook: Lycaon pictus
LONDON RP	Jul 22, 2015	Transponder	276096909069309)	In-Use	

Sex Information

Reported By	<u>Date</u>	<u>Sex</u>	<u>Comments</u>
LONDON RP	Jul 22, 2015	Female	
LYMPNE	Dec 18, 2014	Female	
DORTMUND	Mar 28, 2013	Female	
DUISBURG	Oct 27, 2010	Female	

Parent Info

Reported By	In ZIMS	Parent Info	Type / Probability	Birth Date	Comments
DUISBURG	Yes	MIG12-18594391 [DUISBURG / 5389]	Dam/100%	Jun 05, 2006	
DUISBURG	Yes	MIG12-29370812 [DUISBURG / 5999]	Sire/100%	Nov 04 2007	

Ancestry Information (calculated by Species360 from shared data)

% Pedigree Known	% Pedigree Certain	Taxonomic Inconsistencies	No. Identified Ancestors
18.75%	18.75%	Yes-Subspecies level	35

Specimen Report: MIG12-29371056 | Local ID: LYMPNE / P21479

Species360 12092364

GAN

Lycaon pictus pictus

African hunting dog

Studbooks EAZA, WAZA,

PAAZA, AZA, ZAA

Order Carnivora

Endangered (EN)

Family

Canidae

Local ID: LYMPNE / P96018

CITES

IUCN Start Date Jan 01, 1800

End Date

Aug 08, 2022

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No Local Data Differences Found

Basic Animal Information

Status Sex - Contraception Female -Dead

> May 06, 1995 - 13Y,5M,5D at the Preferred ID time of death

The Anne van Dyk Cheetah

Rearing

LYMPNE / P96018

Centre

Parent

Birth Type Captive Birth/Hatch <u>Sire</u>

MIG12-28304482 (PRET DW /

Hybrid Status

Not Hybrid MIG12-28304483 (PRET DW /

D92565)

<u>Dam</u>

D92568)

Current Collection

Main Institution Animal Collection Collection Trip

Clutch / Litter

Birthdate - Age

Origin

Enclosure

Visit History

Date in Acquisition - Vendor/Local ID Phy Own Reported By Disposition - Recipient/Local ID Phy Own Date Out May 06, 1995 PRET DW / D92628 Sale LYMPNE/P96018 Out Out May 10, 1996 Birth/Hatch In May 10, 1996 Purchase PRET DW/D92628 LYMPNE / P96018 Death Out Out Oct 11, 2008

Identifiers

Reported By	Effective Date	<u>Type</u>	<u>Identifier</u>	Location	Status .	<u>Comments</u>
LYMPNE	May 20, 1996	Transponder	F79		In-Use	
LYMPNE	May 10, 1996	Local ID	P96018		Active	
PRET DW	Mar 02, 1996	Transponder	00-013B-14F5		In-Use	Legacy SLocation: Legacy Comment: TROVAN
WAZA	May 06, 1995	Intl Stdbk#	3057		Active	Studbook: Lycaon pictus
PRET DW	May 06, 1995	Intl Stdbk#	3057		Active	
PRET DW	May 06, 1995	House Name	F079		Active	
EAZA	May 06, 1995	Regional Studbook Number	EAZA/3057		Active	Studbook: Lycaon pictus
ZAA	May 06, 1995	Regional Studbook Number	ZAA/3057		Active	Studbook: Lycaon pictus
PRET DW	May 06, 1995	Local ID	D92628		Active	
LYMPNE	May 06, 1995	Regional Studbook Number	EAZA/3057		Active	Legacy SLocation: EAZA Legacy Comment:
LYMPNE	May 06, 1995	House Name	MASAI		Active	

Sex Information

Reported By	<u>Date</u>	<u>Sex</u>	<u>Comments</u>	
LYMPNE	May 10, 1996	Female		
PRET DW	May 06, 1995	Female		

Parent Info

Reported By	<u>In ZIMS</u>	Parent Info	Type / Probability	Birth Date	<u>Comments</u>
LYMPNE	Yes	MIG12-28304483 [PRET DW / D92568]	Dam/100%	Jan 01, 1992	
LYMPNE	Yes	MIG12-28304482 [PRET DW / D92565]	Sire/100%	Jan 01, 1990	
PRET DW	Yes	MIG12-28304483 [PRET DW / D92568]	Dam/100%	Jan 01, 1992	
PRET DW	Yes	MIG12-28304482 [PRET DW / D92565]	Sire/100%	Jan 01, 1990	

Ancestry Information (calculated by Species360 from shared data)

% Pedigree Known No. Identified Ancestors % Pedigree Certain Taxonomic Inconsistencies 100.00% 100.00% No

Death Information

LYMPNE Reported By **Necropsy Topology** Undetermined **Manner of Death** Undetermined **Necropsy Etiological** Unknown **Death Date** Oct 11, 2008 Relevant Death Information Undetermined **Carcass Disposition** Undetermined **Death In Transit**

Primary Body System Affected

Body Part Institution Recipient ID **Date Sent Date Received Genetic Results**

Specimen Report: 12092364 | Local ID: LYMPNE / P96018

Printed: Aug 08, 2022 02:52 Species360 ZIMS version 2.25.5

Species360 7859433

GAN

Lycaon pictus pictus

African hunting dog

Studbooks EAZA, WAZA, PAAZA, AZA, ZAA

Order Carnivora

Family

Canidae

Local ID: LYMPNE / P97062

IUCN Endangered (EN) **Start Date**

CITES Jan 01, 1800 **End Date**

Aug 08, 2022

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Basic Animal Information Local Data Differences

Status 1983/PRET DW Sex - Contraception Male -Dead Sire Sep 28, 1995 - 12Y,9M,27D at the Preferred ID LYMPNE / P97062 **Dam** Birthdate - Age

time of death

The Anne van Dyk Cheetah

Parent Rearing

913/PRET DW

Origin

Centre

Birth Type <u>Sire</u>

MIG12-28304530 (PRET DW /

Hybrid Status Not Hybrid <u>Dam</u>

MIG12-28304268 (PRET DW /

D92564)

D92486)

Current Collection Main Institution Animal Collection Collection Trip

Captive Birth/Hatch

Clutch / Litter **Enclosure**

Visit History

Phy Own Reported By Date in Acquisition - Vendor/Local ID Disposition - Recipient/Local ID Phy Own Date Out In PRET DW / D92689 Sep 28, 1995 Birth/Hatch Sale LYMPNE/P97062 Out Out Dec 18, 1997 Dec 18, 1997 Purchase PRET DW/D92689 LYMPNE / P97062 Death Out Out Jul 25, 2008

Identifiers

Reported By	Effective Date	<u>Type</u>	<u>ldentifier</u>	<u>Location</u>	<u>Status</u>	<u>Comments</u>
LYMPNE	Sep 25, 1998	Transponder	00-01D9-OAD6		In-Use	
LYMPNE	Jan 19, 1998	House Name	BENGUELA		Active	
LYMPNE	Dec 18, 1997	Local ID	P97062		Active	
PRET DW	Dec 18, 1997	Transponder	00-000D-2BF2		In-Use	Legacy SLocation: L.HIP Legacy Comment: NEW TROVAN TRANSPONDER
PRET DW	Dec 15, 1997	Intl Stdbk#	3081		Active	
PRET DW	Dec 15, 1997	Transponder	00-0142-B9AD		In-Use	Legacy SLocation: L.HIP Legacy Comment: TROVAN - LOST
LYMPNE	Dec 15, 1997	Transponder	00 01 42 B9 AD T /00 00 OD 2B F2 T		In-Use	
PRET DW	Sep 28, 1995	Local ID	D92689		Active	
LYMPNE	Sep 28, 1995	Regional Studbook Number	EAZA/3083		Active	Legacy SLocation: EAZA Legacy Comment:
EAZA	Sep 28, 1995	Regional Studbook Number	EAZA/3083		Active	Studbook: Lycaon pictus
PRET DW	Sep 28, 1995	House Name	M106		Active	
WAZA	Sep 28, 1995	Intl Stdbk#	3083		Active	Studbook: Lycaon pictus

Sex Information

Reported By	<u>Date</u>	<u>Sex</u>	<u>Comments</u>
LYMPNE	Dec 18, 1997	Male	
PRET DW	Sep 28, 1995	Male	

Parent Info

Reported By	In ZIMS	Parent Info	Type / Probability	Birth Date	Comments
LYMPNE	No	PRET DW/913	Dam/100%		
LYMPNE	No	PRET DW/1983	Sire/100%		
PRET DW	Yes	MIG12-28304268 [PRET DW / D92486] Dam/100%	Oct 27, 1990	
PRFT DW	Yes	MIG12-28304530 [PRFT DW / D92564	1 Sire/100%	Sep 08 1989	

Ancestry Information (calculated by Species 360 from shared data)

No. Identified Ancestors % Pedigree Known % Pedigree Certain Taxonomic Inconsistencies

Death Information

Reported By LYMPNE
Manner of Death Undetermined
Death Date Jul 25, 2008

Death In Transit No Primary Body System Affected Necropsy TopologyUndeterminedNecropsy EtiologicalUnknownRelevant Death InformationUndeterminedCarcass DispositionUndetermined

Body Part Institution Recipient ID Date Sent Date Received Genetic Results

Species360 9405583

GAN

Order

Lycaon pictus pictus

African hunting dog

Studbooks EAZA, WAZA,

PAAZA, AZA, ZAA

Carnivora **Family** Canidae

CITES

IUCN Endangered (EN)

Start Date Jan 01, 1800 **End Date** Aug 08, 2022

Status

Copyright, Species360, 2022. All rights reserved.

Basic Animal Information Local Data Differences

Birthdate - Age Jul 31, 1996 - 8Y,3M,5D at the **Preferred ID**

Dead LYMPNE / P97063

Local ID: LYMPNE / P97063

Sire <u>Dam</u> **1984/PRET DW 3015/PRET DW**

The Anne van Dyk Cheetah **Origin**

Sex - Contraception Female -

Centre

Rearing Parent

time of death

Captive Birth/Hatch

Hybrid Status Not Hybrid

<u>Sire</u> D92566)

MIG12-28304884 (PRET DW / <u>Dam</u> MIG12-28304401 (PRET DW /

D92621)

Current Collection

Main Institution Animal Collection Collection Trip

Enclosure

Visit History

Clutch / Litter

Birth Type

Date in Acquisition - Vendor/Local ID Phy Own Reported By Disposition - Recipient/Local ID Phy Own Date Out PRET DW / D92771 Jul 31, 1996 Sale LYMPNE/P97063 Birth/Hatch Out Out Dec 18, 1997 Dec 18, 1997 Purchase PRET DW/D92771 LYMPNE / P97063 Death Out Out Nov 05, 2004

Identifiers

Reported By **Effective Date Identifier Status Comments** Type Location LYMPNE Sep 25, 1998 Transponder 00-01DF-2465 In-Use Active I YMPNF Jan 19, 1998 House Name **ASHANTI** LYMPNE Dec 18, 1997 Local ID P97063 Active PRET DW Sep 30, 1997 Transponder 00-0144-B5 D1 In-Use Legacy SLocation: Legacy Comment: TROVAN LYMPNE Sep 30, 1997 00 01 44 B5 D1 T In-Use Transponder PRET DW Jul 31, 1996 Regional PAAZA/3124 Active Legacy SLocation: PAAZAB Studbook Number Legacy Comment: APPLIED FOR "International Studbook Number" from ARKS 2 records WA7A Jul 31, 1996 Intl Stdbk# Active 3124 Studbook: Lycaon pictus PRET DW Jul 31, 1996 Local ID D92771 Active PRET DW Jul 31, 1996 House Name F131 Active LYMPNE Jul 31, 1996 Regional EAZA/3124 Active Legacy SLocation: EAZA Studbook Number Legacy Comment: **EAZA** Jul 31, 1996 Regional EAZA/3124 Active Studbook: Lycaon pictus Studbook Number

Sex Information

Reported By Comments <u>Date</u> <u>Sex</u> LYMPNE Dec 18, 1997 Female

PRET DW Jul 31, 1996 Female

Parent Info

In ZIMS Parent Info Type / Probability Birth Date Reported By Comments I YMPNF PRET DW/3015 Dam/100% No LYMPNE No **PRET DW/1984** Sire/100% MIG12-28304401 [PRET DW / D92621] Dam/100% PRET DW Yes Dec 14, 1994 MIG12-28304884 [PRET DW / D92566] Sire/100% May 21, 1992 PRET DW Yes

Ancestry Information (calculated by Species 360 from shared data)

% Pedigree Known % Pedigree Certain **Taxonomic Inconsistencies** No. Identified Ancestors

100.00% 100.00%

Death Information

LYMPNE Reported By Respiratory Necropsy Topology **New Growths Manner of Death** Euthanasia, medical **Necropsy Etiological** Nov 05, 2004 **Death Date Relevant Death Information** Undetermined

Sent Out (Part or Whole Body) **Death In Transit Carcass Disposition**

Primary Body System Affected

Body Part Institution **Date Sent** Recipient ID **Date Received Genetic Results EDIN MUS**

Specimen Report: 9405583 | Local ID: LYMPNE / P97063

Printed: Aug 08, 2022 02:54

Port Lympne Wild Animal Park Page: 1 of 1 Re: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Lamberson, Amanda M <amanda_lamberson@fws.gov>

Thu 8/18/2022 1:32 PM

To: Jen Powers < jhb19@cornell.edu>

Hi Jen,

I can give you another 45 days from the date of 08/07/2022 which would be 09/21/22. Were the animals listed in the excel sheet born at Port Lympne Safari Park? If so, I will need an updated signed statement from Jane Hopper including an updated table of animals, sample quantities from each, and total quantity to be imported. I see that a species360 report was also provided for animal P20048. This animal was not listed so please include this animal on the updated table if samples from this animal will be imported. Lastly, a species360 report is still needed for animal H20236. Thank you.

Kind regards,

Amanda Lamberson

From: Jen Powers <jhb19@cornell.edu> Sent: Thursday, August 18, 2022 8:38 AM

To: Lamberson, Amanda M <amanda_lamberson@fws.gov>

Subject: RE: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Hi Amanda,

I have reached out to the PI and it sounds like she has the additional information. I will submit it in FWS. How do I request an extension, if needed?

Thank You, Jen

Jennifer H. Powers
Supervisor, Virology Laboratory
Animal Health Diagnostic Center
New York State Veterinary Diagnostic Laboratory
Cornell University
jhb19@cornell.edu

Phone: 607-253-3900 Phone: 607-253-4458

From: Lamberson, Amanda M <amanda lamberson@fws.gov>

Sent: Thursday, August 18, 2022 8:27 AM **To:** Jen Powers <jhb19@cornell.edu>

Subject: Re: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Good morning Jen,

I am emailing to remind you that the 45 day deadline has been exceeded. Please provide the 360 reports or request an extension if required. Thank you.

Kind regards,

Amanda Lamberson

Re: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Lamberson, Amanda M <amanda_lamberson@fws.gov>

Mon 9/26/2022 3:46 PM

To: Jen Powers <jhb19@cornell.edu>

Good afternoon Jen,

Apologies for the delayed reply. Thank you for the clarification and the updated forms. That should be everything I need. If I do have any further questions I will let you know.

Kind regards,

Amanda Lamberson

From: Jen Powers <jhb19@cornell.edu>
Sent: Tuesday, September 20, 2022 1:42 PM

To: Lamberson, Amanda M <amanda_lamberson@fws.gov>

Subject: RE: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Hello Amanda.

Circling back to the application to make sure we are all set with everything now. I believe the deadline is tomorrow. Please let me know if you need anything else.

Thank You, Jen

Jennifer H. Powers Supervisor, Virology Laboratory Animal Health Diagnostic Center New York State Veterinary Diagnostic Laboratory Cornell University jhb19@cornell.edu

Phone: 607-253-3900 Phone: 607-253-4458

From: Jen Powers

Sent: Tuesday, September 13, 2022 8:45 AM

To: Lamberson, Amanda M <amanda_lamberson@fws.gov>

Subject: RE: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Hi Amanda,

Here is additional information from the PI:

- (1) As requested, I enclose an updated list of animals, sample quantities from each, and total quantity.
- (2) As requested, I also enclose an updated letter from Jane Hopper (Jane this is the same letter sent previously, with a new date!)
- (3) Apologies for the confusion over animal P20048. This ID number is the Port Lympne "local ID" listed on the Species360 Report. But if you look carefully you can see H20236 listed as the local ID of the same animal when it was loaned to Safaripark Beekse Bergen (see the "Disposition" column in the row relating to 5 Oct 2000). So, the "extra" Species360 Report for P20048 is the "missing" report for H20236. To reduce confusion, I have renamed this animal in the updated list.

Is this everything you need?

Thank You, Jen

Jennifer H. Powers Supervisor, Virology Laboratory Animal Health Diagnostic Center New York State Veterinary Diagnostic Laboratory Cornell University jhb19@cornell.edu

Phone: 607-253-3900 Phone: 607-253-4458

From: Lamberson, Amanda M < amanda lamberson@fws.gov >

Sent: Thursday, August 18, 2022 1:33 PM **To:** Jen Powers < jhb19@cornell.edu>

Subject: Re: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Hi Jen,

I can give you another 45 days from the date of 08/07/2022 which would be 09/21/22. Were the animals listed in the excel sheet born at Port Lympne Safari Park? If so, I will need an updated signed statement from Jane Hopper including an updated table of animals, sample quantities from each, and total quantity to be imported. I see that a species360 report was also provided for animal P20048. This animal was not listed so please include this animal on the updated table if samples from this animal will be imported. Lastly, a species360 report is still needed for animal H20236. Thank you.

Kind regards,

Amanda Lamberson

From: Jen Powers < jhb19@cornell.edu>
Sent: Thursday, August 18, 2022 8:38 AM

To: Lamberson, Amanda M < <u>amanda_lamberson@fws.gov</u>>

Subject: RE: [EXTERNAL] RE: Additional Information Required - FWS Application CS0082219

Hi Amanda.

I have reached out to the PI and it sounds like she has the additional information. I will submit it in FWS. How do I request an extension, if needed?

Thank You, Jen

Jennifer H. Powers Supervisor, Virology Laboratory Animal Health Diagnostic Center New York State Veterinary Diagnostic Laboratory Cornell University

jhb19@cornell.edu Phone: 607-253-3900 Phone: 607-253-4458



Vet Dept
Port Lympne Reserve
Aldington Road
Lympne
Nr. Ashford
Kent
CT21 4PD

Telephone: 01303 234175

13 September 2022

Dear Sir

Cornell University Import Permit Application

Cornell University is applying for permission to import blood samples collected from animals born in captivity in the United Kingdom.

I confirm details of these samples as follows

- a Scientific name: Lycaon pictus
- b Common name: African wild dog
- Name and address of the facility where the animal was bred and born:
 Port Lympne Safari Park, Port Lympne Reserve, Hythe, Kent, CT21 4PD, UK.
 A minority of animals were captive bred at other institutions and their places of birth are recorded in the enclosed list (Species 360 reports have been provided separately).
- d Birth date: Listed in the table overleaf
- e Identification information: Listed in the table overleaf
- f Name and address of facility where the parental stock is located: Port Lympne Reserve, Port Lympne Reserve, Hythe, Kent, CT21 4PD, UK.

As Head of Veterinary Services for the Howletts Wild Animal Trust, which owns and runs Port Lympne Reserve, I confirm that the information is correct.

Yours faithfully,

MM

Jane Hopper

Table – Details of the identities of all African wild dogs represented in the set of samples to be exported. Addresses of places of birth: A SafariPark Beekse Bergen, 5081 NJ Hilvarenbeek, Netherlands; B Port Lympne Safari Park, Port Lympne Reserve, Hythe, Kent, CT21 4PD, U.K.; C Dublin Zoo, Phoenix Park, Dublin 8, D08 AC98, Ireland; D Warsaw Zoo, Ratuszowa 1/3, 03-461 Warszawa, Poland; E Friguia Animal Park, GP 1, Aïn Rahma, 4089, Bouficha, Tunisia; F West Midland Safari Park, Spring Grove, Bewdley DY12 1LF, U.K.; G Zoo de Pont Scorff, All. de Kerruisseau, 56620 Pont-Scorff, France; H Ree Park Safari, Stubbe Søvej 15, 8400 Ebeltoft, Denmark; I Zoo Duisburg, Mülheimer Str. 273, 47058 Duisburg, Germany; J Ann Van Dyk Cheetah Centre, R513, Brits, North West Province, 0251 South Africa.

ID number	name/number	place of birth	date of birth	number of vials
P20048	Mzungu/B790	А	08-Jan-98	2
H20237	Kassama/17AB	В	01-Mar-97	4
H20612	Rafiki	В	22-Nov-93	1
H20944	Two Socks/594302	В	04-Dec-06	5
H20946	Blade/599654	В	04-Dec-06	3
P21478	Selous/053881	С	18-Oct-09	3
P21349	Ruaha	С	17-Oct-09	5
P20047	Tsenga	Α	08-Jan-98	3
P20053	Kippa/8DF0	В	13-Nov-00	1
P20077	Shue/B25E	В	13-Nov-00	2
P20078	Rhunt/A079/97F6	В	13-Nov-00	1
P20079	Krane/D481	В	13-Nov-00	4
P20080	Depti/B94F	В	13-Nov-00	1
P20082	Spot/E1AD	В	13-Nov-00	3
P20169	Tatu	D	17-Oct-04	1
P20170	Wili	D	17-Oct-04	5
P20520	Tunis	E	15-Sep-02	1
P20572	NA	В	04-Nov-05	1
P20573	Nyae Nyae/424326	В	04-Nov-05	10
P20574	Whitey/44543	В	04-Nov-05	1
P20680	Sandy/593476	В	04-Nov-05	2
P20684	Tango/599146	В	04-Dec-06	1
	•		04-Dec-06	2
P20686	Blacky/595132	В		1
P20688	NA 05000000/847617	B B	00-Jan-00	2
P20789	956000000/847617		04-Nov-07	3
P20790	Spot-tail	В	04-Nov-07	
P20791	Bandy	В	04-Nov-07	6
P20793	Teye/751	В	04-Nov-07	4
P20796	Tanny	В	04-Nov-07	5
P20799	Pirate/352	В	04-Nov-07	2
P20911	Vango	F	27-Oct-05	7
P21050	Snake/4749	В	24-Nov-01	4
P21052	Domino/E182	В	24-Nov-01	2
P21053	/000606BC5C	В	24-Nov-01	2
P21055	Neleh/FC90	В	24-Nov-01	1
P21056	Eva/88FF	В	24-Nov-01	1
P21057	E03F/0A9F	В	24-Nov-01	2
P21263	Socks	В	10-Nov-12	1
P21264	Scorpion	В	10-Nov-12	3
P21265	Splodge	В	10-Nov-12	2
P21266	Romeo	В	10-Nov-12	4
P21267	Mantler	В	10-Nov-12	2
P21268	Chevron	В	10-Nov-12	2
P21269	Kudu	В	10-Nov-12	4
P21301	Zuri	G	05-Jun-06	1
P21348	Nadifa	Н	12-Nov-11	4
P21350	Chobe	С	18-Oct-09	4
P21360	Ghost	В	15-Dec-13	3
P21361	Two spot	В	15-Dec-13	3
P21464	Sprench	В	12-Nov-14	2
P21465	Flash	В	12-Nov-14	1
P21467	Gecko	В	12-Nov-14	1
P21469	Madi	В	12-Nov-14	3
P21470	Five	В	12-Nov-14	1
P21471	Horseshoe	В	12-Nov-14	1
P21477	Kruger	C	18-Oct-09	3
P21479	Branka	I	27-Oct-10	1
P21480	NA	В	29-Dec-14	2
. 21 100		_		_

ID	name/number	place of	date of	number
number		birth	birth	of vials
P21482	NA	В	29-Dec-14	1
P21483	NA	В	29-Dec-14	1
P21484	NA	В	29-Dec-14	2
P21485	NA	В	29-Dec-14	1
P21486	NA	В	29-Dec-14	1
P21487	NA	В	29-Dec-14	1
P21489	Ace	В	27-Nov-14	1
P21491	Icarus	В	27-Nov-14	2
P21492	Kamana	В	27-Nov-14	1
P21493	Kite	В	27-Nov-14	1
P21494	Cross	В	27-Nov-14	1
P21495	Sickle	В	27-Nov-14	1
P21496	Comma	В	27-Nov-14	1
P21590	Lenny	В	27-Dec-15	1
P21594	S V/Tooth	B B	27-Dec-15	1
P21598	V/Tooth NA	В	27-Dec-15 05-Nov-20	2
P21990 P21994	Threespot	В	05-Nov-20	3
P21994 P21995	Strike	В	05-Nov-20	3
P21996	Widget	В	05-Nov-20	3
P21990 P21997	Shroom	В	05-Nov-20	3
P21997	Zand	В	05-Nov-20	3
P21999	Diamond	В	05-Nov-20	3
P22045	Assegai/B50D	В	02-Nov-02	2
P22049	Falcon/82F1	В	02-Nov-02	2
P22050	Yella/EB07	В	02-Nov-02	2
P22051	Kenya/138E	В	02-Nov-02	2
P22052	Nora/140/1105	В	02-Nov-02	2
P22053	/0006201E4A/141	В	02-Nov-02	2
P22054	Lessa/142/B6AC	В	02-Nov-02	2
P22055	/0006202304/143	В	02-Nov-02	2
P22056	Psyche/02A00	В	02-Nov-02	1
P22057	Saddle/0E42/145	В	02-Nov-02	1
P22059	Bibi/94FE/146	В	02-Nov-02	2
P22070	Flecks	В	05-Nov-20	3
P22071	Kwenna	В	05-Nov-20	3
P22072	Stomp	В	05-Nov-20	3
P22085	/0006205B85	В	02-Nov-02	2
P96018	Masai	J	06-May-95	2
P97005	Kassanga/sccsc	В	01-Mar-97	1
P97011	Kassala/1E18	В	01-Mar-97	1
P97062	Ben(guela)	J	28-Sep-95	3
P97063	Ashanti/2465	J	31-Jul-96	1
P98044	Kang/E1F60	В	15-Jun-98	3
P98047	Tchad/4CF2	В	15-Jun-98	1
P22143	Wicks	В	3-Jan-22	2
P22144	NA	В	3-Jan-22	2
P22145	Spyro	В	3-Jan-22	2
P22146	NA	В	3-Jan-22	2
P22147	Skunk	В	3-Jan-22	2
P22148	NA	В	3-Jan-22	2
P22149	NA	В	3-Jan-22	2
P22150	NA	В	3-Jan-22	2
P22151	NA	В	3-Jan-22	2
P22152	NA	В	3-Jan-22	2
P22153	NA	В	3-Jan-22	2
P22154	NA Decreale	В	3-Jan-22	2
P22155	Bunda	B	3-Jan-22	2
Total anima	als: 117	Iotal	samples:	261

Annex 7d — Details of the identities of all African wild dogs represented in the set of samples to be exported. Addresses of places of birth: A SafariPark Beekse Bergen, 5081 NJ Hilvarenbeek, Netherlands; B Port Lympne Safari Park, Port Lympne Reserve, Hythe, Kent, CT21 4PD, U.K.; C Dublin Zoo, Phoenix Park, Dublin 8, D08 AC98, Ireland; D Warsaw Zoo, Ratuszowa 1/3, 03-461 Warszawa, Poland; E Friguia Animal Park, GP 1, Aïn Rahma, 4089, Bouficha, Tunisia; F West Midland Safari Park, Spring Grove, Bewdley DY12 1LF, U.K.; G Zoo de Pont Scorff, All. de Kerruisseau, 56620 Pont-Scorff, France; H Ree Park Safari, Stubbe Søvej 15, 8400 Ebeltoft, Denmark; I Zoo Duisburg, Mülheimer Str. 273, 47058 Duisburg, Germany; J Ann Van Dyk Cheetah Centre, R513, Brits, North West Province, 0251 South Africa.

ID number	name/number	place of birth	date of birth	number of vials		ID number	name/number	place of birth	date of birth	number of vials
P20048	Mzungu/B790	А	08-Jan-98	2		P21482	NA	В	29-Dec-14	1
H20237	Kassama/17AB	В	01-Mar-97	4		P21483	NA	В	29-Dec-14	1
H20612	Rafiki	В	22-Nov-93	1		P21484	NA	В	29-Dec-14	2
H20944	Two Socks/594302	В	04-Dec-06	5		P21485	NA	В	29-Dec-14	1
H20946	Blade/599654	В	04-Dec-06	3		P21486	NA	В	29-Dec-14	1
P21478	Selous/053881	С	18-Oct-09	3		P21487	NA	В	29-Dec-14	1
P21349	Ruaha	С	17-Oct-09	5		P21489	Ace	В	27-Nov-14	1
P20047	Tsenga	Α	08-Jan-98	3		P21491	Icarus	В	27-Nov-14	2
P20053	Kippa/8DF0	В	13-Nov-00	1		P21492	Kamana	В	27-Nov-14	1
P20077	Shue/B25E	В	13-Nov-00	2		P21493	Kite	В	27-Nov-14	1
P20078	Rhunt/A079/97F6	В	13-Nov-00	1		P21494	Cross	В	27-Nov-14	1
P20079	Krane/D481	В	13-Nov-00	4		P21495	Sickle	В	27-Nov-14	1
P20080	Depti/B94F	В	13-Nov-00	1		P21496	Comma	В	27-Nov-14	1
P20082	Spot/E1AD	В	13-Nov-00	3		P21590	Lenny	В	27-Dec-15	1
P20169	Tatu	D	17-Oct-04	1		P21594	S	В	27-Dec-15	1
P20170	Wili	D	17-Oct-04	5		P21598	V/Tooth	В	27-Dec-15	1
P20520	Tunis	E	15-Sep-02	1		P21990	NA	В	05-Nov-20	2
P20572	NA Name Name (42.4226	В	04-Nov-05	1		P21994	Threespot	В	05-Nov-20	3
P20573	Nyae Nyae/424326	В	04-Nov-05	10		P21995	Strike	В	05-Nov-20	3
P20574	Whitey/44543	В	04-Nov-05	1		P21996	Widget	В	05-Nov-20	3
P20680	Sandy/593476	В	04-Dec-06	2		P21997	Shroom	В	05-Nov-20	3
P20684	Tango/599146	В	04-Dec-06	1		P21998	Zand	В	05-Nov-20	3
P20686	Blacky/595132	В	04-Dec-06	2		P21999	Diamond	В	05-Nov-20	3
P20688	NA	В	00-Jan-00	1		P22045	Assegai/B50D	В	02-Nov-02	2
P20789	956000000/847617	В	04-Nov-07	2		P22049	Falcon/82F1	В	02-Nov-02	2
P20790	Spot-tail	В	04-Nov-07	3		P22050	Yella/EB07	В	02-Nov-02	2
P20791	Bandy	В	04-Nov-07	6		P22051	Kenya/138E	В	02-Nov-02	2
P20793	Teye/751	В	04-Nov-07	4		P22052	Nora/140/1105	В	02-Nov-02	2
P20796	Tanny	В	04-Nov-07	5		P22053	/0006201E4A/141	В	02-Nov-02	2
P20799	Pirate/352	B F	04-Nov-07	2		P22054	Lessa/142/B6AC	В	02-Nov-02	2
P20911	Vango Snake/4749		27-Oct-05	7		P22055	/0006202304/143	В	02-Nov-02	2
P21050 P21052	Domino/E182	B B	24-Nov-01 24-Nov-01	4 2		P22056 P22057	Psyche/02A00 Saddle/0E42/145	B B	02-Nov-02 02-Nov-02	1 1
P21052 P21053	/000606BC5C	В	24-Nov-01 24-Nov-01	2		P22057	Bibi/94FE/146	В	02-Nov-02	2
P21055 P21055	Neleh/FC90	В	24-Nov-01 24-Nov-01	1		P22039	Flecks	В	05-Nov-20	3
P21056	Eva/88FF	В	24-Nov-01	1		P22071	Kwenna	В	05-Nov-20	3
P21057	E03F/0A9F	В	24-Nov-01	2		P22071	Stomp	В	05-Nov-20	3
P21263	Socks	В	10-Nov-12	1		P22085	/0006205B85	В	02-Nov-02	2
P21264	Scorpion	В	10-Nov-12	3		P96018	Masai	J	06-May-95	2
P21265	Splodge	В	10-Nov-12	2		P97005	Kassanga/sccsc	В	01-Mar-97	1
P21266	Romeo	В	10-Nov-12	4		P97011	Kassala/1E18	В	01-Mar-97	1
P21267	Mantler	В	10-Nov-12	2		P97062	Ben(guela)	J	28-Sep-95	3
P21268	Chevron	В	10-Nov-12	2		P97063	Ashanti/2465	J	31-Jul-96	1
P21269	Kudu	В	10-Nov-12	4		P98044	Kang/E1F60	В	15-Jun-98	3
P21301	Zuri	G	05-Jun-06	1		P98047	Tchad/4CF2	В	15-Jun-98	1
P21348	Nadifa	Н	12-Nov-11	4		P22143	Wicks	В	3-Jan-22	2
P21350	Chobe	C	18-Oct-09	4		P22144	NA	В	3-Jan-22	2
P21360	Ghost	В	15-Dec-13	3		P22145	Spyro	В	3-Jan-22	2
P21361	Two spot	В	15-Dec-13	3		P22146	NA	В	3-Jan-22	2
P21464	Sprench	В	12-Nov-14	2		P22147	Skunk	В	3-Jan-22	2
P21465	Flash	В	12-Nov-14	1		P22148	NA	В	3-Jan-22	2
P21467	Gecko	В	12-Nov-14	1		P22149	NA	В	3-Jan-22	2
P21469	Madi	В	12-Nov-14	3		P22150	NA	В	3-Jan-22	2
P21470	Five	В	12-Nov-14	1		P22151	NA	В	3-Jan-22	2
P21471	Horseshoe	В	12-Nov-14	1		P22152	NA	В	3-Jan-22	2
P21477	Kruger	C	18-Oct-09	3		P22153	NA	В	3-Jan-22	2
P21479	Branka	1	27-Oct-10	1		P22154	NA	В	3-Jan-22	2
P21480	NA	В	29-Dec-14	2		P22155	Bunda	В	3-Jan-22	2
P21481	NA	В	29-Dec-14	2	l	Total anin			l samples:	261