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

■New ☐Reissue/Renew ☐Amendment

Complete Sections **A or B**, and C, D, and E of this application. U.S. address may be required in Section C.**

A. Complete if applying as an individual

1.a. Last name	1.b. First name	1.c. Middle name or initial	1.d. Suffix

2 Date of birth (mm/dd/yyyy)	5.a. Telephone number	5.b. Alternate telephone number	6. E-mail address

B. Complete if applying on behalf of a business, corporation, public agency, Tribe, or institution

1.a. Name of business, agency, Tribe, or institution	1.b. Doing business as (dba)
New York State Veterinary Diagnostic Laboratory/Animal Health Diagnostic Center, Cornell University	

2. Tax identification no.	3.a. Description of business, agency, Tribe, or institution	3.b. Website URL (if applicable)
15-0532082	Veterinary Diagnostic Laboratory	https://www.vet.cornell.edu/animal-health-diagnostic-center

4.a. Principal officer (P.O.) last name	4.b. P.O. first name	4.c. P.O. middle initial	4.b. P.O. Title
Elvinger	François		AHDC Executive Director

5. Primary contact name	6. Primary e-mail address
Dr. Diego Diel	dgdiel@cornell.edu

7.a. Business telephone number	7.b. Alternate phone no.	8.a. Primary contact telephone no.
607-253-3900		

C. All applicants complete address information

1.a. Physical address (Street address; Apartment #, Suite #, or Room #; no P.O. Boxes)
240 Farrier Road

1.b. City	1.c. State	1.d. Zip code/Postal code	1.e. County/Province	1.f. Country
Ithaca	NY	14853	Tompkins	USA

2.a. Mailing Address (include if different than physical address; include name of contact person if applicable)

2.b. City	2.c. State	2.d. Zip code/Postal code	2.e. County/Province	2.f. Country

D. All applicants MUST complete

1. Include a check or money order, payable to the U.S. FISH AND WILDLIFE SERVICE, **a nonrefundable processing fee** [50 CFR 13.11(d)(4)]. Federal, Tribal, State, and local government agencies, and those acting on behalf of such agencies, are exempt from the processing fee – ***attach documentation of fee exempt status as outlined in instructions.*** (50 CFR 13.11(d))

2. If you are requesting a reissue/renew/amendment, what is your permit/file number? NA

3. Certification: I hereby certify that I have read and am familiar with the regulations contained in Title 50, Part 13 of the Code of Federal Regulations and the other applicable parts in subchapter B of Chapter I of Title 50, and I certify that the information submitted in this application for a permit is complete and accurate to the best of my knowledge and belief. I understand that any false statement herein may subject me to the criminal penalties of 18 U.S.C. 1001.

François Elvinger

Digitally signed by François Elvinger
Date: 2023.10.25 09:17:55 -04'00'

The individual/principal officer of the business must print and sign the application. (No photocopied or stamped signatures) Date (mm/dd/yyyy)

** Further instructions for the above application may be found on our ePermits website. See the last page for information on the Privacy Act, Paperwork Reduction Act, Estimated Burden, and Freedom of Information Act aspects of this application form.

Mail your application(s) to Division of Management Authority, Branch of Permits, MS:IA 5275 Leesburg Pike, Falls Church, VA 22041-3803.

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 list of CITES species	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 3-200-52

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

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

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

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.

   NYS Veterinary Diagnostic Laboratory/AHDC Cornell University
   240 Farrier Road, Ithaca, NY 14853

2. Point of contact if we have questions about the application (name, phone number, and email).

   Jennifer Powers, 607-253-4458, jhb19@cornell.edu

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:	State Veterinary Services Laboratory
Address:	Leopard street
City:	Skukuza
State/Province:	Kruger National Park Mpumalanga
Postal Code:	1350
Country:	South Africa

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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: Mmadi Mogolodi B. Reuben
Address: 71 Suni Road,
City: Skukuza
State/Province: Kruger National Park
Mpumalanga
Postal Code: 1350
Country: South Africa

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
Lycaon pictus	African wild dog	64 serum samples; 64 nas	Wild	02/2023 to 09/202	serum in cryovials; frozen dry
Lycaon pictus	African wild dog	32 serum samples	Wild	02/2024 to 05/202	serum in cryovials
				g. TOTAL # of all samples in the shipment:	Shipment 1: 192 samples Shipment 2: 32 samples

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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;
    b. 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:
   i. 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 list of designated ports (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 Resolution Conf. 5.10 (Rev CoP15).
- 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.

OCKERT LOUIS VAN SCHALKWYK

Contact

Address:

[redacted]

Phone:

[redacted]

Email:

[redacted]

Bio

[redacted]

Languages

[redacted]

Skills highlights

Problem solver
Epidemiology
Wildlife immobilisation
Animal tracking
Spatial analyses
R Programming

[redacted]

ORCID profile
Google Scholar

Summary

Wildlife veterinarian with experience in wildlife disease surveillance & investigations as well as chemical immobilisation and physical restraint of African wildlife. Strong skills in spatial epidemiology and risk analysis. Fluent in R Statistical language, in particular spatial methods and data visualisation. Experienced in setting up laboratories in remote locations and ISO17025 Standard. Special interest in animal & bird of prey tracking and movement analysis.

Education

- Bachelor of Veterinary Science: 2002, University of Pretoria
- Master of Science (*cum laude*): 2004, University of Pretoria
- Doctor of Philosophy: 2015, University of Pretoria

Employment

National Department of Agriculture, Land Reform & Rural Development:

- ***State Veterinarian**, Kruger National Park, South Africa*

March 2012 – present

University of Pretoria:

- **Research Station Manager**, Hans Hoheisen Research Station, Centre for Veterinary Wildlife Studies, Orpen, Kruger National Park

January 2010 – February 2012

- **Lecturer**, Hans Hoheisen Research Station, Centre for Veterinary Wildlife Studies, Orpen, Kruger National Park

June 2008 – February 2012

- **Clinical assistant**, Department of Production Animal Studies, Faculty of Veterinary Science

January 2004 – September 2004

Peace Parks Foundation, Trans-frontier Conservation Area Veterinary Programme (TFCA-VP):

- **Coordinator** (Peace Parks Foundation)**:** TFCA-VP

June 2007 – May 2008

- **Veterinarian:** TFCA-VP

September 2004 – May 2007
(based at Hans Hoheisen Research Station from May 2005)

Affiliations

Max Planck Society:

- **Affiliated Scientist:** Institute of Animal Behaviour

November 2020 – present

University of Pretoria:

- **Extraordinary lecturer:** Department of Veterinary Tropical Diseases

January 2021 – present

Achievements

- Pat Fletcher Wild Dog Conservation Award, Endangered Wildlife Trust, 2019
- National Individual Award in recognition of 'spearheading the largest wild dog conservation and research project in the history of the Kruger National Park', Wildlife and Environment Society of South Africa, 2019
- Managing Executive Special Award, Kruger National Park, 2018 & 2019

Professional Membership & Participation

- *South African Veterinary Council:* Registered member (D02/4511)
- *Wild Dog Advisory Group:* Veterinary Group chair
- *International Conference on Animal Health Surveillance 2022* Scientific Committee
- *Elephants Alive Ethics Committee* Member
- *Anthrax Advisory Group:* Convener/Chairman
- *South African Society for Veterinary Epidemiology & Preventive Medicine:* Member
- *Bovine Tuberculosis Study Group:* Member
- *South African Veterinary Foundation:* Director (2012 – 2014)
- *Contemplate Wild non*-profit organisation Founder & Director

Selected Publications

Full list available at *https://orcid.org/0000-0003-4365-4904*

C MEIRING, H SCHURZ, P VAN HELDEN, E HOAL, G TROMP, C KINNEAR, L KLEYNHANS, B GLANZMANN, **OL VAN SCHALKWYK**, M MILLER, M MÖLLER. 2022. African wild dogs (*Lycaon pictus*) from the Kruger National Park, South Africa, are not inbred but have low genomic diversity. *Scientific Reports 12:14979*. DOI: 10.1038/s41598-022-19025-7

K KOEPPEL, P GEERTSMA, B KUHN, **OL VAN SCHALKWYK**, P THOMPSON. 2022. Antibody response to Raboral VR-G® oral rabies vaccine in captive and free-ranging black-backed jackals (*Canis mesomelas*). *Onderstepoort Journal of Veterinary Research 89(1), a1975.* DOI: 10.4102/ojvr.v89i1.1975

KN KOEPPEL, **OL VAN SCHALKWYK**, PN THOMPSON. 2022. Patterns of rabies cases in South Africa between 1993-2019, including the role of wildlife. *Transboundary and Emerging Diseases 69:836–848.* DOI: 10.1111/tbed.14080

C MARNEWECK, **OL VAN SCHALKWYK**, DG MARNEWECK, G BEVERLEY, HT DAVIES-MOSTERT, DM PARKER. 2021. Reproductive state influences the degree of risk tolerance for a seasonally breeding mesopredator. *Behavioral Ecology* 32(4):717–727. DOI: 10.1093/beheco/arab018

C MEIRING, R HIGGITT, W GOOSEN, **OL VAN SCHALKWYK**, L DE KLERK-LORIST, P BUSS, P VAN HELDEN, SDC PARSONS, M MÖLLER, M MILLER. 2021. Shedding of *Mycobacterium bovis* in respiratory secretions of free-ranging wild dogs (*Lycaon pictus*): Implications for intraspecies transmission. *Transboundary and Emerging Diseases*. 2021 Apr. DOI: 10.1111/tbed.14125

EC NETHERLANDS, C STROEBEL, LH DU PREEZ, N SHABANGU, PT MATJILA, **OL VAN SCHALKWYK**, BL PENZHORN. 2021. Molecular confirmation of high prevalence of speciesof Hepatozoon infection in free-ranging African wild dogs (*Lycaon pictus*) in the Kruger National Park, South Africa, 2021. *International Journal for Parasitology: Parasites and Wildlife:* In Press. DOI: 10.1016/j.ijppaw.2021.03.002

N SHABANGU, BL PENZHORN, M OOSTHUIZEN, I VOSTER, **OL VAN SCHALKWYK**, R HARRISON-WHITE, PT MATJILA. 2021. A shared pathogen: *Babesia rossi* in domestic dogs, black-backed jackals (*Canis mesomelas*) and African wild dogs (*Lycaon pictus*) in South Africa. *Veterinary Parasitology*. S0304-4017(21)00041-8. DOI: 10.1016/j.vetpar.2021.109381.

C MEIRING, R HIGGITT, A DIPPENAAR, E ROOS, P BUSS, J HEWLETT, D COOPER, P ROGERS, L DE KLERK-LORIST, **OL VAN SCHALKWYK**, G HAUSLER, P VAN HELDEN, M MÖLLER, R WARREN, M MILLER. 2020. Characterizing epidemiological and genotypic features of *Mycobacterium bovis* infection in wild dogs (*Lycaon pictus*). *Transboundary and Emerging Diseases* 00:1–10. DOI: 10.1111/tbed.13947

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*. DOI: 10.1007/s00442-019-04512-y

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).* DOI: 10.7589/2018-03-089

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. *Mycobacterium bovis* infection in African Wild Dogs, Kruger National Park, South Africa. *Emerging Infectious Diseases* 25(7):1425-1427. DOI: 10.3201/eid2507.181653

Post graduate supervision & refereeing

Supervisor

M Reuben: *PhD* (ongoing): .

J Steenkamp: *MMedVet(Fer)* (2013): Predictive ecological suitability modeling for anthrax in the Kruger National Park, South Africa.
https://repository.up.ac.za/handle/2263/23358

Co-supervisor

KN Koeppel: *PhD* (2021): Spatiotemporal analysis of rabies in South Africa, the role of black-backed jackals (*Canis mesomelas*) and aspects of its control by oral rabies vaccination.

O Pretorius: *MSc* (2019): The quantification of cattle movement in the Bushbuckridge Local Municipality, Mpumalanga, and implications for trade and disease control.
https://repository.up.ac.za/handle/2263/76769

D Lazarus: *MSc* (2014): Improved FMD vaccination schedules in the GLTP interface.
https://repository.up.ac.za/handle/2263/69253

I Rossouw: *MSc* (2011): The intra- and inter-population relatedness of bovine tuberculosis-infected and -uninfected African buffaloes (Syncerus caffer caffer) in the Kruger National Park.
https://repository.up.ac.za/handle/2263/25714

External Examiner

HJ Swanepoel: *MSc* (2020): A scoping review of viral diseases in African ungulates. Department of Veterinary Tropical Diseases, University of Pretoria.

L Gaudex: *MSc* (2014): A health and demographic surveillance system of cattle on communal rangelands in Bushbuckridge, South Africa: baseline census and population dynamics over 12 months. Department of Veterinary Tropical Diseases, University of Pretoria.

M Broekman: *MSc* (2011): Detection of hyperthermia during capture in wild antelope. School of Physiology, University of Witwatersrand.

J Sawicka: *MSc* (2010): Cooling methods to treat capture-induced hyperthermia in Blesbok (*Damaliscus dorcas phillipsi*). School of Physiology, University of Witwatersrand.

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

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

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

☐New ☐Reissue/Renew ☐Amendment

Complete Sections **A or B**, and C, D, and E of this application. U.S. address may be required in Section C.**

A. Complete if applying as an individual

1.a. Last name	1.b. First name	1.c. Middle name or initial	1.d. Suffix

2 Date of birth (mm/dd/yyyy)	5.a. Telephone number	5.b. Alternate telephone number	6. E-mail address

B. Complete if applying on behalf of a business, corporation, public agency, Tribe, or institution

1.a. Name of business, agency, Tribe, or institution	1.b. Doing business as (dba)
New York State Veterinary Diagnostic Laboratory/Animal Health Diagnostic Center, Cornell University	

2. Tax identification no.	3.a. Description of business, agency, Tribe, or institution	3.b. Website URL (if applicable)
15-0532082	Veterinary Diagnostic Laboratory	https://www.vet.cornell.edu/animal-health-diagnostic-center

4.a. Principal officer (P.O.) last name	4.b. P.O. first name	4.c. P.O. middle initial	4.b. P.O. Title
Elvinger	François		AHDC Executive Director

5. Primary contact name	6. Primary e-mail address
Dr. Diego Diel	dgdiel@cornell.edu

7.a. Business telephone number	7.b. Alternate phone no.	8.a. Primary contact telephone no.
607-253-3900		

C. All applicants complete address information

1.a. Physical address (Street address; Apartment #, Suite #, or Room #; no P.O. Boxes)
240 Farrier Road

1.b. City	1.c. State	1.d. Zip code/Postal code	1.e. County/Province	1.f. Country
Ithaca	NY	14853	Tompkins	USA

2.a. Mailing Address (include if different than physical address; include name of contact person if applicable)

2.b. City	2.c. State	2.d. Zip code/Postal code	2.e. County/Province	2.f. Country

D. All applicants MUST complete

1. Include a check or money order, payable to the U.S. FISH AND WILDLIFE SERVICE, **a nonrefundable processing fee** [50 CFR 13.11(d)(4)]. Federal, Tribal, State, and local government agencies, and those acting on behalf of such agencies, are exempt from the processing fee – ***attach documentation of fee exempt status as outlined in instructions.*** (50 CFR 13.11(d))

2. If you are requesting a reissue/renew/amendment, what is your permit/file number?

3. Certification: I hereby certify that I have read and am familiar with the regulations contained in Title 50, Part 13 of the Code of Federal Regulations and the other applicable parts in subchapter B of Chapter I of Title 50, and I certify that the information submitted in this application for a permit is complete and accurate to the best of my knowledge and belief. I understand that any false statement herein may subject me to the criminal penalties of 18 U.S.C. 1001.

François Elvinger — 10/25/2023

The individual/principal officer of the business must print and sign the application. (No photocopied or stamped signatures) — Date (mm/dd/yyyy)

** Further instructions for the above application may be found on our ePermits website. See the last page for information on the Privacy Act, Paperwork Reduction Act, Estimated Burden, and Freedom of Information Act aspects of this application form.

Mail your application(s) to Division of Management Authority, Branch of Permits, MS:IA 5275 Leesburg Pike, Falls Church, VA 22041-3803.

8.**For each biological sample taken from an animal in the wild**, provide:

a. Scientific name (genus, species, and *if applicable*, subspecies),

Lycaon pictus

b. Common name,

African Wild Dog

c. Specific location (e.g., county, state, province, country) where the samples were taken from the wild,

Kruger National Park, Mpumalanga, South Africa

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.

Animal Immobilisation was done by Dr. Louis van Schalkwyk (SAVC reg. number: D02/4511)

Sample collection procedure done by Dr. Louis van Schalkwyk and Dr. Mmadi M. Reuben.

Authorisation documents included are,

- South African National Parks AUCC approval,
- University of Pretoria AEC approval,
- DALRRD Section 20 permit approval,
- TOPS permit.

e. Method of collection: sampling protocol, approximate length of time held in captivity, any injury and/or mortality experienced during collection, transport, or holding.

i. Immobilisation of animals

All animals were darted in the field, sampled, treated, and reversed. Free darting is done from a vehicle using Dan-Inject dart projector and Dan-inject 1.5 ml dart syringe with a barbed needle. The darting distance is 15 meters on average.

Drug combination:

Dart mix: 1.5 mg Medetomidine, 10 mg Butorphanol, 5 mg Midazolam

Reversal: Atipamezole 10 mg, Naltrexone 30 mg (iv)

The downtime was approximately 5 minutes post darting; The animals are handled for an average of 40 minutes before being reversed. Post injection of antidote, animals get to their feet within 2 – 5 minutes.

Animals generally rejoin their packs within an hour of reversal. There has not been any injuries or mortalities in the animals sampled so far (see sample collection table below)

ii. Sampling: Blood sampling is done from femoral vein using a 21" or 18" vacutainer needle. Serum tubes are used for blood collection. Swabs from the nasal sinus and rectum are collected using swab stick.

Table: Sample collection

Tranche	Pre-treatment sampling	30-day post treatment sampling	12 months post treatment sampling
1. LP-BN-04M 2. LP-BN-05M 3. LP-CS-02F 4. LP-CS-03F 5. LP-CF-02M 6. LP-CF-03M 7. LP-BJ-06F 8. LP-BJ-07M	completed	completed	pending
9. LP-BL-05F 10. LP-BL-06F 11. LP-BO-06F 12. LP-BO-07F 13. LP-CV-03F 14. LP-CV-04F 15. LP-BN-06F 16. LP-BN-07F	completed	completed	pending
17. LP-CM-02F 18. LP-CM-03M 19. LP-BS-04M 20. LP-BS-05F 21. LP-BT-02M 22. LP-BT-03F 23. LP-BF-08F 24. LP-BF-09M	completed	completed	pending
25. LP-CG-02F 26. LP-CG-03F 27. LP-CY-01F 28. LP-CY-02F 29. LP-CQ-01F 30. LP-CQ-02F 31. LP-CO-02M 32. LP-CO-03M	completed	pending	pending

f. Information related to any remuneration, either financial or in-kind, provided for acquiring the sample(s).

1. Dr. Louis van Schalkwyk is state veterinarian in Kruger National Park and an Extra-ordinary lecturer at University of Pretoria. He is the main supervisor for this academic study. He is a collaborator and not being paid for sample collection.

2. Dr. Mmadi Reuben is a PhD (vet science) candidate at the University of Pretoria, and he is doing this trial as part of his academic work.

g. Efforts to use captive specimens (e.g., captive-born, captive-held) in lieu of taking samples from wild animals.

This study is aimed at addressing canine distemper disease risk in free ranging African wild dogs; therefore, it is targeting the free ranging population to evaluate CDV vaccine safety and efficacy.

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),

Study Proposal

Hypothesis and Objectives

The study aims to assess the safety, efficacy, and the practicality of vaccinating free ranging African Wild Dogs (AWD) against canine distemper. The outcomes of the study will be to (i) provide safe, effective canine distemper vaccination protocol for free ranging African Wild Dogs and (ii) quantify impacts on extinction risk in African wild dog populations using an existing mathematical model, and then use Structured Decision Making (SDM) framework to develop a plan for management of infectious diseases in the study population (Kruger National Park). The study findings will contribute towards development of guidelines for the management of infectious diseases in free ranging African wild dogs.

Study Design and Methods

The field trial is being conducted in Kruger National Park, South Africa. It will evaluate whether free-ranging wild dogs mount a strong and protective immune response to modified live vaccine of canine distemper after a single handling event, and whether vaccinated individuals survive as well as unvaccinated pack-mates. Although the vaccine have been tested in captivity, the field study will reflect guidance on designing “first in man” trials, 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 to 22 days post-vaccination. 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 a single dose vaccine 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. 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 $\alpha=0.05$, 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 period52.
To measure 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-vaccination. We use similar methods to compare vaccine titres 6-12 months post-vaccination, providing some information on 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.

b. How long the research has been (or will be) conducted,
The field trial will last for 15 months; it will come to end in May 2024. Analysis of the results will follow thereafter and academic writeup.

c. Detailed information on sampling methods including:
- i. who will be taking the samples
- ii. equipment and methods used
- iii. measures taken to prevent injuries and mortalities during collection

See attached University of Pretoria Animal Ethics Committee application form.

d. A copy of the study's Institutional Animal Care and Use Committee (IACUC) form (*if applicable*),
Application form for AEC and approval from the University of Pretoria is attached.

e. Peer-reviewed scientific papers published from this research (*if applicable*),
No publication yet as the study is ongoing.

f. An explanation of whether similar research has already been conducted or is currently being conducted.

This is the first field trial conducted in the free ranging wild dog population to evaluate safety and efficacy of Canine Distemper virus modified live vaccine. It has the potential to advance knowledge in field vaccination of African wild dogs against canine distemper. There is currently no defined CDV vaccination protocol which can be used in case of canine distemper outbreak to protect wild dog population.

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.

Conservation Impact

Conservation managers have been using vaccination to manage canine distemper and rabies in wild dogs. Rabies vaccination is effective; vaccine safety and efficacy studies have been done to validate the procedure in wild carnivores, on the contrary there is no documented effective canine distemper vaccination protocol for free ranging wildlife. The current use of canine distemper vaccines is based on proven protective levels in domestic dogs or in wildlife species under captive environment. Previous canine distemper vaccinations in free ranging populations have not demonstrated promising results; in some cases lack of vaccine effectiveness was recognized in retrospect after vaccinated individuals succumb to canine distemper. There is an urgent need to have a protocol in place to mitigate canine distemper risks to the population in case of an outbreak.

Species conservation plans and strategies have been developed at international, regional, and national levels in response to declining numbers of African wild dogs. These conservation documents keep evolving with recognition of new threats to the species, integration of infectious disease management to the species conservation plan is now a priority since many range states have experienced disease impacts. Conservation decisions are complex as they require one to integrate scientific evidence, human values and economic factors during the decision making process. This research work will apply a decision science framework, Structured Decision Making to develop guidelines for managing infectious diseases in Kruger wild dog population.

Impact 1: Provide practical guidance to conservation managers on key considerations for field vaccination of African wild dogs against canine distemper.

Impact 2: Apply prove of concept in Structured Decision Making to develop infectious disease management plan for Kruger Wild Dog population. This will form a case study document which other conservation areas would refer to in addressing infectious diseases challenge to their wild dog populations.

Impact 3: Contribute towards development of IUCN SSC Canid specialist group guidelines in management of infectious diseases in African Wild Dogs. These will be a resource to all conservation stakeholders involved in species conservation at national and site level.

The outcomes will allow conservation managers to confidently use the developed protocol following best practice to prevent CD occurrence in wild dogs or to mitigate local species extinction risks during an ongoing CD outbreak. This will be first study ever done to evaluate field use of CDV-MLV and many conservation managers facing CDV threats in wild dogs awaits the findings of this study as there is currently no product for use during outbreak.

The African wild dog is the second most endangered canid species of the African continent after the Ethiopian wolves. The two species are closely related, and work done in either may have a direct benefit on the other. The guidelines on the Management of Infectious Diseases will benefit both species.

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.

    Dr. O. L. van Schalkwyk's CV attached.

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.

    Samples will be shipped in two batches within one year.

14. How will the samples be imported or exported (e.g., personally carried or shipped)?

    The samples will be transported by air freight using an international courier company licensed and experienced in transporting such type of cargo.

15. If personally carried, please specify the individual(s) who will be transporting the samples.

    An international air freight courier will be used to transport samples.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

13 October 2022

Approval Certificate
New Application

AEC Reference No.: REC078-22
Title: Can vaccination protect African wild dogs from canine distemper? Addressing a conservation emergency
Researcher: A/Pr RW Woodroffe
Student's Supervisor: Dr OL van Schalkwyk

Dear A/Pr RW Woodroffe,

The **New Application** as supported by documents received between 2022-08-22 and 2022-09-26 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2022-09-26.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	Number
Wild Dogs - KNP	32
Samples	Number
Blood (Samples from live animals)	96
Nasal swabs (Samples from live animals)	32
Rectal swabs (Samples from live animals)	32

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2023-10-13.

3. Please remember to use your protocol number (REC078-22) on any documents or correspondence with the AEC regarding your research.

4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.

6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

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Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

We wish you the best with your research.

Yours sincerely

Prof Andrew McKechnie
Acting Chairperson: UP-Animal Ethics Committee

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Fax +27 12 529 8321
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ANIMAL USE AND CARE COMMITTEE: APPROVAL CERTIFICATE

A. PROJECT DETAILS

Project Title	**Can vaccination protect African wild dogs from canine distemper? Addressing a conservation emergency.**		
Researcher	**R Woodroffe**	SANParks Reference No.	**03-22**

B. CONDITIONS OF APPROVAL

- There must be an approved and signed research contract with SANParks prior to implementation of this project.
- Ethics approval is valid for the duration of the SANParks research contract.
- Any changes to the original research protocol must be submitted in the appropriate format to the AUCC for evaluation and approval.
- The AUCC must be informed of mortalities or injuries beyond those expected in the approved research protocol.

Submission Date:	**17 May 2022**	APPROVED
AUCC Approval Date:	**22 August 2022 2022**	Signature:

Note: In accordance with the South African National Standard (SANS 10386-2008): "The Care and Use of Animals for Scientific Purposes", an animal is regarded as being "live, sentient non-human vertebrate, including eggs, foetuses and embryos, that is, fish, amphibians, reptiles, birds and mammals, including domestic animals, purpose-bred animals, farm animals, wildlife and higher invertebrates such as advanced members from the Cephalopoda and Decapoda".

addo elephant
agulhas
lAi-lAis / richtersveld
augrabies
bontebok
camdeboo
garden route
golden gate highlands
groenkloof
karoo
kgalakgadi transfronti
kruger
mapungubwe
marakele
mokala
mountain zebra
namaqua
table mountain
tankwa karoo
west coast

University of Pretoria
Faculty of Veterinary Sciences
Department of Veterinary Tropical Disease

Can Vaccination Protect African Wild Dogs from Canine Distemper? Addressing a Conservation Emergency.

for the degree – PhD (Veterinary science)(Veterinary Tropical Diseases)

(08260272)

Mmadi Mogolodi B. Reuben

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Cell: [redacted]

E-mail [redacted]

Supervisor: Dr. Louis van Schalkwyk

Co-supervisor: Prof. Leith Meyer

Principal Investigator: Prof. Rosie Woodroffe

Date: 10th July 2022

Can Vaccination Protect African Wild Dogs from Canine Distemper? Addressing a Conservation Emergency.

Executive Summary

The African wild dog is a globally endangered large carnivore species, with fewer than 700 packs remaining in the wild. The major causes of the species decline were historically habitat fragmentation and retaliatory killing by humans due to species predation on livestock. Canine Distemper Virus (CDV) was assumed to pose little risk to the species, because epidemiological studies in many areas within the species range had found healthy animals with antibodies to the virus, suggesting that wild dogs often survived exposure to the virus. CDV is now recognized as a re-emerging disease affecting vaccinated canine populations globally as well as expanding its wildlife host range. Wild canids, particularly the African Wild dog account for over half of the wildlife cases. Recently, six separate fatal CDV outbreaks have been recorded, with the worst wiping out the largest African Wild Dog (AWD) population in the northern hemisphere, demonstrating the emergence of CDV as an extinction risk factor for the species. The control of CDV in free ranging AWD present a challenge due to the virus' multi-hosts within a given ecosystem. Mass vaccination of domestic dogs does not adequately manage disease risk in AWD and vaccination of the species need to be considered where CDV risks are most acute.

Although species vaccination appears to be a promising disease prevention measure, there is no safe, effective, and practical CDV vaccination protocol available for use on free-ranging AWD populations. This study aims to identify such a protocol, to inform urgent conservation efforts. The study will test the hypothesis that extinction risks to African wild dog populations can be reduced by vaccination against CDV using a Modified Live Vaccine.

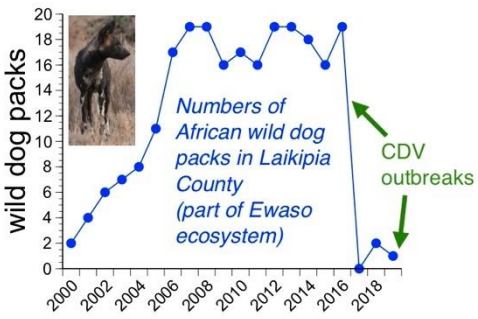
The study is designed to inform time-sensitive conservation decisions for this endangered species. Two peer-reviewed papers will be produced and the outcome will support the development of CDV management guidelines to be shared with conservationists throughout the species range in Africa. The project has enormous potential to improve both animal health and wildlife population viability. Conservation managers from Kenya

and South Africa are partners on the project, poised to implement its positive and practical recommendations as soon as they become available. The approach could also influence disease management planning process for other endangered species under in-situ conservation.

1. Introduction and literature review *

1.1 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) is a growing threat. CDV is now called canine morbillivirus. Habitat loss and deliberate killing have extirpated the species across 93% of its historic range (Woodroffe et al., 2017): climate change now compounds these threats to the less than 700 packs that remain . 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 1991 (Gascoyne et al.,1993), and several subsequent whole-pack deaths linked to rabies (Alexander et al., 2010; Flacke et al., 2013; Hofmeyr et al., 2004, 2000), led to 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 animals (Alexander et al., 2010; Berentsen et al., 2013; Creel and Creel, 1998; K. C. Prager et al., 2012; Woodroffe et al., 2012). Although sporadic whole-pack deaths were reported (Alexander et al., 1996; Goller et al., 2010), the only major confirmed outbreak was in a captive breeding center (van de Bildt et al., 2002). However, in 2016 CDV killed whole packs at three separate sites in South Africa (Du Plessis, 2016; Loots et al., 2018), 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 packs (Mutinda, 2017). 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.



Canine Distemper virus is a *Morbillivirus* belonging to the family Paramixoviradae which causes severe systemic disease in dogs (Martella et. al 2008). There has been a global increase of CDV related disease occurrence in canine populations with evidence of the virus being able to adapt to new host species (Blixenkrone-Möller et al., 1992; Martella et al., 2008). CDV has a broad host range within the mammalian species including the families Canidae, Felidae, Mustelidae, Procyonidae, Ursidae and Viverridae making the disease challenging to manage at ecosystem level. It is an enveloped virus

which is sensitive to environmental exposure. The main transmission mode is by direct animal contact and aerosol (Alexander et al., 2010; Martella et al., 2008). Various biotypes of the CDV exist owing to genetic variability of hemagglutinin H-gene. However, there is no significant antigenic variation and the available vaccines provide protection against all biotypes of the virus when administered appropriately (Tizard, 2021). Land use approach alone may not be effective at protecting endangered species from pathogen exposure, more pragmatic approaches are needed if the extinction risks for the species is to be reduced (Alexander et al., 2010).

Because CDV is primarily a canine pathogen, there have been several attempts to reduce wildlife CDV risks by vaccinating domestic dogs (Mutinda, 2017; Viana et al., 2015). However, this approach may have limited effectiveness, since;-

(i) Domestic dog populations may not act as maintenance population for CDV due to landscape factors. Although domestic dog is a known reservoir host for CDV, the population size is critical in determining whether the canine population is able to maintain the pathogen or not (Alexander et al., 2010). A CDV maintenance community may develop where a large community of interacting susceptible species within an ecosystem facilitates maintenance of the pathogen by creating effective pathogen transmission between different species (Alexander et al., 2010). Mass dog vaccination around the Serengeti reduced CDV incidence in dogs but not in wild lions (Viana et al., 2015), 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 dog (Gilbert, 2016). Disease transmission study done within the Ewaso ecosystem showed that CDV was not persisting in local domestic dogs (Prager et al., 2012), and that wild dogs with greater opportunities for domestic dog contact were not more likely to have been exposed to CDV (Woodroffe et al., 2012).

(ii) Even in situations where domestic dogs do act as CDV maintenance population, controlling infection would be challenging because CDV, like other *morbilliviruses* such as measles (Keeling and Grenfell, 1997) and phocine distemper virus(Swinton et al.,1998), may persist only on very large geographic scales, and control requires vaccination coverage of ≥95% (Rikula, 2008).

(iii) While governments are committed to eradicating dog-mediated rabies by 2030 (WHO/FAO/OIE, 2018), 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 have limited impact in reducing CDV threats to wild dogs, in some circumstances vaccination of wild dogs may need to be considered. Recurrence of canine distemper outbreaks in captive carnivores had led to some zoos vaccinating susceptible species for CDV (Sadler et al., 2016). Vaccination of free-ranging wild dogs is been widely practiced in populations where CDV risk is high, even though such protocols have not been evaluated and in some instances leading to breakthrough outbreaks in vaccinated population (Ewaso case Kenya, van Schalkwyk personal com).

1.2 Choice of CDV vaccine

Vaccines use have proved to be an effective and adequate solution in the control of dog CDV for over three decades (Chappuis, 1995). Three categories of vaccine are currently available: inactivated, modified-live, and recombinant.

Modified-live vaccines (MLVs) are highly effective in domestic dogs (Chappuis, 1995; Rikula, 2008) and where maternal antibodies have faded, a single vaccine injection has been shown to trigger protective antibodies that persist for up to three years, MLV can prompt seroconversion in captive African wild dogs (van Heerden et al., 2002). Nevertheless MLVs have occasionally induced clinical distemper in a number of nondomestic carnivores (Carpenter et al., 1976; Henke, 1977), including African wild dogs (Durchfeld et al., 1990; McCormick, 1983; van Heerden et al., 1989) and their inappropriate use nearly caused extinction of the endangered black-footed ferret (Tizard, 2021). Risks appear to be low for the species (Woodroffe, 2021), and MLVs are widely used on captive African wild dogs in Europe.

Inactivated vaccines generally give inferior protection and have been used on African wild dogs in captivity to avoid all risk of vaccine-induced distemper (Woodroffe, 2021). However, they have consistently failed to provoke protective serological responses (van Heerden et al., 2002; Visee et al., 1997), and failed to prevent CDV from killing 49 of 52 wild dogs in a captive facility in Tanzania (van de Bildt et al., 2002).

Recombinant vaccines likewise cannot induce distemper, because they do not contain a complete viral genome; they produce immunity comparable to MLVs in domestic dogs (Tizard, 2021). Such vaccines have induced seroconversion in African wild dogs (Connolly et al., 2013), and other sensitive species (Bronson et al., 2007). However, a trial in captive tigers showed that recombinant vaccines produced weaker immune responses than MLVs (Sadler et al., 2016). Moreover, use of the recombinant CDV vaccine on free-ranging wild dogs in an outbreak situation might be difficult, because the import of Genetically Modified Organisms is forbidden in some African countries and requires time-consuming permitting in others (Birhanu, 2010). Moreover, the vaccine has faced repeated supply problems (Hines, 2015; Lau, 2012).

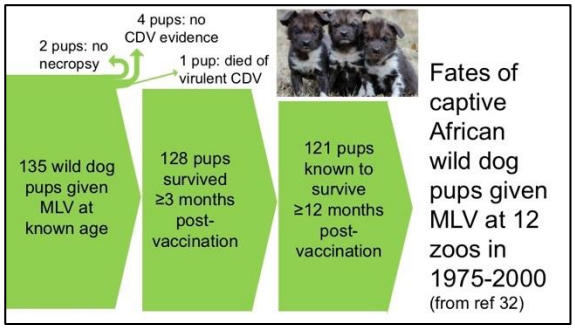
As MLV (Onderstepoort strain) appears to be immunogenic, low risk, and widely available in Africa, it is a strong candidate for use in protecting free-ranging populations of African wild dogs threatened by canine distemper. However, there is currently no established vaccination protocol suitable for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

1.3 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 vaccine (Ford et al., 2017). To ensure adequate seroconversion, doses are repeated at 2 - 4 weeks intervals until 16 weeks of age (Ford et al., 2017). Because vaccination of free-ranging wild dogs would require darting, it would have to target older animals, as darting may injure young pups. If a domestic dog receives its first vaccinations at >20 weeks, after maternal antibodies have waned, a single MLV dose is protective (Ford et al., 2017). 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 MLV (Spencer and Burroughs, 1992) or inactivated (Van Heerden et al., 2002) CDV vaccine. If a single dose proved insufficient, immune responses might be strengthened by giving multiple doses simultaneously, as in rabies control (Connolly et al., 2015; Warell et al., 1985). We anticipate that a double dose would be safe, because the dose for a 5-month pup is 2ml/15.9kg (Thomas et al., 2006) which 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 adjuvant (Neo Tech, 2009) which some have tentatively linked to adverse vaccine reactions in small domestic dog breeds (Moore et al., 2005). It may thus be helpful to evaluate both single and double doses of MLV in African wild dogs.

1.4 Preliminary Data

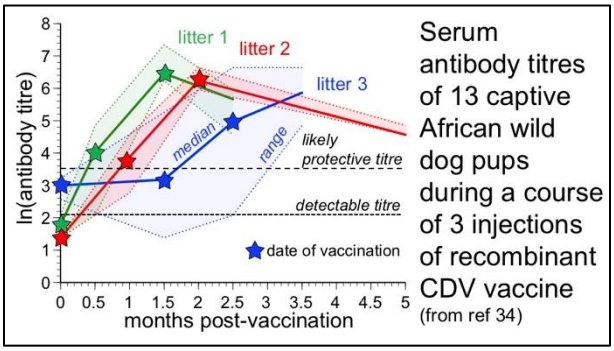
The safety of modified live CDV vaccine in captive African wild dogs have previously been evaluated by evaluating zoos' vaccination records for the period 1975-2000, and comparing individual survival using studbook data (Rhodes et al., 2007; Verberkmoes and Verberkmoes 2007). This work (Woodroffe, 2021) 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



Fates of captive African wild dog pups given MLV at 12 zoos in 1975-2000 (from ref 32)

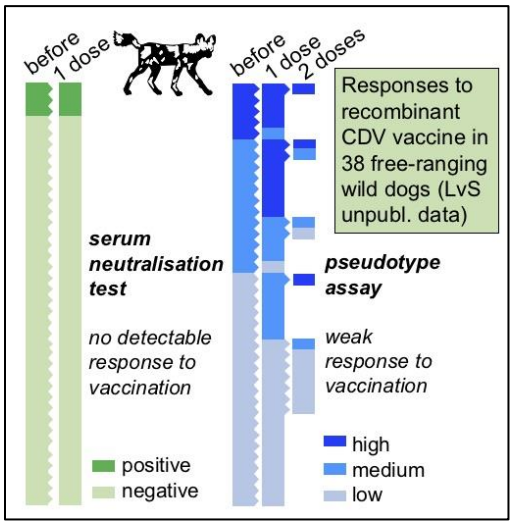
assumed to have died of vaccine-induced distemper, the risk would be 2.2% (CI 0.5-6.4%).

Evaluation of antibody responses to recombinant CDV vaccine in captive wild dog pups, showed that this vaccine is safe and immunogenic in captivity, if delivered by a parenteral route (Connolly et al., 2013). 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 doses (Connolly et al., 2013).

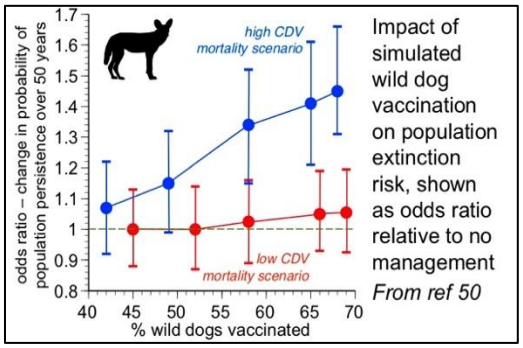


Serum antibody titres of 13 captive African wild dog pups during a course of 3 injections of recombinant CDV vaccine (from ref 34)

However, 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, 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 previous page left below). These (unpublished) data raise concerns about the utility of recombinant CDV vaccine for free-ranging wild dogs.



Nevertheless, the ZSL 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 dynamics), vaccination was associated with >40% reductions in extinction risk if CDV could cause high mortality (Smallwood, 2020; Woodroffe et al., 2019).



Impact of simulated wild dog vaccination on population extinction risk, shown as odds ratio relative to no management *From ref 50*

2. Aim(s) and objectives

2.1 Hypothesis

This study aims to test the hypothesis that canine distemper virus – modified live vaccine is safe and likely effective for use in free-ranging African wild dog populations, and is therefore likely to reduce population extinction risks.

2.2 Study objectives

The study objectives are:

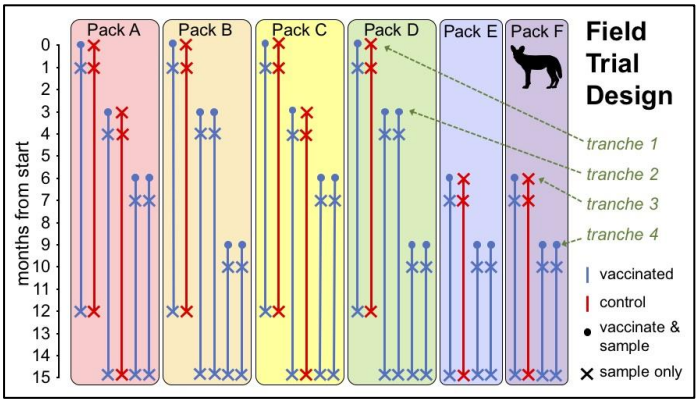
1. To work with free-ranging African Wild Dogs, to assess the safety, efficacy, and practicality of vaccination with CDV-MLV.

2. To parameterize an existing model of CDV dynamics and control using data from findings of objective 1 and vaccination work done in captive African wild dogs, to quantify impacts on extinction risks. The outcomes will be used to i. development infectious diseases management plan for Kruger wild dog population, ii. develop guidelines for CDV management in African wild dog populations through a Structured Decision Making (SDM) process.

3. Methods

3.1 Study design

The study will be conducted in Kruger National Park. 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 has already been tested in captivity, the field study will reflect guidance on designing "first in man" trials (European Medicines Agency, 2007), 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 cases of suspected (but not confirmed) vaccine-induced distemper emerged 10-22 days post-vaccination

(Woodroffe, 2021). Animals will be recruited to the trial in four tranches. For tranche 1, two animals will be darted in each of four packs, with one of each pair randomly selected to receive vaccine 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 approximately 1 month and 12 months later. Mortality shall be monitored 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 among 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 the world CDV reference laboratory at Cornell University. If CDV is detected in a vaccinated animal, vaccinations will be paused pending discussions within the team, and more broadly 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 $\alpha=0.05$, this study design (24 vaccinated and eight controls) 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 wild dog-month monitoring period (Dhand and Khatkar, 2014).

3.2 Study Setting

The study animals will be monitored within their natural habitat once they have received the allocated treatments. The animals will be captured by darting at time of first handling, one month later and 12 months later for sample collection. During darting procedure, the animal will be handled for approximately 40 minutes before being reversed and allowed to rejoin the pack which will often be within a reasonable distance.

3.3 Study population and sampling

3.3.1 Study population

The Kruger National Park wild dogs is a self-sustaining and viable population in South Africa. The study animals are born in the park, the risk of interaction with domestic dog population is only at the park boundary. The Kruger NP measures 19,623 km^2 and sustains a variety of wildlife species. The wild dogs are exposed to both intra and inter-species competition within their habitat. The main diet for the wild dog is impala, however the wild dogs can also catch larger antelope species such nyala, kudu, wildebeest, waterbuck and reedbuck.

The study will target packs which are identified to be closer to the communities and hence at a higher risk of CDV exposure. The one year old animals are preferred study subjects for a number of reasons; 1) they are easier to safely dart than the juvenile and in the event that they get injured during handling, it won't destabilise the pack, 2) they have fully competent immune system and maternally derived antibodies completely faded, 3) they are less likely to have been exposed to wild type virus strain at time of first handling.

3.3.2 Sampling method

At least six wild dog packs from the population will be selected for sampling. The selection of the packs will be based on their proximity to human settlements where possible. A pair of yearling animals will be selected from each pack for treatments at a time and a maximum of 3 pairs selected per pack. Each pair darted from a pack will be randomly allocated to a treatment (vaccination or no-vaccination) by a toss of coin.

3.3.3 Sampling size

There will be a total of 32 yearling wild dogs of mixed sex, 24 of which will be given a Modified Live Vaccine of canine distemper and 8 not vaccinated. The sample size has been determined to provide statistically significant results for vaccine safety and efficacy evaluations.

3.4 Measurements

The study will measure the following variables;-

Independent variable

Treatment: vaccination or control.

Dependent variables

1. Animal survival (in days) over a 30 day period.
   Mortality will be monitored on daily basis by mortality sensing collars. Animals will be visualized every 2-3 days for health checks.
2. CDV Serum antibody level at 0, 1 and 12 months post-vaccination.
   Antibody levels will be measured by Serum Neutralization test at Cornell University laboratory. Serum-neutralisation test is highly sensitive and specific for picking CDV antibodies (Loots et. al., 2017)
   Blood will be collected from the jugular or femoral veins of anaesthetized animal using a serum tube. Sample will be centrifuged and serum decanted for storage at minus 20 degrees until time of testing.

4. Data Management and Analysis

4.1 Data capture

Capture form will be developed and used for animal handling event data collection. Field monitoring data sheets will be designed and used for data collection. Data from the forms will be transferred into excel spreadsheet and backed-up for safe keeping at regular intervals.

4.2 Data analysis

Vaccine safety

The field trial will generate an estimate of wild dog survival post-vaccination, which will be compared with that of unvaccinated controls. This comparison will be made by fitting a Cox proportional hazards model to the data, measuring the survival (in days post-vaccination) of vaccinated and unvaccinated animals. This approach is preferred because it analyses survival as a function over time, rather than a simple binary outcome (alive/dead). Covariates (e.g. age, dispersal status) can also be included in time-dependant models, and pack identity can also be accounted for.

Vaccine efficacy

The study will generate estimates of CDV antibody titres in vaccinated vs control animals, and at different times relative to vaccination. Antibody titres can be difficult to analyse because serial dilutions mean they are not normally distributed. Basic analyses will therefore use logistic regression models, specifying a cut-off to classify individuals as seropositive/seronegative at specific time points.

Statistical analyses will be conducted under the guidance of Prof Christl Donnelly FRS (Imperial College London and University of Oxford).

5. Ethical and legal considerations

5.1 Approval of study by the relevant departments

1. SANParks AUCC Application – submitted
2. DAFF Section 20 permit – submitted
3. Zoological Society of London ethical committee application – approved.
4. TOPS permit for working with endangered species
5. Animal Ethics Committee application

5.2 Privacy of information/confidentiality

The intellectual property for the study will be jointly owned by University of Pretoria and Zoological Society of London. Data generated from the study will be kept for 10 years in line with University requirements.

5.3 Potential harms and benefits

Scheduled wildlife immobilisation drugs poses a hazard to the handler and team, they will be handled in accordance with veterinary and pharmacological legislation by a qualified, competent and authorised person, trained in first aid of accidental exposure to these drugs. The study will not pose any biohazard to other animals or staff. Contaminated materials generated during animal handling events will be destroyed by incineration.

The study has the potential benefit of providing immediate protection against CDV on study subjects if satisfactory seroconversion occurs. The research has the potential to provide useful outcomes for conservation managers on how to better manage CDV in wild dogs and related species under similar environment. The outcome will further provide scientific evidence to support development of guidelines on Canine Distemper management for African wild dog populations.

5.4 Conflict of interest

There is no conflict of interest identified between the research team and the University of Pretoria with regard to this study.

6. Logistics and time schedule

6.1 Responsibilities of staff and/or investigators

Individual	Responsibility
Dr. Louis van Schalkwyk (Supervisor and project veterinarian)	Project supervisor and responsible for veterinary care of the animal. Capture animals, deploy tracking collars and collect biological samples from the animals. Also undertakes autopsy in the event of mortality.
Prof. Leith Meyer (Co-supervisor)	Project supervisor
Prof. Rosie Woodroffe (Co-Supervisor)	Principal Investigator and project supervisor
Dr. Mmadi Reuben (PhD student)	Monitoring health of study animals and reporting any ill-health problems to the veterinarian.
Dr. Peter Buss (SANParks veterinarian)	Responsible for veterinary care of the animal. Capture animals, deploy tracking collars and collect biological samples from the animals. Undertakes autopsy in the event of mortality.

Dr. Lufuno Netshitavhadulu (SANParks veterinarian)	Responsible for veterinary care of the animal. Capture animals, deploy tracking collars and collect biological samples from the animals. Also undertakes treatment of ill animals and autopsy in the event of mortality.
Grant Beverley (Co-researcher)	Monitoring health of study animals and reporting any ill-health problems to the veterinarian

5.2 Project Management Timetable

TIMELINE																																	
Planned Research Activity	2022									2023												2024											
Month	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Literature review																																	
Apply for Research permits																																	
Vaccinations																																	
Field monitoring																																	
Sampling																																	
Samples export Permit application																																	
Sample shipment and analysis																																	
First publication																																	
Parameterise model																																	
Second publication																																	
Thesis writing																																	
Thesis submission to Supervisors																																	
Final Submission																																	

7. Budget/ Resources

7.1 Available resources

There are three collaborators in the project

The study is funded by Morris Animal Foundation through the Zoological Society of London (ZSL).

1. ZSL will provide telemetry and some field equipment for animal monitoring, immobilizing drugs and vaccines, transport cost for field monitoring, sample shipment and diagnostic testing cost.
2. Endangered Wildlife Trust will support animal monitoring through provision of transport for the project, EWT has an ongoing wild dog monitoring project within Kruger National Park.
3. SANParks will avail the veterinarians for the capture exercise and other required veterinary interventions.

8. Reporting of results

The findings will be reported through the following;-

1. Collation of results into a thesis.
2. Publication of results in peer reviewed journal (3 articles).
3. Presentation of findings at conferences.
4. Presentation of findings to conservation managers.

9. References

1. Alexander, K.A., Kat, P.W., Munson, L.A., Kalake, A. and Appel, M.J., 1996. Canine distemper-related mortality among wild dogs (Lycaon pictus) in Chobe National Park, Botswana. *Journal of Zoo and Wildlife Medicine,* **27**(3), pp.426-427.

2. Alexander, K.A., McNutt, J.W., Briggs, M.B., Standers, P.E., Funston, P., Hemson, G., Keet, D., van Vuuren, M., 2010. Multi-host pathogens and carnivore management in southern Africa. *Comp. Immunol. Microbiol. Infect. Dis.* **33**, 249–265.

3. Berentsen, A.R., Dunbar, M.R., Becker, M.S., M'soka, J., Droge, E., Sakuya, N.M., Matandiko, W., McRobb, R., Hanlon, C.A., 2013. Rabies, Canine Distemper, and Canine Parvovirus Exposure in Large Carnivore Communities from Two Zambian Ecosystems. *Vector-Borne and Zoonotic Diseases,* **13**, 643–649.

4. Birhanu, F.M., 2010. Genetically Modified Organisms in Africa: Regulating a Threat or an Opportunity?, in: *The Regulation of Genetically Modified Organisms: Comparative Approaches.* Oxford University Press, Oxford.

5. Blixenkrone-Möller, M., Svansson, V., Appel, M., Krogsrud, J., Have, P., Örvell, C., 1992. Antigenic relationships between field isolates of morbilliviruses from different carnivores. *Arch. Virol.* **123**, 279–294. https://doi.org/10.1007/BF01317264

6. Bronson, E., Deem, S.L., Sanchez, C., Murray, S., 2007. Serologic response to a canarypox-vectored canine distemper virus vaccine in the giant panda (*Ailuropoda melanoleuca*). *Journal of Zoo and Wildlife Medicine*, 38, 363–366.

7. Carpenter, J.W., Appel, M.J., Erickson, R.C., Novilla, M.N., 1976. Fatal vaccine-induced canine distemper virus infection in black-footed ferrets. *Journal of the American Veterinary Medical Association,* **169,** 961–964.

8. Chappuis, G., 1995. Control of canine distemper. *Veterinary Microbiology,* **44**, 351–358.

9. Connolly, M., Thomas, P., Woodroffe, R. and 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**(4), 882-888.

10. Connolly, M., Thomas, P., Woodroffe, 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.

11. Creel, S., Creel, N.M., Munson, L., Sanderlin, D. and Appel, M.J., 1997. Serosurvey for selected viral diseases and demography of African wild dogs in Tanzania. *Journal of Wildlife Diseases*, **33**(4), 823-832.

12. Dhand, N.K. and Khatkar, M.S., 2014. Statulator: An online statistical calculator. Sample Size Calculator for Comparing Two Independent Proportions. (http://statulator.com/SampleSize/ss2P.html)

13. Du Plessis, C., 2016. Canine distemper virus inoculations at HIP. (https://wildlifeact.com/blog/canine-distemper-virus-inoculations-hip/)

14. Durchfeld, B., Baumgärtner, W., Herbst, W. and Brahm, R., 1990. Vaccine-associated canine distemper infection in a litter of African hunting dogs (*Lycaon pictus*). *Journal of Veterinary Medicine*, Series B, **37**(1-10), 203-212.

15. European Medicines Agency, 2007. Guidelines on requirements for First-in-Man clinical trials for potential high-risk medicinal products. (http://www.emea.europa.eu/pdfs/human/swp/2836707en.pdf)

16. Ford, R.B. et al., 2017. AAHA Canine Vaccination Guidelines. (https://www.aaha.org/globalassets/02-guidelines/canine-vaccination/vaccination_recommendation_for_general_practice_table.pdf.

17. Gascoyne, S.C., King, A.A., Laurenson, M.K., Borner, M., Schildger, B., Barrat, J., n.d. Aspects of rabies infection and control in the conservation of the African wild dog (Lycaon pictus) in the Serengeti region, Tanzania. *Onderstepoort Journal of Veterinary Research* **60**, 415-420.

18. Gilbert, M., 2016. Understanding and managing canine distemper virus as a disease threat to Amur tigers. (PhD thesis, University of Glasgow)

19. Goller, K.V., Fyumagwa, R.D., Nikolin, V., East, M.L., Kilewo, M., Speck, S., Müller, T., Matzke, M. and Wibbelt, G., 2010. Fatal canine distemper infection in a pack of African wild dogs in the Serengeti ecosystem, Tanzania. *Veterinary microbiology*, **146**(3-4), 245-252.

20. Henke, S.E., 1997. Effects of modified live-virus canine distemper vaccines in gray foxes. *Journal of Wildlife Rehabilitation*, **20**(2), 3-7.

21. Hines, R., 2015. Vaccination of zoo animals, wild animals and exotic pets. https://www.2ndchance.info/vaccination.htm

22. Hofmeyr, M., Bingham, J., Lane, E.P., Ide, A., Nel, L., 2000. Rabies in African wild dogs (Lycaon *pictus*) in the Madikwe Game Reserve, *South African Veterinary Records* **146**, 50–52.

23. Hofmeyr, M., Hofmeyr, D., Nel, L., Bingham, J., 2004. A second outbreak of rabies in African wild dogs (Lycaon pictus) in Madikwe Game Reserve, South Africa, demonstrating the efficacy of vaccination against natural rabies challenge. *Animal Conservation.* 7, 193–198.

24. Keeling, M.J., Grenfell, B.T., 1997. Disease extinction and community size: Modeling the persistence of measles. *Science* **275**, 65–7.

25. Lau, E., 2012. Merial: PureVax for ferrets coming back this week. https://news.vin.com/vinnews.aspx?articleId=22494

26. Loots, A.K., Mokgokong, P.S., Mitchell, E., Venter, E.H., Kotze, A., Dalton, D.L., 2018. Phylogenetic analysis of canine distemper virus in South African wildlife. *PLOS ONE* **13**, e0199993.

27. Martella, V., Elia, G., Buonavoglia, C., 2008. Canine Distemper Virus. *Vet. Clin. North Am. Small Anim. Pract., Emerging and Reemerging Viruses in Dogs and Cats* 38, 787–797.

28. McCormick, A.E., 1983. Canine distemper in African cape hunting dogs (*Lycaon pictus*): possibly vaccine induced. *The journal of zoo animal medicine*, **14**(2), pp.66-71.

29. Moore, G.E., Guptill, L.F., Ward, M.P., Glickman, N.W., Faunt, K.K., Lewis, H.B., Glickman, L.T., 2005. Adverse events diagnosed within three days of vaccine administration in dogs. *Journal of American Veterinary Medical Association,* **227**, 1102–1108.

30. Mutinda, M et al., 2017. Canine distemper outbreak in wild and domestic carnivores in Laikipia ecosystem of Kenya. (Kenya Wildlife Service)

31. NeoTech Vaccines, 2009. Material Safety Data Sheet - NeoVac-D. (https://neotechvaccines.com/images/MSDS-NeoVac-D.pdf)

32. Prager, K. C., Mazet, J.A.K., Dubovi, E.J., Frank, L.G., Munson, L., Wagner, A.P., Woodroffe, R., 2012. Rabies Virus and Canine Distemper Virus in Wild and

Domestic Carnivores in Northern Kenya: Are Domestic Dogs the Reservoir? *EcoHealth* **9**, 483–498.

33. Prager, K.C., Mazet, J.A.K., Munson, L., Cleaveland, S., Donnelly, C.A., Dubovi, E.J., Szykman Gunther, M., Lines, R., Mills, G., Davies-Mostert, H.T., Weldon McNutt, J., Rasmussen, G., Terio, K., Woodroffe, R., 2012. The effect of protected areas on pathogen exposure in endangered African wild dog (Lycaon pictus) populations. *Biological Conservation* **150**, 15–22.

34. Rhodes, S et al., 2007. North American regional studbook – African wild dog (*Lycaon pictus*). (Association of Zoos and Aquariums/Chicago Zoological Society)

35. Rikula, U.K., 2008. Canine distemper in Finland – vaccination and epidemiology. (PhD thesis, University of Helsinki)

36. Sadler, R.A., Ramsay, E., McAloose, D., Rush, R., Wilkes, R.P., 2016. Evaluation of two canine distemper virus vaccines in captive tigers (*Panthera tigris*). *Journal of Zoo and Wildlife Medicine,* **47**, 558–563.

37. Scheepers, J.L. and Venzke, K.A.E., 1995. Attempts to reintroduce African wild dogs Lycaon pictus into Etosha National Park, Namibia. *South African Journal of Wildlife Research*, **25**(4),138-140.

38. Smallwood, T., 2020. Modelling multi-host viral pathogens for African wild dog conservation. (PhD thesis, Imperial College London)

39. Spencer, J., Burroughs, R., 1992. Antibody Response to Canine Distemper Vaccine in African Wild Dogs. *Journal of Wildlife Diseases,* **28**, 443–444.

40. Swinton, J., Harwood, J., Grenfell, B.T. and Gilligan, C.A., 1998. Persistence thresholds for phocine distemper virus infection in harbour seal (*Phoca vitulina*) metapopulations. *Journal of Animal Ecology*, **67**, 54-68.

41. Thomas, P.R., Powell, D.M., Fergason, G., Kramer, B., Nugent, K., Vitale, C., Stehn, A.M., Wey, T., 2006. Birth and simultaneous rearing of two litters in a pack of captive African wild dogs (*Lycaon pictus*). *Zoo Biology,* 25, 461–477.

42. Tizard I. R., 2021. Canine vaccines. *Vaccines for Veterinarians.*153-166.e1.https://doi.org/10.1016/B978-0-323-68299-2.00022-8. Accessed 07 Jul, 2022, WSAVA-vaccination Guidelines Group

43. van de Bildt, M.W.G., Kuiken, T., Visee, A.M., Lema, S., Fitzjohn, T.R., Osterhaus, A.D.M.E., 2002. Distemper Outbreak and Its Effect on African Wild Dog Conservation. *Emerging Infectious Diseases* **8**, 212–213.

44. van Heerden, J., Bainbridge, N., Burroughs, R.E.J., Kriek, N.P.J., 1989. Distemper-like disease and encephalitozoonosis in wild dogs (*Lycaon pictus*). *Journal of Wildlife Diseases,* **25**, 70–75.

45. van Heerden, J., Bingham, J., Van Vuuren, M., Burroughs, R.E.J., Stylianides, E., 2002. Clinical and serological response of wild dogs (*Lycaon pictus*) to vaccination against canine distemper, canine parvovirus infection and rabies. *Journal of South African Veterinary Association,* **73**, 8–12.

46. Verberkmoes, W & Verberkmoes, H., 2007. European regional studbook – African wild dog (*Lycaon pictus*). (GaiaPark, Kerkrade Zoo)

47. Viana, M., Cleaveland, S., Matthiopoulos, J., Halliday, J.O., Packer, C., Craft, M.E., Hampson, K., Czupryna, A., Dobson, A.P., Dubovi, E.J. and Ernest, E., 2015. Dynamics of a morbillivirus at the domestic–wildlife interface: Canine distemper virus in domestic dogs and lions. *Proceedings of the National Academy of Sciences*, **112**(5), 1464-1469.

48. Visee, A.M. et al., 1997. The Mkomazi Project African wild dog Report. (George Adamson Wildlife Preservation Trust, Netherlands)

49. Warrell, M.J. et al., 1985. Economical multiple-site intradermal immunization with human diploid-cell strain vaccine is effective for post-exposure rabies prophylaxis. *Lancet* **1,** 1059-1062

50. WHO/FAO/OIE, 2018. Zero by 30: The global strategic plan to end human deaths from dog-mediated rabies by 2030. (https://apps.who.int/iris/bitstream/handle/10665/272756/9789241513838-eng.pdf?ua=1)

51. Woodroffe, R. and Sillero-Zubiri, C., 2013. African wild dog red list assessment. Gland, Switzerland: IUCN.

52. Woodroffe, R., 2021. Modified live distemper vaccines carry low mortality risk for captive African wild dogs, Lycaon pictus. *Journal of Zoo and Wildlife Medicine* **52**(1), 176-184. https://doi.org/10.1638/2020-0045

53. Woodroffe, R., Groom, R., McNutt, J.W., 2017. Hot dogs: High ambient temperatures impact reproductive success in a tropical carnivore. *Journal of Animal Ecology* **86**, 1329–1338.

54. Woodroffe, R., O'Neill, H.M. and Rabaiotti, D., 2020. Within-and between-group dynamics in an obligate cooperative breeder. *Journal of Animal Ecology*, **89**(2), 530-540

55. 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.

STANDING PERMIT

(Issued in terms of the provisions of the National Environmental Management: Biodiversity Act 2004, Act 10 of 2004)

PERMIT NUMBER: S 65757

NAME OF ISSUING AUTHORITY	
NAME	DEPT OF ENVIRONMENTAL AFFAIRS
ADDRESS	PRIVATE BAG X 447
	PRETORIA 0001
PROVINCE	NATIONAL DEPARTMENT

	PROVINCIAL DEPARTMENT	NATIONAL DEPARTMENT	
	PROTECTED AREA MANAGEMENT AUTHORITY	VETERINARIAN	X
	REGISTERED CAPTIVE BREEDING OPERATION	REGISTERED SCIENTIFIC INSTITUTION	X
	REGISTERED SANCTUARY	REGISTERED REHABILITATION FACILITY	
	REGISTERED COMMERCIAL EXHIBITION FACILITY	REGISTERED GAME FARM	
	REGISTERED WILDLIFE TRADER	REGISTERED NURSERY	

UNIQUE REGISTRATION NUMBER N78/770713 5156 084

DETAILS OF HUNTER

NAME	University of Pretoria	ID NO.	770713 5156 084
SURNAME	C/o Vinny Naidoo	PASSPORT NO.	

	POSTAL ADDRESS	RESIDENTIAL ADDRESS
ADDRESS	University of Pretoria	University of Pretoria
ADDRESS	Faculty of Veterinary Science	Faculty of Veterinary Science
ADDRESS	Private Bag X04	Old soutpan Road
TOWN	Onderstepoort	Onderstepoort
POSTAL CODE	0110	0110
PROVINCE	Gauteng	Gauteng

PROPERTY WHERE RESTRICTED ACTIVITIES WILL BE CARRIED OUT

NAME AND SURNAME OF APPLICANT	University of Pretoria
NAME AND SURNAME: RESPONSIBLE PERSON	Vinny Naidoo
NAME AND SURNAME OF AGENT	
PHYSICAL ADDRESS OF FACILITY	
PHYSICAL ADDRESS OF FACILITY	See attached annexure A
DISTRICT	
PROVINCE	
REGISTERED NAME AND NUMBER (in the case of game farm)	

DETAILS OF SPECIES INVOLVED

SPECIES		SEX (if known)	QUANTITY	MARKING (if applicable)
COMMON NAME	SCIENTIFIC NAME			
All avian, reptile, mammalian and fish species listed as threaned or protected in terms of section 56 of the National Environmental management Biodiversity Act (Act No 10 of 2004)				

DETAILS OF RESTRICTED ACTIVITIES INVOLVED

Collection, gathering, receiving, transport, moving, receiving as donation, giving, temporary possession, exercise physical control over, Darting and Capturing.

PERMIT VALIDATION

PERIOD OF VALIDITY	FROM:	11-02-2022	TO:	02-02-2025
RECEIPT NUMBER				

DEPT VAN OMGEWINGSAKE
PRIVAATSAK / PRIVATE BAG X 447
PRETORIA 0001
DEPT OF ENVIRONMENTAL AFFAIRS

SIGNATURE ISSUING OFFICER:

SIGNATURE PERMIT HOLDER:

DATE STAMP: 2022 -02- 11

STANDING PERMIT CONDITIONS

1. This permit is not transferable.
2. Any unauthorised alteration to this permit shall render it invalid.
3. This permit is subject to the provisions of any applicable law in force during the period of validity of the permit.
4. This permit is valid only within the province where it was issued,
5. The holder of this permit shall, at the request of a person authorised in terms of applicable legislation so to demand, forthwith produce such permit to such person.
6. This permit shall be invalid until such time that it is signed by the permit holder.
7. This permit shall be deemed invalid when it is lost or destroyed and no copy thereof shall be issued.
8. This permit may be withdrawn by an authorised person if the execution of any activity may be' detrimental to the welfare of any wild animal or the safety of any person, provided that the permit holder is given notice of such intention and be granted the opportunity to appeal to such withdrawal.
9. The prescribed fees paid for the issuing of this permit shall not be refunded.
10. If the holder of this permit contravenes or fails to comply with any permit condition or requirement to which this permit is subject, he or she shall be guilty of an offence.
11. This permit shall be subject to any applicable norms and standards in existence at the time of issuance of this permit.

DEPT. VAN OMGEWINGSAKE
PRIVAATSAK / PRIVATE BAG X 447
2022 -02- 11
PRETORIA 0001
DEPT. OF ENVIROMENTAL AFFAIRS

SPECIAL CONDITIONS

1. The issuing authority shall determine the species and restricted activities for which this permit will apply.
2. If this permit applies to a registered game farm, the holder of this permit must:
   a. Have a copy of this permit authorising the hunt, on his or her person during the hunt;
   b. Within 21 days after the hunt, furnish the issuing authority with a written return on the hunt stating:
      (i) the permit number and date of issuance of the permit;
      (ii) the species, sex and number of animals hunted; and
      (iii) the location where the hunt took place.
   c. Return all used copies of the game farm hunting permits within 3 weeks after the end of the calendar year following the issuance of the game farm hunting permit, to the Issuing Authority.

ANNEXURE A

STANDING PERMIT

(Issued in terms of the provisions of the National Environmental Management: Biodiversity Act 2004, Act 10 of 2004)

Standing permit no: S 65757

Registration certificate no:02251

NAME OF ISSUING AUTHORITY	
NAME	DEPARTMENT OF FORESTRY, FISHERIES AND THE ENVIRONMENT
ADDRESS	PRIVATE BAG X 447
	PRETORIA 0001
PROVINCE	NATIONAL DEPARTMENT

DETAILS OF SPECIES INVOLVED

All avian, reptile mammalian and fish species that are listed as threatened or protected in terms of section 56 of the National Environmental Management: Biodiversity Act (Act No.10 of 2004).

DETAILS OF RESTRICTED ACTIVITIES INVOLVED AND SPECIAL CONDITIONS

1. This permit authorizes the Faculty of Veterinary Science in the University of Pretoria to carry out the following restricted activities:

   Collection/gathering of samples
   Receiving;
   Transporting or moving;
   Receiving as donation;
   Giving;
   Temporary possession/ exercise physical control over;
   Darting and
   Capturing

2. This permit authorizes the Faculty of Veterinary Science of the University of Pretoria to carry out the restricted activities as specified in paragraph 1 of this permit, involving blood and tissue samples of listed threatened or protected avian, reptiles, fish and mammalian species, for diagnostic analysis and research purposes.

3. This permit authorizes the Faculty of Veterinary Science of the University of Pretoria to receive, and temporary keep live specimens of listed threatened or protected mammalian, avian, fish and reptile species for treatment purposes.

4. This permit authorizes the carrying of the restricted activities as specified in paragraph 2 and 3 of this permit, only by employees and registered students carrying out restricted activities on behalf of the Faculty of Veterinary Science of the University of Pretoria.

5. This permit authorizes the restricted activities, and for the purpose as specified in paragraph 2 of this permit within the boundaries of the Republic of South Africa.

6. The holder of this permit may give/receive blood and tissue samples of listed threatened or protected avian, reptiles, mammalian and fish species, only to or from a person who is in possession of a permit issued in terms of the National

Environmental Management: Biodiversity Act, 2004 (Act No.10 of 2004), that authorizes the possession and receiving/giving/donation of such specimens.

7. Blood or tissue samples collected/received in terms of this permit may not be offered for sale.

8. In the event that the permit holder receives specimens of listed threatened or protected avian, reptiles, mammalian and fish species, the permit holder must report in writing, by the end of each year during the period of validity of this permit, to the Department of Forestry, Fisheries and the Environment (DFFE) the following information:

   (a) Name and physical address of person who the specimens have been received from;
   (b) Permit number of the person who the specimens have been received from (where applicable); and
   (c) Particulars of the species, number and type of specimens received and markings (where applicable).

9. The permit holder must make the report available to the DFFE, upon request from DFFE.

10. All restricted activities involving rhinoceros must be carried out in compliance with the Norms and Standards for the marking of the rhinoceros and rhinoceros' horn and for the hunting of the rhinoceros for trophy hunting purposes as published in terms of the National Environmental Management: Biodiversity Act, 2004 (Act No. 10 of 2004).

11. The permit holder must comply with the National Norms and Standards for the Management of Elephants in South Africa as published in terms of the National Environmental Management: Biodiversity Act, 2004 (Act No. 10 of 2004).

12. The carrying out of the restricted activities referred to in paragraph 1 by the permit holder is subject to the provisions of the Threatened or Protected Species regulations.

13. This permit does not absolve the permit holder to obtain any permit that may be required in terms of any other applicable legislation.

PERMIT VALIDATION				
PERIOD OF VALIDITY	**FROM :**	11-02-2022	**TO:**	02-02-2025
SIGNATURE OF ISSUING OFFICER			**SIGNATURE PERMIT HOLDER**	

DATE STAMP:

DEPT. VAN OMGEWINGSAKE
PRIVAATSAK / PRIVATE BAG X 447
2022 -02- 11
PRETORIA 0001
DEPT. OF ENVIROMENTAL AFFAIRS

November 3, 2023

To whom it may concern,

The species needed for this permit is African wild dog, *Lycaon pictus.* This species is not listed in the dropdown. I inquired with FWS and was told to pick a different species and submit an attachment with the species that I actually need. The support case number is EPS0102622.

Sincerely,

Jennifer Powers Jennifer Powers

Template for UP online Research and Animal Ethics committee applications

1. **Project title**
   a. *Project title: Can vaccination protect African wild dogs from canine distemper? Addressing a conservation emergency*.

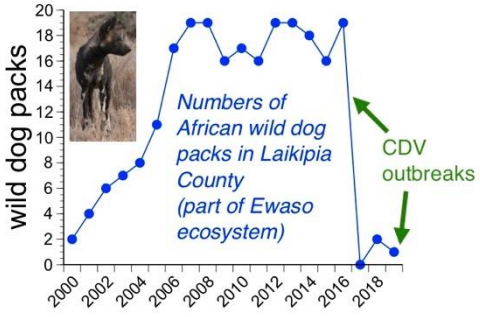
   b. Short description: *The African wild dog (Lycaon pictus) is a globally endangered species, with fewer than 700 packs remaining in the wild. Canine Distemper Virus (CDV) was assumed to pose little risk to the species, because field studies in many parts of Africa had found healthy animals with antibodies to the virus, suggesting that wild dogs often survived the disease. Recently, six separate fatal CDV outbreaks have been recorded, with the worst all but wiping out the largest population in the northern hemisphere. Previous work demonstrates 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 research work aims to identify such a protocol, to inform urgent conservation efforts*.
   c. Is study related to another study Y/N (if yes, which study number) *Yes*

2. **Short literature review that justifies the project**
   a. Short literature review - Refer to the Project Proposal for details

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 range[1], and climate change now compounds these threats to the <700 packs that remain[2]. 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 1991[3], and several subsequent whole-pack deaths linked to rabies[4-7], led to 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 animals[7-11]. Although sporadic whole-pack deaths were reported[12,13], the only major confirmed outbreak was in a captive breeding centre[14]. 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 packs[17]. 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*

African wild dog dying of confirmed Canine Distemper Virus. Ewaso ecosystem, Kenya, 12 Oct 2019. Photo: Shivani Bhalla.



(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 lions[18], 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 dogs[19]. MAF-funded research within the Ewaso ecosystem showed that CDV was not persisting in local domestic dogs[20], and that wild dogs with greater opportunities for domestic dog contact were not more likely to have been exposed to CDV[9].

(ii) Even if domestic dogs did act as a CDV reservoir, controlling infection would be challenging because CDV, like other morbilliviruses (e.g. measles[21], phocine distemper virus[22]), 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 2030[24], 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 dogs[25],[23], and can prompt seroconversion in captive African wild dogs[26]. Nevertheless MLVs have occasionally induced clinical distemper in a number of nondomestic carnivores[27,28], including African wild dogs[29-31]. Risks appear to be low, however[32], 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 distemper[32]. 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 Tanzania[14].

Recombinant vaccines likewise cannot induce distemper, because they do not contain a complete viral genome. Such vaccines have induced seroconversion in African wild dogs[34], and other sensitive species[35]. However, a trial in captive tigers showed that recombinant vaccines produced weaker immune responses than MLVs[36]. 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 others[37]. Moreover, the vaccine has faced repeated supply problems[38,39].

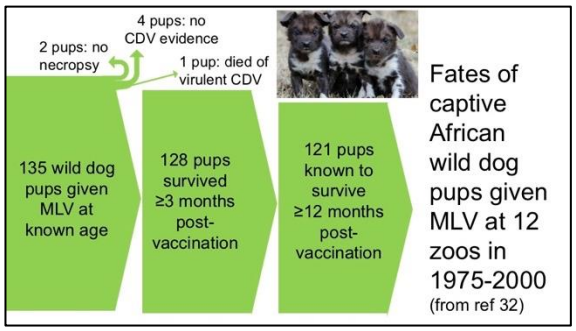
As MLV appears to be immunogenic, low risk, and widely available in Africa, it is a strong candidate for use in protecting free-ranging populations of African wild dogs threatened by canine distemper. However, there is currently no established vaccination protocol suitable for field use.

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 vaccine[40]. To ensure vaccine "take", doses are repeated at 2-4 week intervals until 16 weeks of age[40]. 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 protective****[40]. 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 MLV[41] or inactivated[26] 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/15.9kg[44] 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 adjuvant[45] which some have tentatively linked to adverse vaccine reactions in small domestic dog breeds[46]. It may thus be helpful to evaluate both single and double doses of MLV in African wild dogs.*

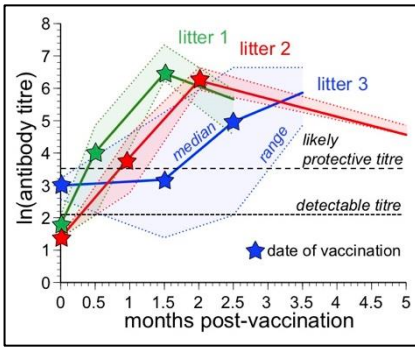
iii. Preliminary Data

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 work[32] 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%).*

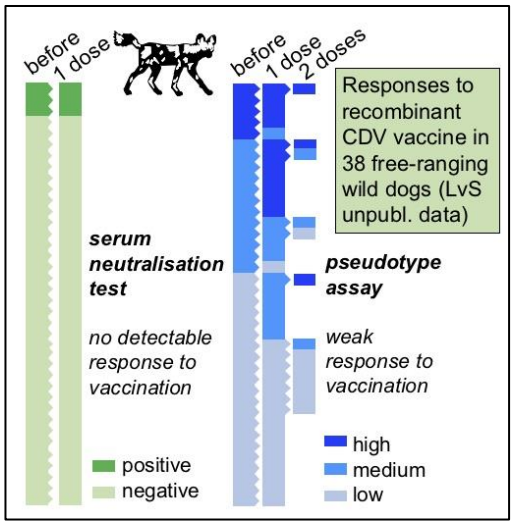


*We have also evaluated antibody responses to **recombinant CDV vaccine** in **captive** wild dog pups, showing that this vaccine is safe and immunogenic in captivity, if delivered by a parenteral route[34]. All pups without detectable maternal antibodies at the start of vaccination showed strong, rising titres after a single dose, although those with maternal antibodies required multiple doses[34].*

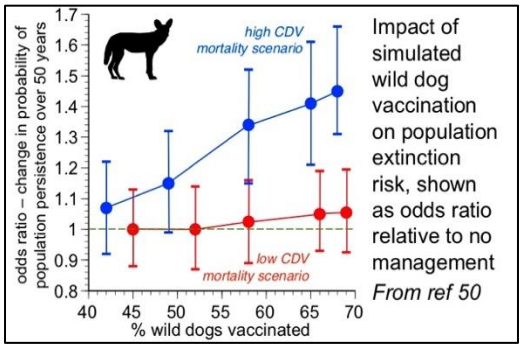
*However, our evaluation of immune responses to **recombinant CDV vaccine** in **free ranging wild dogs** showed a much less promising immune response (van Schalkwyk, unpubl. data). Wild dogs in 20 packs given recombinant vaccine in Kruger National Park, South Africa, showed no immune response detectable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



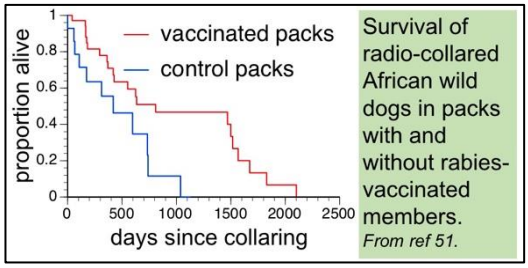
Serum antibody titres of 13 captive African wild dog pups during a course of 3 injections of recombinant CDV vaccine (from ref 34)



*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 ref 50) simulating wild dog population dynamics (including within-and between-pack dynamics[49]), vaccination was associated with >40% reductions in extinction risk if CDV could cause high mortality[50].*



Impact of simulated wild dog vaccination on population extinction risk, shown as odds ratio relative to no management *From ref 50*



Survival of radio-collared African wild dogs in packs with and without rabies-vaccinated members. *From ref 51.*

*Our team has previously used **field trials** to evaluate vaccine safety, including a trial at a site in Kenya[51] which showed that rabies vaccination was safe for use in African wild dogs (see left).*

3. **Aims and Objectives of the project**
   a. Give a brief description

   The study aims to assess the safety, efficacy and the practicality of vaccinating free ranging African Wild Dogs (AWD) against CDV. The outcome of the study will be to (i) provide safe, effective CDV vaccination protocol for free ranging African Wild Dogs and (ii) quantify impacts on extinction risk to develop guidelines for CDV management in African wild dog populations using an existing mathematical model, findings from the study and other work done in captive wild dog populations.

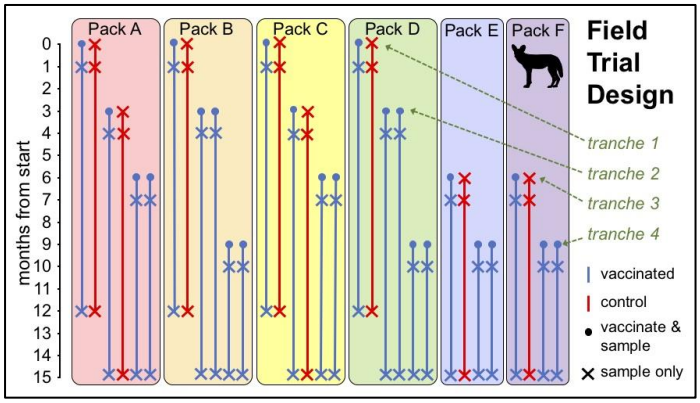
4. **Materials and Methods**
   a. Full description of all materials and methods

   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" trials[52], 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-vaccination[29-31]. 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 period[53].*

*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-vaccination[53]. 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.*



5. No details required

6. **Duration of project**
   a. Proposed commencement date: *The anticipated start is August 2022*
   b. Proposed finalisation date: *Completed December 2023*

7. **Research environment - Where will the study be conducted?**

a. Drop down menu with options (select **other or private owner** for field work)
The study will be conducted at Kruger National Park using six (6) free ranging African wild dog packs.

8. Research team

a. For each person in the following details are required.

i. Role – drop down list i.e. supervisor, principle investigator, student
ii. Formal Name: *Dr. Mmadi Mogolodi Reuben*
iii. UP employee / Student / External number: *22963155*
iv. Highest Qualification: *MSc*
v. UP Department: *Tropical Veterinary Diseases, Faculty of Veterinary Science*
vi. Email Address: *mmadireuben@gmail.com*

vii. Role – drop down list i.e. supervisor, principle investigator, student
viii. Formal Name: *Dr. Louis van Schalkwyk*
ix. UP employee / Student / External number
x. Highest Qualification: *PhD*
xi. UP Department:
xii. Email Address*: lvs0836332203@gmail.com*

xiii. Role – drop down list i.e. supervisor, principle investigator, student
xiv. Formal Name: *Prof. Leith Meyer*
xv. UP employee / Student / External number:
xvi. Highest Qualification: *PhD*
xvii. UP Department: *Centre for Veterinary Wildlife Research, Faculty of Veterinary Science*
xviii. Email Address: *leith.meyer@up.ac.za*

xix. Role – drop down list i.e. supervisor, principle investigator, student
xx. Formal Name: *Prof. Rosie Woodroffe*
xxi. UP employee / Student / External number
xxii. Highest Qualification: *DPhil*
xxiii. UP Department
xxiv. Email Address: *Rosie.Woodroffe@ioz.ac.uk*

xxv. Role – drop down list i.e. supervisor, principle investigator, student
xxvi. Formal Name:
xxvii. UP employee / Student /
xxviii. Highest Qualification:
xxix. UP Department
Email Address:

*** Click on the dropdown arrow**

*** Select the relevant role**

*** EMPLID -** **Search the person by clicking on the (Internal and External) magnifying glass**

Add your Research Team involved in the project, for example:

*** Student Supervisor (only applicable for Degree purpose)**
*** Principal Investigators (usually yourself)**
*** Internal Co-Researchers**
*** External Co-Researchers**
*** Postdoctoral fellows**
*** Postgraduate students**
*** Assistants**

To add more than one Team Member:

*** Click on the + sign at the end of the line**

To add External Team Members:

*** Click on the External Persons menu function on the left**
*** Add all the External Persons' information**
*** Add the External Persons as Team members on the Form by:**
1) Chosing an External Role
2) Click on the magnifying glass - This will show the External Persons
3) Select the correct person

9. **Agreements between researchers**
    a. The following boxes need to be ticked by each researcher listed
        i. Right to use the results in a dissertation or thesis (*Dr. Mmadi Mogolodi Reuben)*
        ii. Right to present the results at a Conference (*All*)
        iii. Right to publish the results in a Science Journal (*All- Dr. Reuben given first option*)
        iv. Right to publish the results through a Non-Science medium (*All*)
        v. Right to Co-Authorship (*All*)

10. **Funders of the project**
    a. Project funded Y/N *Yes*
    b. If yes drop down with following request for details
        i. Funders Organization: *Morris Animal Foundation*
        ii. Funder / Contact person: *Prof. Rosie Woodroffe/ Prof. Leith Meyer*
        iii. Email Address: *leith.meyer@up.ac.za*
        iv. Cell Phone (Format: *082 574 2896*):
        v. Land line phone number (Format: *0125454785*):
        vi. Postal address line 1
        vii. Postal address line 2
        viii. Postal address line 3
        ix. City
        x. Postal Code
        xi. Total amount at local site
        xii. Obligations towards funder
        xiii. Contract Number
        xiv. Does funding depend on the approval by the Ethics Commitee? *No*
        xv. Date funding received
        xvi. Registered for VAT

11. **Involvement of people as participants (only if human subjects are needed)**
    a. Will people be recruited as research participants? Y/N *No*
    b. If yes then a drop down menu with additional questions are asked (see system)

12. No details required
13. No details required
14. No details required
15. No details required

16. **Data recorded, archived and stored**
    a. Data/samples recorded/collected at the point of measurement (drop down menu)

How will data /samples be recorded at the point of measurement:

*** Click on the dropdown arrow**
*** Select the relevant choice**

To add another selection:

*** Click on the + sign at the end of the row**

*** Click on the dropdown arrow**

*** Select the relevant choice**

When you choose "Other form":

*** Please describe the details in the field provided**

b. Data / samples stored and archived after conclusion of study (drop down menu)

POLICY FOR THE PRESERVATION AND RETENTION OF RESEARCH DATA

2.1.4Data is required to be stored for a minimum period of ten years after the completion of the original project but if intellectual property is involved, or if there are particular statutory or contractual requirements, a longer period may well be required. Special consideration about length of storage should also be given to cases where a potential conflict of interest or misconduct is involved. In some cases, and in particular where experiments with humans are concerned, funding bodies may require that all raw data be kept indefinitely.

Please familiarise yourself with the full content of the "Policy for the preservation and retention of research data" ** Also note that the Final Signed Informed Consent forms also need to be stored for the entire period.

17. Secondary data

Secondary data consist of both external and internal data.

External data are generated by sources external to an organization, i.e. data that were not collected by the organization, or individuals in the organization. Examples are data from government, banks, businesses or commercially provided information.

Internal data refer to data that are generated by the company, i.e. procured and consolidated from different units or individuals within the organization. In the context of UP

internal data will for example be data obtained from BIRAP.

a. Will secondary data be used in this research Y/N *Yes*
b. If Yes drop down menu and more detail required. *Data from captive wild dogs study from European Zoos*.

18. Intellectual property (IP)

The PI declares the interests in the IP of this research project by the participating institutions (university or research, financial or other institution), or persons (project supervisor, research leader, student or other persons).

Examples:

a) Data provided by FNB to study poverty in Gauteng. FNB relinquishes all IP to the UP.

b) Prof XYZ is the project supervisor, while Mr J is the PhD candidate analysing the data with the assistance of the IT Dept. The participants agree that the IP resides equally with all persons and that the PhD candidate has the first option to be principal author of research papers.

c) Project is fully sponsored by an external entity and there is an agreement that all IP is owned by that external entity.

d) External parties may generate IP in the project and a benefit sharing agreement exist between the parties.

a. Will all intellectual property be owned by UP? Y/N *No* if no drop down menu with more detail required with Y/N answers (No)
    i. Relinquishes IP to UP conditionally
    ii. IP jointly owned by another entity (Yes)
    iii. Collate results in student's thesis with no public access
    iv. Consult with the owner for any publication of the results
    v. Other conditions
b. Conflict of interest with respect to IP (if yes describe the conflict of interest) *No conflict exist.*

Do any researchers have a conflict of interest, such as directorships or shares in the entity sponsoring the research? *No*

State the name of the persons and entities. *N/A*

19. No details required
20. No details required

21. **Benefits associated with the research study**

a. Describe benefits associated with the research project/study
The research will provide (i) a formal evaluation of the safety and likely efficacy of modified-live CDV vaccination in free-ranging wild dogs and hence (ii) evidence-based CDV management guidelines for this endangered species, for South Africa specifically but also for wild dog populations' elsewhere in Africa. While free ranging African wild dog populations are at high risk of CDV related mortality, there currently is no identified safe and effective vaccination protocol to manage these risks under field conditions.

22. Planned application of results

a. Drop down menu with various options i.e.
   i. *publication in scientific journal*
   ii. *Establishment of a safe, efficient and practical CDV vaccination protocol in free ranging wild dog population.*
   iii. *Development of guidelines for management of Canine Distemper Virus in African wild dog populations*.

23. Additional approval or formal permissions

a. Does the study require any other approvals / permissions? Y/N *Yes*
b. If yes then - When and how will these approvals / permissions be obtained?
SANParks Research application – approved.
SANParks AUCC Application – submitted.
DLRRD Section 20 permit application – submitted
Endangered Wildlife Trust ethical committee application - submitted.
Zoological Society of London ethical committee application – approved.

24. Confidentiality clause and Pty Ltd issues

a. Are there any confidentiality clauses or Pty Ltd issues? Y/N *No*
b. If yes, describe the confidentiality clauses/Pty Ltd issues. *N/A*

25. Environmental impact and hazardous materials

Does the project pose a potential bio-hazard as a result of treatment or material waste, or any other outcome? *No*

a. Does the study require the use of hazardous materials? Y/N *No*
b. If yes biohazard drop down menu and additional biohazard information is asked for
   i. Precautionary measures in the case of existing bio-hazards
      1. Protect Staff
      2. Protect Public
      3. Protect Environment
      4. Will biohazards be imported? Y/N

c. Does the doing of the research have an environmental impact? Y/N *No*
d. If yes - Please describe the environmental impact.

26. Animals for research or testing purpose

Research Category: Pain/Discomfort/Stress classification (select 1)

☐ Experiments on embryonated eggs or cephalopods and decapods or stored samples previously collected from animals.

☐ Studies on vertebrate animals during the course of routine examination, teaching procedures and treatment.

☑ Procedures on vertebrate species that are expected to

produce stress but no pain requiring anaesthesia.

☐ Experiments that produce minor or short-duration pain

requiring the use of pain relieving drugs.

☐ Experiments that involve significant but unavoidable stress

or pain requiring anaesthesia or a humane endpoint.

☐ Procedures that involve inflicting severe pain at or above

the pain tolerance threshold and the use of pain relievers are contra-indicated.

a. Hypothesis

f a hypothesis is being tested give the postulate/s (null hypothesis and alternates) to aid the reviewers in following the rationale of the proposed study.

This project aims to test the hypothesis that extinction risks to African wild dog populations can be reduced by vaccination against Canine Distemper Virus.

b. Animal requirements (Please complete this section for each species by clicking on the + sign)

i. Animal Species (Please state whether domesticated or not); *free ranging African Wild Dog* (*Lycaon pictus*)
ii. Specify for Training / Usage: *Vaccine safety and efficacy evalutation on the species under open system management.*
iii. Strain:
iv. Total number required: 32
v. Will the same original animals be used? *Yes*
vi. Gender used: *Males and Females*
vii. Body mass: *20 - 30 kg*
viii. Age: *> 1 year*
ix. Microbial status: N/A.
x. Source of animals: *Kruger National Park free ranging wild dog population*

c. Samples Derived from Animals

i. Info asked for (Sample Type, Number, Volume, Species, Previous AEC approval, Location / Country)

For example:

Sample Type: *Blood(serum)/Nasal & Rectal swabs*
Number: *96 tubes/32/32*
Volume: *20ml blood/sampling event*
Species: *African Wild dog* (*L. pictus*)
Previous AEC approval: None
Location/Country: *Kruger National Park, RSA*

d. Justification for the use of sentient animals

Briefly justify the use of animals, the choice of species, the numbers to be used. If there is limited availability, or large numbers are to be used, provide additional rationale

for their selection and numbers. State also what non-sentient model/s or non-animal models were considered
and on what grounds they were rejected.

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.

This work aims to develop evidence-based guidelines for managing the risks of canine distemper virus (CDV) to endangered African wild dogs, using a structured decision making (SDM) approach. The due diligence for developing these guidelines requires field research on at least six free-ranging packs of wild dogs. For the study to help inform wild dog conservation, it is important that the six packs are exposed to the array of challenges (predators, competitors, pathogens, prey, environmental stressors) typical of free-ranging wild dogs. Within South Africa, these conditions and this number of packs are available only within Kruger National Park. Canine distemper is a recognised threat to Kruger's wild dogs, so the work will directly benefit wild dog conservation within Kruger itself.

The study subjects will be 32 African wild dogs in six or more packs. Younger animals, in their natal packs, will be targeted for two reasons. First, in the very unlikely event of an animal being harmed by the handling procedures, packs which lose a subdominant natal animal remain intact, whereas those which lose an alpha (breeding) individual may break up[21]. Second, younger animals are less likely to have been previously exposed to CDV, and hence to have positive antibody titres before vaccination, making results clearer. The study will target the packs within Kruger already identified as being closest to communities and potentially at highest risk of CDV exposure. This approach should help to ensure that the project yields immediate benefits in terms of CDV protection as well as longer term benefits in terms of research outcomes.

e. Reduction of the number of animals to a minimum to achieve scientific objectives

Describe how this was determined either by calculation (statistical design) or by specification (i.e. use of a validated testing protocol) or any other strategy.

In performing our power calculations (described below), we have 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.

Using continuity correction and $\alpha=0.05$, 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 period[47]. 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-vaccination[47]. 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.

f. Animal housing and care

Briefly describe how the animals will be housed (penned, stabled, caged or confined in any other way, kept in metabolic crates or cages, etc.), their nutrition (feeding and watering) and what provisions have been made for the physical and psychological wellbeing i.e. comfort, socialisation, behavioural needs and enrichment of their immediate environment.

The experimental animals will be free ranging wild dogs within Kruger National Park.

g. Facilities and Sample Storage Facilities (NB! Leave blank if not applicable)

i. Name of facility used: *State Veterinary Laboratory*.
ii. SAVC Registration Number (Look up SAVC Registration Number – For Animal Research facilities or Veterinary Laboratory facilities):
iii. Physical Address: *Leopard street, Skukuza*, *Kruger National Park*.
iv. Emergency Contact Number:

h. Statement of animal care competence, expertise and experience

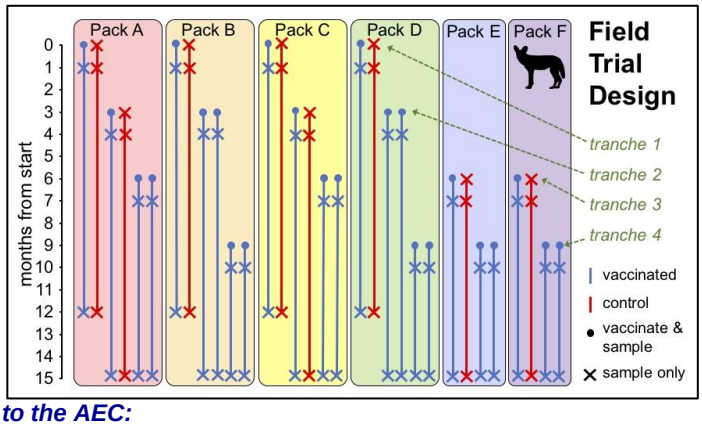
Provide a short statement of the scientific knowledge competence and experience of the person(s) appointed to ensure the comfort, health and humane treatment of the animal subjects in this study and provide their registration credentials either with the South African Veterinary Council, the Health Professions Council of South Africa or the South African Council for Natural Sciences Professions and any in-house accreditation obtained.

Dr. Louis van Schalkwyk

Name	Contact Number	e-mail address	Contact Address
Louis van Schalkwyk	+27(0)836332203	LvS@vodamail.co.za	PO Box 12, Skukuza, 1350

Qualifications	BVSc, MSc, PhD
Appropriate experience in animal research	Kruger National Park (KNP) wild dog health survey & vaccination campaign 2016-2019, KNP wild dog monitoring platform 2020-present, including associated analyses. Qualified as veterinarian for 20 years, all of which involved wildlife-related work and 10 of which have involved clinical wildlife work in the Kruger National Park. > 200 wild dog immobilisations for snare removals, vaccination, disease investigation, sample taking and collar fitment.
Professional registration or authorisation number (e.g. SAVC/HPCA)	SAVC D02/4511

i. **Experimental design (please upload flow diagram under the document icon, if applicable)**



For all studies submitted to the AEC:

Explain the reasoning behind the study design and experimental planning, with particular reference to determination of sample size and statistical analysis. The use of flow charts is recommended. The information should be presented in an easily accessible manner.

For studies involving animals also:

Describe how the animals will be allocated to experimental and control groups and where applicable, how the experimental treatments will be assigned to each group.

j. Restraint of the animals

Describe the methods of physical (manual procedures and use of special restraint equipment) or chemical restraint to be used on the animals and state who the animal handler/s will be.

Animals involved in the research 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 CO_2-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 short distance (<100m), 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.

Methods used for chemical restraint will be at the discretion of the veterinarian conducting the darting, although medetomidine, butorphanol and ketamine, with the medetomidine reversed using atipamezole and butorphanol reversed with naltrexone is a likely drug combination of choice.

Immobilizations will be conducted by either Dr Louis Van Schalkwyk, Dr Peter Buss, and Dr Lufuno Netshitavhadulu.

k. Experimental animal procedures

For all studies submitted to the AEC:

Explain the reasoning behind the study design and experimental planning, with particular reference to determination of sample size and statistical analysis. The use of flow charts is recommended. The information should be presented in an easily accessible manner.

For studies involving animals also:

Describe how the animals will be allocated to experimental and control groups and where applicable, how the experimental treatments will be assigned to each group.

l. Administration of all medicines / substances

i. Responsible person (Leave blank if N/A). *Dr. Louis van Schalkwyk*
ii. Qualification: *BVSc. MSc. PhD*.

List ALL substance administrations to the animals and give routes or administration, dosages per body mass including anaesthetics, analgesics and euthanasing agents. State who

is legally responsible for prescribing and directing the administration of the controlled Scheduled 3 - 6 medicinal substances and other controlled substances and provide their acceptance of this responsibility by signature.
Please note that it expected that animals experiencing painful conditions will be given appropriate analgesic and / or anaesthetic support.

Drug combinations considered and that has been used safely before:
Combination of choice:
Butorphanol 0.3mg/kg + Midazolam 0.15mg/kg + Medetomidine 0.05mg/kg
>Reversal: Naltrexone 1-2x butorphanol dose in mg + Atipamezole 5x Medetomidine dose in mg
OR
Butorphanol 0.3mg/kg + Azaperone, 0.12mg/kg; and Medetomidine, 0.12mg/kg (BAM) (+ Ketamine 0.5mg/kg at lower BAM dose)
>Reversal: Naltrexone 1-2x Butorphanol dose in mg + Atipamezole 5x Medetomidine dose in mg
OR
Fentanyl 0.05-0.1mg/kg (2.5mg maximum) + Xylazine 0.5-1mg/kg (40mg maximum)
>Reversal: Naltrexone 3x fentanyl dose in mg + Yohimbine 0.1-0.2mg/kg
OR
Ketamine 5mg/kg + Medetomidine 0.1mg/kg
>Reversal: Atipamezole 5x Medetomidine dose in mg
OR
Zoletil (Tiletamine / Zolazepam) 0.5mg/kg + Medetomidine 0.03-0.05mg/kg
>Reversal: Atipamezole 5x Medetomidine dose in mg

m. Severity of effects of the experimental procedures on the animals

List the procedures that may case deprivation, fear, distress and pain. Describe what sensations the animal may feel. Categorise these as minimal, intermediate or high.
***Give their likely duration in time.**
Describe what specific steps will be taken to alleviate these conditions through the use of ataractics, dissociative agents, analgesics, anaesthetics or other methods. Estimate how effective these are likely to be.

Capture techniques (minimal)
The animals could become injured by a misplaced dart, experiencing pain and fear. This is avoided by exercising extreme caution when darting, firing only at short range and with appropriate pressure, 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. With these mitigation measures, the pain associated with darting is likely to be minimal and very brief (typically <5mins from darting to recumbency).
There is a small risk of groups of animals being broken up by darting, experiencing stress and fear. However, this has never been reported from the multiple wild dog projects, involving hundreds of darting events, in which the study leaders are involved. 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) anaesthesia (minimal)
Animals 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 stable immobilization, and reviewing drug doses on an ongoing basis. Reversal agents are

kept on hand through immobilization. Animals' pulse and respiratory rates, and SpO_2 where possible, are monitored through immobilization, with early reversal or administration of respiratory stimulant (e.g. Dopram V) possible should this appear necessary. Such harm would involve animals which were anaesthetised so, while there would be a risk of harm, the animals would be unconscious and probably unaware of any sensation. 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.

(c) vaccination (minimal)
In principle, animals might be harmed as a result of adverse reactions to the vaccine; indeed, this possibility is a major reason for conducting the study. If the CDV-MLV vaccine were to cause clinical distemper, its welfare consequences would be severe: study animals would suffer life-threatening illness over a period of several days, probably ending in death. However, as detailed above, evidence from captivity indicates an extremely low probability of such harmful effects. The study is carefully designed to avoid and minimise such harm, first through a thorough evaluation in captivity prior to the field trial, and second through the staggered recruitment of animals to the trial, so that additional animals are vaccinated only if no harmful effects are observed in earlier recruits.

(d) collaring (minimal)
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. We are not aware of any reports of collar injuries in wild dogs. With appropriate collar design, any discomfort associated with wearing a collar should be minimal, although it would be likely to last for months or years until the collar is removed.

(e) sampling (minimal)
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. With care taken to minimise blood collection volume, immobilised animals are unlikely to experience any sensation from having had blood collected.

(f) recovery (minimal)
Animals might over-heat during recovery if they move out of the shade while still somewhat disoriented, experiencing fear and discomfort. 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 appear disorientated for approximately 10-15 mins on average. This period may be longer in animals given top-up doses of immobilising drugs (e.g., due to incomplete injection from the dart); this can be minimised by appropriate choice of drug doses and careful dart placement. The stress associated with this period of disorientation is minimised by moving people and vehicles away from the animal before reversal, observing quietly from a distance.
Animals are monitored carefully during recovery to allow intervention should ill effects be detected. However, no such problems have been encountered in hundreds of past wild dog immobilizations conducted by team members.

(g) release (minimal)
At release in the field, animals which are disoriented for longer periods (e.g. one to two hours if a drug top-up was administered), and might be harmed by larger carnivores (e.g. lions and hyaenas), or by people. Minimising the length of the recovery period through careful choice of drug doses (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 larger carnivores. 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.

(h) subsequent monitoring (minimal)
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.

n. Fate of animals and their disposal at the end of the study

Briefly state the fate of the experimental animals at the end of the study (e.g. rehabilitation and release, return to stock, euthanasia; released into its natural environment. What method of euthanasia is to be used, what humane rationale supports this choice and how the animals or animal carcasses are to be disposed of in a responsible and ecologically sound manner.

No euthanasia of study animals is anticipated as part of the study protocol. SANParks veterinarians would make decisions about euthanasia of animals in the field trial; for example, they might choose to euthanise an animal with clinical evidence of distemper, which was causing suffering and from which it was unlikely to recover. Separate from the study objectives, veterinarians might euthanise an animal on both conservation and welfare grounds if it was showing signs of neurological disease which might be rabies or wild-type distemper. Visual observations will be conducted every 2-3 days to check for such signs. 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).

o. Statistical analysis

Describe briefly how the data obtained from the study will be analysed statistically, explain this decision and state by whom the analyses will be performed.

The field trial will provide two key outcome variables, each analysed in a different way.

First, the field trial will generate an estimate of wild dog survival post-vaccination, which will be compared with that of unvaccinated controls. This comparison will be made by fitting a Cox proportional hazards model to the data, measuring the survival (in days post-vaccination) of vaccinated and unvaccinated animals. This approach is preferred because it analyses survival as a function over time, rather than a simple binary outcome (alive/dead). Covariates (e.g. age, dispersal status) can also be included in time-dependant models, and pack identity can also be accounted for.

Second, the field trial will generate estimates of CDV antibody titres in vaccinated vs control animals, and at different times relative to vaccination. Antibody titres can be difficult to analyse because serial dilutions mean they are not normally distributed. Basic analyses will therefore use logistic regression models, specifying a cut-off to classify individuals as seropositive/seronegative at specific time points.

Statistical analyses will be conducted under the guidance of Prof Christl Donnelly FRS, who is a prominent statistician in the UK, who has an honorary position at the University of Pretoria, and a long-term collaboration with project leader Prof Rosie Woodroffe.

p. Refinement

Describe the specific steps that have been taken to refine the experimental procedures to make them as humane as possible i.e. minimising the impact of the proposed procedures on the animals' wellbeing e.g. use of enrichment aids, analgesia, etc.

We have refined all elements of the project protocols to ensure they are as humane as possible

(i) *Our study is carefully designed to yield data to inform the conservation of wild dog populations while minimising the risks of harm to individual wild dogs. The vaccine to be used has been evaluated in captivity prior to this proposal for a field trial, and the field trial commences with a small number of animals, building a sample size sufficient to deliver statistical power only as confidence grows that the first animals have experienced no ill-effects. Numbers of vaccinates and controls have been carefully chosen to maximise statistical power while darting the minimum number of animals.*

(ii) *Darting and anaesthesia protocols have been refined over years to minimise the risk of harm, including careful selection of dart types, habituation of animals to vehicles to minimise stress and allow darting at close range to maximise dart accuracy, and refinement of drug types and doses.*

(iii) *Collar designs have been refined over the years to minimise both the weight and bulk of collars, reducing the strain on animals.*

(iv) *Handling protocols have been refined to minimise stress, for example including the use of eye shades.*

(v) *The vaccine brand to be used (Nobivac puppy DP) has been chosen to contain the safest vaccine strain (the Onderstepoort strain), which is judged to be least likely to revert to virulence.*

(vi) *The administration of reversal agents is carefully timed to minimise recovery time, with most animals alert and moving within 10 minutes of reversal.*

(vii) *All animals are carefully monitored to allow rapid intervention in the unlikely event of adverse effects being observed.*

(viii) *Visual observations are made from vehicles at a safe distance, approaching animals slowly and carefully to minimise the risk of disturbance.*

q. Monitoring of experimental animals

Describe who will be responsible for the care of the animals during the experimental period, and provide an indication of their experience and competence. Briefly state what clinical, physiological and/or behavioural criteria will be specifically monitored to access the animal's wellbeing e.g. weight gain/loss, food intake, vital parameters, etc. Please note that any study that has the potential to interfere with growth or cause weight loss, will need a minimum of weekly weight monitoring.

Mmadi Reuben will be primarily responsible for monitoring of experimental animals. He has 13 years' experience working in wildlife field projects including capture and deployment of telemetry collars. In the wild all monitoring will be conducted entirely from vehicles. 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.

On each sighting, the health of study animals will be assessed through visual observation, recording the presence or absence of clinical signs which might indicate distemper, such as ocular or nasal discharge, neurological signs, diarrhoea, etc.

r. End points for experiments in animals

Provide the points at which this study will be terminated

for welfare reasons e.g. percentage weight loss, injury, animals showing distress, pain, animals becoming moribund. Also provide how the monitoring towards these endpoints will be undertaken e.g. weekly weights, twice daily observations.

s. General veterinary care

Provide details, including emergency contact details, of the veterinarian who will be responsible to provide the general veterinary care and who will have the authority to enforce the endpoints stipulated under the previous point. The veterinarian must be registered or authorised to undertake procedures limited to that a treating veterinarian by the SAVC and is preferably independent of the research group. It is the responsibility of the veterinarian to arrange for a locum if he/she is not available.

i. Person responsible for veterinary care (Leave blank if N/A): *Dr. Louis van Schalkwyk*
ii. Emergency contact details: *+27 83 633 2203*
iii. Veterinarian resident Y/N; *Yes*
iv. Schedule of visits if not resident at study or research site: Resident at research site.

Schedule of visits if not resident at study or research site (e.g. weekly; for emergencies only; only available telephonically) and for off-site studies the distance of the study to the veterinarian

v. Management of injured animals

At inception study animals will be deployed with either VHF or Satellite collars which have mortality alert function. Post vaccination for each tranche monitoring team will endeavour to visualise the animals every 2 to 3 days for the first month where emphasis of will be on health of the individuals. Any animal showing signs of ill health will be reported to the veterinarian to attend. In the event of mortality effort will be made to recover the carcasses for autopsy.

t. Personnel activities

Describe the specific responsibilities and duties of EACH PERSON who will be involved with the procedures on animals, preferably in a tabular format.

Individual	Responsibility
Supervisor (Louis van Schalkwyk)	Responsible for veterinary care of the animal. Capture animals, deploy tracking collars and collect biological samples from the animals. Also undertakes treatment of ill animals and autopsy in the event of mortality.
SANParks Veterinarian (Dr. Peter Buss)	Responsible for veterinary care of the animal. Capture animals, deploy tracking collars and collect biological samples from the animals. Also undertakes treatment of ill animals and autopsy in the event of mortality.
SANParks Veterinarian (Dr. Lufuno Netshitavhadulu)	Responsible for veterinary care of the animal. Capture animals, deploy tracking collars and collect biological samples from the animals. Also undertakes treatment of ill animals and autopsy in the event of mortality.
Principal Investigator & PhD student (Dr. Mmadi Reuben)	Monitoring health of study animals and reporting any ill-health problems to the veterinarian.
Kruger Wild dog monitoring team members(Grant Beverley)	Monitoring health of study animals and reporting any ill-health problems to the veterinarian

u. Biohazard statement

Does the project pose any hazards to other animals and/or staff from the use of infective agents, toxic substances, carcinogenic agents or ionising radiation? If it does, state the specific safety procedures to be followed to contain these hazards and provide an approval statement from the Institutional Safety Officer. If available, you may append the laboratory's relevant SOPs and policies.

Scheduled immobilisation drugs will be handled according to local veterinary & pharmacological legislation by a qualified and authorised person, trained in first aid of accidental exposure to these drugs.
The study will not pose any biohazard to other animals or staff.
Contaminated materials will be destroyed by incineration.

i. Faculty (Leave blank if N/A)
ii. Name of officer

v. Declaration for studies needing external/other approval

Please provide a list of external/other approval that this following project requires e.g. section 16 approval from DAFF for research with animal disease; Section 17 approval from the MCC for any study involving an unregistered *medicine; TOPS approval*

Approval from the relevant Nature Conservation organisation and/or Provincial Authority to work with wild (SANParks research and AUCC approval)
species; Medical Ethics Approval
Section 20 approval from DLRRD

27. Genetically modified organisms (Y/N)

There will be no use of genetically modified organisms in the research project. A commercially available modified live vaccine for canine distemper virus and rabies will be used.

28. References

a. Reference list

[1]Woodroffe, R & Sillero-Zubiri, C. *African wild dog Red List Assessment*. (IUCN, 2013);

[2]Woodroffe, R *et al.* Hot dogs: high ambient temperatures influence reproductive success in a tropical mammal. *Journal of Animal Ecology* **86**, 1329-1338 (2017);

[3]Gascoyne, SC *et al.* Aspects of rabies infection and control in the conservation of the African wild dog (*Lycaon pictus*) in the Serengeti region, Tanzania. *Onderstepoort Journal of Veterinary Research* **60**, 415-420 (1993);

[4]Scheepers, JL & Venzke, KAE. Attempts to reintroduce African wild dogs *Lycaon pictus* into Etosha National Park, Namibia. *South African Journal of Wildlife Research* **25**, 138-140 (1995);

[5]Hofmeyr, M *et al.* Rabies in African wild dogs (*Lycaon pictus*) in the Madikwe Game Reserve, South Africa. *Vet. Rec.* **146**, 50-52 (2000);

[6]Hofmeyr, M *et al.* A second outbreak of rabies in African wild dogs (Lycaon pictus) in Madikwe Game Reserve, South Africa, demonstrating the efficacy of vaccination against natural rabies challenge. *Anim. Conserv.* **7**, 193-198 (2004);

[7]Alexander, KA *et al.* Multi-host pathogens and carnivore management in southern Africa. *Comp. Immunol. Microbiol. Infect. Dis.* **33**, 249-265 (2010);

[8]Prager, KC *et al.* The effect of protected areas on pathogen exposure in endangered African wild dog (Lycaon pictus) populations. *Biological Conservation* **150**, 15-22 (2012);

[9]Woodroffe, R *et al.* Contact with domestic dogs increases pathogen exposure in endangered African wild dogs (Lycaon pictus). *PLoS ONE* **7** (2012);

[10]Berentsen, AR *et al.* Rabies, canine distemper, and canine parvovirus exposure in large carnivore communities from two Zambian ecosystems. *Vector-Borne and Zoonotic Diseases* **13**, 643-649 (2013);

[11]Creel, S *et al.* Serosurvey for selected viral diseases and demography of African wild dogs in Tanzania. *Journal of Wildlife Diseases* **33**, 823-832 (1997);

[12]Alexander, KA *et al.* Canine distemper-related mortality among wild dogs (*Lycaon pictus*) in Chobe National Park, Botswana. *Journal of Zoo & Wildlife Medicine* **27**, 426-427 (1996);

[13]Goller, KV *et al.* Fatal canine distemper infection in a pack of African wild dogs in the Serengeti ecosystem, Tanzania. *Veterinary Microbiology* **146**, 245-252 (2010);

[14]van de Bildt, MWG *et al.* Distemper outbreak and its effect on African wild dog conservation. *Emerging Infectious Diseases* **8**, 211-213 (2002);

[15]Loots, AK *et al.* Phylogenetic analysis of canine distemper virus in South African wildlife. *PLOS ONE* **13**, e0199993 (2018);

[16]Du Plessis, C. *Canine distemper virus inoculations at HIP*. (https://wildlifeact.com/blog/canine-distemper-virus-inoculations-hip/, 2016);

[17]Mutinda, M *et al. Canine distemper outbreak in wild and domestic carnivores in Laikipia ecosystem of Kenya*. (Kenya Wildlife Service, 2017);

[18]Viana, M *et al.* Dynamics of a morbillivirus at the domestic–wildlife interface: Canine distemper virus in domestic dogs and lions. *Proceedings of the National Academy of Sciences* **112**, 1464-1469 (2015);

[19]Gilbert, M. *Understanding and managing canine distemper virus as a disease threat to Amur tigers*. (PhD thesis, University of Glasgow, 2016);

[20]Prager, KC *et al.* Rabies and canine distemper virus in wild and domestic carnivores in northern Kenya: Are domestic dogs the reservoir? *EcoHealth* **9**, 483 (2013);

[21]Keeling, MJ & Grenfell, BT. Disease extinction and community size: modeling the persistence of measles. *Science* **275**, 65-67 (1997);

[22]Swinton, J *et al.* Persistence thresholds for phocine distemper virus infection in harbour seal *Phoca vitulina* metapopulations. *Journal of Animal Ecology* **67**, 54-68 (1998);
[23]Rikula, UK. *Canine distemper in Finland – vaccination and epidemiology*. (PhD thesis, University of Helsinki, 2008);

[24]WHO/FAO/OIE. *Zero by 30: The global strategic plan to end human deaths from dog-mediated rabies by 2030*. (https://apps.who.int/iris/bitstream/handle/10665/272756/9789241513838-eng.pdf?ua=1, 2018);

[25]Chappuis, G. Control of canine distemper. *Veterinary Microbiology* **44**, 351-358 (1995);

[26]van Heerden, J *et al.* Clinical and serological response of wild dogs (Lycaon pictus) to vaccination against canine distemper, canine parvovirus infection and rabies. *J. S. Afr. Vet. Assoc.-Tydskr. Suid-Afr. Vet. Ver.* **73**, 8-12 (2002);

[27]Carpenter, JW *et al.* Fatal vaccine-induced canine distemper virus infection in black-footed ferrets. *Journal of the American Veterinary Medical Association* **169**, 961-964 (1976);

[28]Henke, SE. Effects of modified live-virus canine distemper vaccines in Gray Foxes. *J. Wildl. Rehabil.* **20**, 3-7 (1997);

[29]McCormick, AE. Canine distemper in African hunting dogs (*Lycaon pictus*) – possibly vaccine induced. *Journal of Zoo Animal Medicine* **14**, 66-71 (1983);

[30]Durchfeld, B *et al.* Vaccine-associated canine distemper infection in a litter of African hunting dogs (*Lycaon pictus*). *Zentralblatt für Veterinrmedizin B* **37**, 203-212 (1990);

[31]van Heerden, J *et al.* Distemper-like disease and encephalitozoonosis in wild dogs (*Lycaon pictus* Temminck, 1820). *Onderstepoort Journal of Veterinary Research* **48**, 19-21 (1989);

[32]Woodroffe, R. Assessing the risks of vaccination for captive African wild dogs. (in review);

[33]Visee, AM *et al.* The Mkomazi Project African wild dog Report 1997. (George Adamson Wildlife Preservation Trust, Netherlands, 1997).

[34]Connolly, M *et al.* Comparison of oral and intramuscular recombinant canine distemper vaccination in African wild dogs (*Lycaon pictus*). *Journal of Zoo and Wildlife Medicine* **44**, 882-888 (2013);

[35]Bronson, E *et al.* Serologic response to a canarypox-vectored canine distemper virus vaccine in the giant panda (Ailuropoda melanoleuca). *Journal of Zoo and Wildlife Medicine* **38**, 363-366 (2007);

[36]Sadler, RA *et al.* Evaluation of two canine distemper virus vaccines in captive tigers (*Panthera tigris*). *Journal of Zoo and Wildlife Medicine* **47**, 558-563 (2016);

[37]Birhanu, FM. in *The regulation of genetically modified organisms: comparative approaches* (eds L Bodiguel & M Cardwell) (Oxford University Press, 2010);

[38]Lau, E. Merial: PureVax for ferrets coming back this week. *https://news.vin.com/vinnews.aspx?articleId=22494* (2012);

[39]Hines, R. Vaccination of zoo animals, wild animals and exotic pets. *https://www.2ndchance.info/vaccination.htm* (2015);

[40]Ford, RB *et al. 2017 AAHA Canine Vaccination Guidelines*. (https://www.aaha.org/globalassets/02-guidelines/canine-vaccination/vaccination_recommendation_for_general_practice_table.pdf, 2017);

[41]Spencer, J & Burroughs, R. Antibody response to canine distemper vaccine in African wild dogs. *Journal of Wildlife Diseases* **28**, 443-444 (1992);

[42]Connolly, M *et al.* Single- versus double-dose rabies vaccination in captive African wild dogs (*Lycaon pictus*). *Journal of Zoo and Wildlife Medicine* **46**, 691-698 (2015);

[43]Warrell, MJ *et al.* Economical multiple-site intradermal immunization with human diploid-cell strain vaccine is effective for post-exposure rabies prophylaxis. *Lancet* **1**, 1059-1062 (1985);
[44]Thomas, PR *et al.* Birth and simultaneous rearing of two litters in a pack of captive African wild dogs (*Lycaon pictus*). *Zoo Biology* **25**, 461-477 (2006);

[45]NeoTech Vaccines. *Material Safety Data Sheet - NeoVac-D*. (https://neotechvaccines.com/images/MSDS-NeoVac-D.pdf, 2009);

[46]Moore, GE *et al.* Adverse events diagnosed within three days of vaccine administration in dogs. *Journal of the American Veterinary Medical Association* **227**, 1102-1108 (2005);

[47]Rhodes, S *et al. North American regional studbook – African wild dog (Lycaon pictus)*. (Association of Zoos and Aquariums/Chicago Zoological Society, 2007);

[48]Verberkmoes, W & Verberkmoes, H. *European regional studbook – African wild dog (Lycaon pictus)*. (GaiaPark, Kerkrade Zoo, 2007);

[49]Woodroffe, R *et al.* Within- and between-group dynamics in an obligate cooperative breeder. *Journal of Animal Ecology*, doi: 10.1111/1365-2656.13102. (2019);

[50]Smallwood, T. *Modelling multi-host viral pathogens for biodiversity conservation*. (PhD thesis, Imperial College London, in prep);

[51]Woodroffe, R *et al.* Effects of double-dose rabies vaccination on endangered African wild dogs (Lycaon pictus): a field trial. (in prep.);

[52]European Medicines Agency. *Guideline on requirements for First-in-Man clinical trials for potential high-risk medicinal products*. (http://www.emea.europa.eu/pdfs/human/swp/2836707en.pdf, 2007);

[53]Dhand, NK & Khatkar, MS. *Statulator: An online statistical calculator. Sample Size Calculator for Comparing Two Independent Proportions*. (http://statulator.com/SampleSize/ss2P.html, 2014);

[54]McNutt, JW *et al.* Ambient temperature provides an adaptive explanation for seasonal reproduction in a tropical mammal. *Journal of Zoology*, doi: 10.1111/jzo.12712 (2019).

29. Declaration (note copies of permits will need to be attached)

- **a. The research will be done in accordance with all relevant policies of the University of Pretoria. (Y/N)***Yes*
- **b. Has the research begun without ethical approval (Y/N)***No*
- **c. Approval for Section 20 (Animal Disease Act 1984) from DAFF for research with animal diseases (Y/N).** *Submitted pending approval***.**
- **d. Approval for Section 21 from the MCC for any studies involving an unregistered medicine (Y/N).** *N/A***.**
- **e. TOPS permit for approval to work with endangered species (Y/N).***Yes***.**
- **f. Approval from the relevant Nature Conservation organization and/or Provincial Authority to work with wild species (Y?N).***Yes***,** *SANParks research permit and AUCC approval***.**

31. Attach documents

a. Suggested documents to attach in so far relevant

b. Please note that if you choose not to attach the mentioned documents, it will delay your application substantially and you will most likely be requested to attach the documents in any case. If you still choose not to attach the relevant documents, please provide an explanation for the omission of the information.

Personalize | Find | | First **1-7 of 7** Last

Document Type	Description	Add Docs	Required	Omission Reason
Research	Research proposal			
OthCountry	Local approval of Another Country			
TOPS	Approval permit from TOPS (endangered species)			
Nature Con	Approval letter from Nature Conservation (wild species)			
Prov Auth	Approval letter from Provincial Authority (wild species)			
DAFF	Approval letter from DAFF (diseases)			
MCC	Approval letter from MCC (unregistered medicine)			

c. Add other relevant documents

Galarreta, Angela A

From: Galarreta, Angela A
Sent: Wednesday, February 7, 2024 5:44 PM
To: jhb19@cornell.edu
Subject: CS3888509 - 3-200-37e: Import/Export/Re-Export of biological specimens (CITES/ESA) for scientific research

Hello,

My name is Angela and I am the biologist assigned to your ESA application, CS3888509. First, I want to apologize for missing your communications through ePermits. It seems that the email notifications were being redirected another folder. However, I have reviewed your application and find that we require additional information to move forward with our review. Please provide the following:

1. Per question 6.f., the description of packaging, please provide an estimate for the size/dimensions of packaging and the estimated amount of sample each package will contain (e.g, 5-mL glass vials containing up to 3 mL whole blood). Also, please note if the number of samples is not consistent with the amount of packaging. For example, if each vial contains two nasal swabs and you have a total of 32 nasal swabs then the number of vials used for the nasal swab samples would be 16.
2. In response to question 8.d., the name of the individual(s) who collected the animals/samples, the application stated "Sample collection procedure done by Dr. Louis van Schalkwyk and Dr. Mmadi M. Reuben." However, in response to question 12, CV or resume of the researchers and field technicians collecting samples, the application only included a CV for Dr. van Schalkwyk. Additionally, we note that some of the documents provided indicated that Dr. Peter Buss and Dr. Lufuno Netshitavhadulu would also be responsible for collecting biological sample. If any individuals other than Dr. van Schalkwyk collected samples requested for import then we will need their CV or resume including experience with the species.
3. Also in response to 8.d., the application indicated that there were several authorizations which were supposed to attached including the "South African National Parks AUCC approval, University of Pretoria AEC approval, DALRRD Section 20 permit approval, TOPS permit." It is clear which document is the AUCC approval but it's not clear to me based on the documents or their file names which is the AEC approval or Section 20 permit or TOPS permit. Please clarify or provide the documents with file names to indicate which is which.

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, **March 23, 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.*

Thank you,

Angela Galarreta, M.S. (she/her)
Senior Biologist
Division of Management Authority
U.S. Fish and Wildlife Service
5275 Leesburg Pike, MS: IA
Falls Church, Virginia, 22041-3803, USA

https://www.fws.gov/program/international-affairs
https://fwsepermits.servicenowservices.com/fws/

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

13 October 2022

Approval Certificate
New Application

AEC Reference No.: REC078-22
Title: Can vaccination protect African wild dogs from canine distemper? Addressing a conservation emergency
Researcher: A/Pr RW Woodroffe
Student's Supervisor: Dr OL van Schalkwyk

Dear A/Pr RW Woodroffe,

The **New Application** as supported by documents received between 2022-08-22 and 2022-09-26 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2022-09-26.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	Number
Wild Dogs - KNP	32
Samples	Number
Blood (Samples from live animals)	96
Nasal swabs (Samples from live animals)	32
Rectal swabs (Samples from live animals)	32

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2023-10-13.

3. Please remember to use your protocol number (REC078-22) on any documents or correspondence with the AEC regarding your research.

4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.

6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

We wish you the best with your research.

Yours sincerely

Prof Andrew McKechnie
Acting Chairperson: UP-Animal Ethics Committee

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

ANIMAL USE AND CARE COMMITTEE: APPROVAL CERTIFICATE

A. PROJECT DETAILS

Project Title	**Can vaccination protect African wild dogs from canine distemper? Addressing a conservation emergency.**		
Researcher	**R Woodroffe**	SANParks Reference No.	**03-22**

B. CONDITIONS OF APPROVAL

- There must be an approved and signed research contract with SANParks prior to implementation of this project.
- Ethics approval is valid for the duration of the SANParks research contract.
- Any changes to the original research protocol must be submitted in the appropriate format to the AUCC for evaluation and approval.
- The AUCC must be informed of mortalities or injuries beyond those expected in the approved research protocol.

Submission Date:	**17 May 2022**	APPROVED
AUCC Approval Date:	**22 August 2022 2022**	Signature:

Note: In accordance with the South African National Standard (SANS 10386-2008): "The Care and Use of Animals for Scientific Purposes", an animal is regarded as being "live, sentient non-human vertebrate, including eggs, foetuses and embryos, that is, fish, amphibians, reptiles, birds and mammals, including domestic animals, purpose-bred animals, farm animals, wildlife and higher invertebrates such as advanced members from the Cephalopoda and Decapoda".

addo elephant
agulhas
lAi-lAis / richtersveld
augrabies
bontebok
camdeboo
garden route
golden gate highlands
groenkloof
karoo
kgalakgadi transfronti
kruger
mapungubwe
marakele
mokala
mountain zebra
namaqua
table mountain
tankwa karoo
west coast

University of Pretoria, Faculty of Vet. Scie., Dept. of Vet. Tropical Diseases
P/Bag X04 Onderstepoort 0110, Pretoria
+267 72628121/+27765801676
[redacted]

MMADI MOGOLODI BOKANG REUBEN

PROFILE

A veterinarian experienced in disease surveillance & investigations as well as wildlife & conservation medicine. I also have experience in public administration with good skills in conservation policy analysis and conservation planning. I have undertaken international large scale conservation projects (endangered wild animals' translocation and range expansion) and collaborated in extensive wildlife research work. I have special interest in veterinary infectious diseases, immunology, human-wildlife conflict, and the application of decision science in conservation.

Bio

[redacted]

Languages

[redacted]

SKILLS & ABILITIES

Problem solving
Leadership
Adaptability
Policy analysis and Implementation
Communication
Strategic Planning
Decision science
Research methods
Animal tracking

EMPLOYMENT HISTORY

Department of Wildlife and National Parks:

- ***Chief Veterinary Officer***, *May 2021- present*
- ***Principal Veterinary Officer I***, *July 2012 – May 2021*
- ***Principal Veterinary Officer II***, *May 2010 – June 2012*
  - Head of Veterinary Wildlife Division,
  - Coordinates monitoring, investigation, and management of diseases in open wildlife populations,
  - Advice on policy formulation and implementation,
  - Innovate on human-wildlife conflict mitigation measures,
  - Coordinate wildlife capture and translocation,
  - Coordinate the country's endangered species recovery plan,
  - Foster strategic partnerships between department and stakeholders,
  - Represent department in different forums,
  - Public education,
  - Customer service,
  - Responsible for Divisional budget.

- ***Veterinarian,*** *Oct 2008 – May 2010*

Veterinary and Agricultural Consultants (Clinical practice):

- ***Veterinarian:*** *May 2004 – Sept 2008*
  - *Investigation and treatment of diseases in domestic animals*
  - *Provide advisory services for livestock businesses.*
  - *Accredited mentor for Citizen Entrepreneurial Development Agency funded agrobusiness startups.*
  - *Staff supervision*
  - *Customer care*

AFFILIATIONS

Zoological Society of London: Institute of Zoology

- *Postgraduate Scholar: Science directorate*

March 2022 -present

University of Pretoria: Faculty of Veterinary Science

- *PhD Candidate:*
  1. *Department of Veterinary Tropical Diseases*
  2. *Centre for Veterinary Wildlife Research*

March 2022 – present

EDUCATION

• *Bachelor of Veterinary Medicine: 2004*, **University College Dublin, Ireland**

• *MSc (Animal Science – Breeding and Reproduction):2019*, **University of Botswana and Botswana University of Agriculture and Natural Resources.**

CONTINUOUS PROFESSIONAL DEVELOPMENT

• Training on Highly Pathogenic Avian Influenza outbreak response: Eswatini 2009

• Convergence of Veterinary Science, Public Health and Trade for Sustainable Livelihood in Sub-Saharan Africa: Uganda 2009

• Wild birds and wildlife capture & disease surveillance: Zim 2010

• Strategic Planning course: IDM 2015

• Chemical and Physical Restraint of African Wildlife: Zim 2016

• Advanced course in Wildlife Chemical Immobilisation and Field Practice: South Africa 2016

• Leadership and Management Development: IDM 2016

• Induction workshop for Board member: 2017

• Leadership and Management Theories: Uni. Of South Wales: 2019

• Strategic Analysis; Tools and Techniques: Uni. Of South Wales: 2019

• Conservation Planning Specialist Group's Facilitating Species Conservation Planning Workshops: 2022

PROFESSIONAL MEMBERSHIP

• Botswana Veterinary Surgeons Council

Registered member (200405070)

• Botswana Veterinary Surgeons Council

Chair Private Practice Licensing (2008 -2021)

• Wild Dog Advisory Group

Member (2020 - present)

• IUCN: African Rhino Specialist Group

Member (2017 - present)

• IUCN: African Elephant Specialist Group

Member (current)

• Botswana Livestock Improvement Board

Vice Chair (2020 -2022)

• IUCN: Wildlife Health Specialist Group

Former member (2015-2020)

SPECIAL ASSIGNMENTS

- Secretariat Member to Botswana Cabinet sub-committee on Social Dialogue on hunting (2018),
- Secretary to the Reference Group on development of the Botswana National Elephant Action Plan (2018),
- Coordinator – Mass rhino relocation project to Botswana (2012 – 2016),
- Botswana National Rhino coordinator (2018 – 2022),
- Committee member for the drafting of Game ranching regulations (2012)
- Committee secretary for development of captive carnivore guidelines in Botswana (2015),
- Committee Chair on development of Veterinary Private Practice Standards for Botswana (2008-2021),
- Subcommittee member for the development of the African Rhino Conservation Framework (2022),

REFERENCES

Dr. James K. Sento Ms. Malebogo Somolekae Dr. Kobedi Segale

agriculture, land reform
& rural development
Department:
Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Land Reform and Rural Development Private Bag X138, Pretoria 0001
Enquiries: Ms Marna Laing • Tel: +27 12 319 7442 • Fax: +27 12 319 7470 • E-mail: MarnaL@dalrrd.gov.za
Reference: 12/11/1/1/6 (2678 AC)

Dr Mmadi Mogolodi Reuben
Department Veterinary Tropical Diseases
University of Pretoria
E-mail: rosie.woodroffe@ioz.ac.uk [redacted]

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)

Dear Dr Reuben,

Your fax / memo / letter/ Email received 2022-09-12, requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers. I am pleased to inform you that permission is hereby granted to perform the following research/study, with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. The research project is approved as per the application form received 2022-09-12 and the correspondence thereafter. Written permission from the Director: Animal Health must be obtained prior to any deviation from the conditions approved for this research project under this Section 20 permit. Please apply in writing to MarnaL@dalrrd.gov.za;
3. Ethics approval must be obtained prior to the start of the study;
4. Permission in terms of the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No 36 of 1947) and/or the Medicines and Related Substances Control Act, 1965 (Act No 101 of 1965) may be needed prior to the start of the study;
5. Only the locally registered Nobivac DP vaccine may be used for the purposes of this study to vaccinate free-roaming wild dogs (*Lycaon pictus*) in the Kruger National Park, as indicated;
6. Blood samples, nasal swabs and faecal samples may be collected from the wild dogs (*Lycaon*

pictus) in the Kruger National Park, for which a state veterinary letter has been received. Samples may be sent to the Skukuza State Veterinary Laboratory for processing and storage, before being sent to Cornell University for testing, or the SANParks biobank for long term storage;

7. Samples must be packaged and transported in accordance with International Air Transport Association (IATA) requirements and/or the National Road Traffic Act, 1996 (Act No. 93 of 1996);
8. Wild dog samples (as per point 6) may be sent to the Animal Health Diagnostic Centre, Cornell University, USA (screening for CDV antibodies and CDV Antigen testing). The researcher must ensure that the samples comply with the import conditions as set by the importing country;
9. The relevant movement permit must be obtained from the responsible state veterinarian to move the collected samples from the Skukuza State Veterinary Laboratory directly to OR Tambo international airport, for export to the USA;
10. No challenge studies may be conducted as part of this study;
11. All potentially infectious material utilised or collected during the study is to be destroyed at the completion of the study using incineration, as indicated. Records must be kept for five years for audit purposes;
12. Aliquots of serum and whole blood samples may be stored under access control in the freezers of the SANParks Biobank, KNP;
13. Samples or material may not be outsourced or used for further/ other research without prior written approval from the Director: Animal Health;
14. If required, an application for an extension must be made by the responsible researcher at least one month prior to the expiry of this Section 20 permit. Please apply in writing to MarnaL@dalrrd.gov.za;
15. This Section 20 approval is valid until December 2023.

Title of research/study: "*Can vaccination protect African wild dogs from canine distemper? Addressing a conservation emergency*"
Researcher: Dr Mmadi Mogolodi Reuben
Institution: Department Veterinary Tropical Diseases, University of Pretoria in collaboration with the Institute of Zoology, Zoological Society of London, UK, and the Animal Health Diagnostic Centre, Cornell University, USA
Your Ref./ Project Number: SANParks AUCC approval 03-22
Our ref Number: 12/11/1/1/6 (2678 AC)

Kind regards,

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date: 2022 -09- 27

Galarreta, Angela A

From: Mmadi Mogolodi Bokang Reuben <[redacted]
Sent: Tuesday, February 27, 2024 7:35 AM
To: Jen Powers
Cc: Rosie Woodroffe
Subject: Re: Application for FWS permit- RSA wild dog samples to Cornell University
Attachments: AUCC_Aproval_Certificate_SANParks.pdf; AEC_Approval_Certificate.pdf; TOPS_Permit.pdf; DALRRD_Section_20_PermitApproval.pdf; CV_Mmadi_2024.pdf

Hie Jan, sorry for the delayed response, wanted to contact the courier company on how they would do the final packaging.

Per question 6.f., the description of packaging, please provide an estimate for the size/dimensions of packaging and the estimated amount of sample each package will contain (e.g, 5-mL glass vials containing up to 3 mL whole blood). Also, please note if the number of samples is not consistent with the amount of packaging. For example, if each vial contains two nasal swabs and you have a total of 32 nasal swabs then the number of vials used for the nasal swab samples would be 16.
There will be only one shipment for all samples, I was hoping by February the permit would be ready and send the first batch of samples.
The packaging;
A GDI 30-liter box.
Inside the GDI box will be a 25-liter Biobag.
Inside a 25 liter Biobag; 96 x 1.8 ml (each filled with 1.8 ml serum) cryovials, 64 x 5 ml (each containing one swab) cryovials and 15 kg of dry ice.

In response to question 8.d., the name of the individual(s) who collected the animals/samples, the application stated "Sample collection procedure done by Dr. Louis van Schalkwyk and Dr. Mmadi M. Reuben." However, in response to question 12, CV or resume of the researchers and field technicians collecting samples, the application only included a CV for Dr. van Schalkwyk. Additionally, we note that some of the documents provided indicated that Dr. Peter Buss and Dr. Lufuno Netshitavhadulu would also be responsible for collecting biological sample. If any individuals other than Dr. van Schalkwyk collected samples requested for import then we will need their CV or resume including experience with the species.
During planning, we included members of the vet team for SANParks as they could assist with animal immobilisations. But in the end all animals were immobilised and sampled by Louis van Schalkwyk and Mmadi Reuben (I have attached my CV).

Also in response to 8.d., the application indicated that there were several authorizations which were supposed to attached including the "South African National Parks AUCC approval, University of Pretoria AEC approval, DALRRD Section 20 permit approval, TOPS permit." It is clear which document is the AUCC approval but it's not clear to me based on the documents or their file names which is the AEC approval or Section 20 permit or TOPS permit. Please clarify or provide the documents with file names to indicate which is which.
I have renamed the attached documents as required

Kind regards
Mmadi

On Thu, Feb 8, 2024 at 5:14 PM Jen Powers <jhb19@cornell.edu> wrote:

> Hi Mmadi,

I heard back from US Fish and Wildlife on your permit application. They are requesting the following information/clarification:

1. Per question 6.f., the description of packaging, please provide an estimate for the size/dimensions of packaging and the estimated amount of sample each package will contain (e.g, 5-mL glass vials containing up to 3 mL whole blood). Also, please note if the number of samples is not consistent with the amount of packaging. For example, if each vial contains two nasal swabs and you have a total of 32 nasal swabs then the number of vials used for the nasal swab samples would be 16.
2. In response to question 8.d., the name of the individual(s) who collected the animals/samples, the application stated "Sample collection procedure done by Dr. Louis van Schalkwyk and Dr. Mmadi M. Reuben." However, in response to question 12, CV or resume of the researchers and field technicians collecting samples, the application only included a CV for Dr. van Schalkwyk. Additionally, we note that some of the documents provided indicated that Dr. Peter Buss and Dr. Lufuno Netshitavhadulu would also be responsible for collecting biological sample. If any individuals other than Dr. van Schalkwyk collected samples requested for import then we will need their CV or resume including experience with the species.
3. Also in response to 8.d., the application indicated that there were several authorizations which were supposed to attached including the "South African National Parks AUCC approval, University of Pretoria AEC approval, DALRRD Section 20 permit approval, TOPS permit." It is clear which document is the AUCC approval but it's not clear to me based on the documents or their file names which is the AEC approval or Section 20 permit or TOPS permit. Please clarify or provide the documents with file names to indicate which is which.

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, **March 23, 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.*

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: Mmadi Mogolodi Bokang Reuben [redacted]>
Sent: Wednesday, October 18, 2023 5:04 AM
To: Jen Powers <jhb19@cornell.edu>
Cc: Rosie Woodroffe <Rosie.Woodroffe@ioz.ac.uk>
Subject: Re: Application for FWS permit- RSA wild dog samples to Cornell University

Hie Jen

Please receive the application documents for the export permit. I have tried to include as much information as possible including supporting documents.

And one issue to clarify;

I have included dry nasal and rectal swabs for CDV antigen screening. These are only frozen, no transport media, would these be suitable for your tests?

Kind regards

Mmadi

On Tue, Oct 10, 2023 at 8:55 PM Jen Powers <jhb19@cornell.edu> wrote:

> Hi Mmada,
>
> I am well, thank you. Sorry for the delay in response. A few questions to refresh my memory.
>
> 1. Is this application for the collection of samples from animals in the field in South Africa?
>
> 2. Do you still plan to submit samples under the first permit from the captive animals in the UK? I don't believe we have received them.
>
> 3. Recommendations from the permit reviewer regarding the new permit application: I would recommend they be thorough in describing methods of collection including measures to mitigate injury and mortality. Just for their awareness, they can also request the permit be multi-use if multiple imports of samples will be needed.
>
> I have attached the permit application to get us started. Please disregard page 1 as that will need to be completed on our end.

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: Mmadi Mogolodi Bokang Reuben [redacted]>
Sent: Thursday, October 5, 2023 5:49 AM
To: Jen Powers <jhb19@cornell.edu>; Rosie Woodroffe <Rosie.Woodroffe@ioz.ac.uk>
Subject: Application for FWS permit- RSA wild dog samples to Cornell University

Hie Jen

Hope you are well, I am at that stage where I feel ready to start the application process for African Wild dog samples exportation to the US.

All study animals have been recruited so at this stage I have all the ID's for the animals under observation. I also have paired one-month samples for most animals. The remainder of the one-month samples will be collected by the end of this month (October).

I wish to apply for a multiple-use permit to be able to export samples in two batches, the first batch will be ready for dispatch from mid-November onwards. The second dispatch will be ready for dispatch beginning of June 2024.

Thanks for help.

Kind regards

> Mmadi Reuben

--

Mmadi Reuben

--
Mmadi Reuben

STANDING PERMIT

(Issued in terms of the provisions of the National Environmental Management: Biodiversity Act 2004, Act 10 of 2004)

PERMIT NUMBER: S 65757

NAME OF ISSUING AUTHORITY	
NAME	DEPT OF ENVIRONMENTAL AFFAIRS
ADDRESS	PRIVATE BAG X 447
	PRETORIA 0001
PROVINCE	NATIONAL DEPARTMENT

	PROVINCIAL DEPARTMENT	NATIONAL DEPARTMENT	
	PROTECTED AREA MANAGEMENT AUTHORITY	VETERINARIAN	X
	REGISTERED CAPTIVE BREEDING OPERATION	REGISTERED SCIENTIFIC INSTITUTION	X
	REGISTERED SANCTUARY	REGISTERED REHABILITATION FACILITY	
	REGISTERED COMMERCIAL EXHIBITION FACILITY	REGISTERED GAME FARM	
	REGISTERED WILDLIFE TRADER	REGISTERED NURSERY	

UNIQUE REGISTRATION NUMBER N78/770713 5156 084

DETAILS OF HUNTER

NAME	University of Pretoria	ID NO.	770713 5156 084
SURNAME	C/o Vinny Naidoo	PASSPORT NO.	

	POSTAL ADDRESS	RESIDENTIAL ADDRESS
ADDRESS	University of Pretoria	University of Pretoria
ADDRESS	Faculty of Veterinary Science	Faculty of Veterinary Science
ADDRESS	Private Bag X04	Old soutpan Road
TOWN	Onderstepoort	Onderstepoort
POSTAL CODE	0110	0110
PROVINCE	Gauteng	Gauteng

PROPERTY WHERE RESTRICTED ACTIVITIES WILL BE CARRIED OUT

NAME AND SURNAME OF APPLICANT	University of Pretoria
NAME AND SURNAME: RESPONSIBLE PERSON	Vinny Naidoo
NAME AND SURNAME OF AGENT	
PHYSICAL ADDRESS OF FACILITY	
PHYSICAL ADDRESS OF FACILITY	See attached annexure A
DISTRICT	
PROVINCE	
REGISTERED NAME AND NUMBER (in the case of game farm)	

DETAILS OF SPECIES INVOLVED

SPECIES		SEX (if known)	QUANTITY	MARKING (if applicable)
COMMON NAME	SCIENTIFIC NAME			
All avian, reptile, mammalian and fish species listed as threaned or protected in terms of section 56 of the National Environmental management Biodiversity Act (Act No 10 of 2004)				

DETAILS OF RESTRICTED ACTIVITIES INVOLVED

Collection, gathering, receiving, transport, moving, receiving as donation, giving, temporary possession, exercise physical control over, Darting and Capturing.

PERMIT VALIDATION

PERIOD OF VALIDITY	FROM:	11-02-2022	TO:	02-02-2025
RECEIPT NUMBER				

DEPT VAN OMGEWINGSAKE
PRIVAATSAK / PRIVATE BAG X 447
PRETORIA 0001
DEPT OF ENVIRONMENTAL AFFAIRS

SIGNATURE ISSUING OFFICER:

SIGNATURE PERMIT HOLDER:

DATE STAMP: 2022 -02- 11

STANDING PERMIT CONDITIONS

1. This permit is not transferable.
2. Any unauthorised alteration to this permit shall render it invalid.
3. This permit is subject to the provisions of any applicable law in force during the period of validity of the permit.
4. This permit is valid only within the province where it was issued,
5. The holder of this permit shall, at the request of a person authorised in terms of applicable legislation so to demand, forthwith produce such permit to such person.
6. This permit shall be invalid until such time that it is signed by the permit holder.
7. This permit shall be deemed invalid when it is lost or destroyed and no copy thereof shall be issued.
8. This permit may be withdrawn by an authorised person if the execution of any activity may be' detrimental to the welfare of any wild animal or the safety of any person, provided that the permit holder is given notice of such intention and be granted the opportunity to appeal to such withdrawal.
9. The prescribed fees paid for the issuing of this permit shall not be refunded.
10. If the holder of this permit contravenes or fails to comply with any permit condition or requirement to which this permit is subject, he or she shall be guilty of an offence.
11. This permit shall be subject to any applicable norms and standards in existence at the time of issuance of this permit.

DEPT. VAN OMGEWINGSAKE
PRIVAATSAK / PRIVATE BAG X 447
2022 -02- 11
PRETORIA 0001
DEPT. OF ENVIROMENTAL AFFAIRS

SPECIAL CONDITIONS

1. The issuing authority shall determine the species and restricted activities for which this permit will apply.
2. If this permit applies to a registered game farm, the holder of this permit must:
   a. Have a copy of this permit authorising the hunt, on his or her person during the hunt;
   b. Within 21 days after the hunt, furnish the issuing authority with a written return on the hunt stating:
      (i) the permit number and date of issuance of the permit;
      (ii) the species, sex and number of animals hunted; and
      (iii) the location where the hunt took place.
   c. Return all used copies of the game farm hunting permits within 3 weeks after the end of the calendar year following the issuance of the game farm hunting permit, to the Issuing Authority.

ANNEXURE A

STANDING PERMIT

(Issued in terms of the provisions of the National Environmental Management: Biodiversity Act 2004, Act 10 of 2004)

Standing permit no: S 65757

Registration certificate no:02251

NAME OF ISSUING AUTHORITY	
NAME	DEPARTMENT OF FORESTRY, FISHERIES AND THE ENVIRONMENT
ADDRESS	PRIVATE BAG X 447
	PRETORIA 0001
PROVINCE	NATIONAL DEPARTMENT

DETAILS OF SPECIES INVOLVED

All avian, reptile mammalian and fish species that are listed as threatened or protected in terms of section 56 of the National Environmental Management: Biodiversity Act (Act No.10 of 2004).

DETAILS OF RESTRICTED ACTIVITIES INVOLVED AND SPECIAL CONDITIONS

1. This permit authorizes the Faculty of Veterinary Science in the University of Pretoria to carry out the following restricted activities:

   Collection/gathering of samples
   Receiving;
   Transporting or moving;
   Receiving as donation;
   Giving;
   Temporary possession/ exercise physical control over;
   Darting and
   Capturing

2. This permit authorizes the Faculty of Veterinary Science of the University of Pretoria to carry out the restricted activities as specified in paragraph 1 of this permit, involving blood and tissue samples of listed threatened or protected avian, reptiles, fish and mammalian species, for diagnostic analysis and research purposes.

3. This permit authorizes the Faculty of Veterinary Science of the University of Pretoria to receive, and temporary keep live specimens of listed threatened or protected mammalian, avian, fish and reptile species for treatment purposes.

4. This permit authorizes the carrying of the restricted activities as specified in paragraph 2 and 3 of this permit, only by employees and registered students carrying out restricted activities on behalf of the Faculty of Veterinary Science of the University of Pretoria.

5. This permit authorizes the restricted activities, and for the purpose as specified in paragraph 2 of this permit within the boundaries of the Republic of South Africa.

6. The holder of this permit may give/receive blood and tissue samples of listed threatened or protected avian, reptiles, mammalian and fish species, only to or from a person who is in possession of a permit issued in terms of the National

Environmental Management: Biodiversity Act, 2004 (Act No.10 of 2004), that authorizes the possession and receiving/giving/donation of such specimens.

7. Blood or tissue samples collected/received in terms of this permit may not be offered for sale.

8. In the event that the permit holder receives specimens of listed threatened or protected avian, reptiles, mammalian and fish species, the permit holder must report in writing, by the end of each year during the period of validity of this permit, to the Department of Forestry, Fisheries and the Environment (DFFE) the following information:

   (a) Name and physical address of person who the specimens have been received from;
   (b) Permit number of the person who the specimens have been received from (where applicable); and
   (c) Particulars of the species, number and type of specimens received and markings (where applicable).

9. The permit holder must make the report available to the DFFE, upon request from DFFE.

10. All restricted activities involving rhinoceros must be carried out in compliance with the Norms and Standards for the marking of the rhinoceros and rhinoceros' horn and for the hunting of the rhinoceros for trophy hunting purposes as published in terms of the National Environmental Management: Biodiversity Act, 2004 (Act No. 10 of 2004).

11. The permit holder must comply with the National Norms and Standards for the Management of Elephants in South Africa as published in terms of the National Environmental Management: Biodiversity Act, 2004 (Act No. 10 of 2004).

12. The carrying out of the restricted activities referred to in paragraph 1 by the permit holder is subject to the provisions of the Threatened or Protected Species regulations.

13. This permit does not absolve the permit holder to obtain any permit that may be required in terms of any other applicable legislation.

PERMIT VALIDATION				
PERIOD OF VALIDITY	**FROM :**	11-02-2022	**TO:**	02-02-2025
SIGNATURE OF ISSUING OFFICER			**SIGNATURE PERMIT HOLDER**	

DATE STAMP:

DEPT. VAN OMGEWINGSAKE
PRIVAATSAK / PRIVATE BAG X 447
2022 -02- 11
PRETORIA 0001
DEPT. OF ENVIROMENTAL AFFAIRS

Galarreta, Angela A

From: Jen Powers <jhb19@cornell.edu>
Sent: Wednesday, February 28, 2024 9:53 AM
To: Galarreta, Angela A
Subject: [EXTERNAL] RE: CS3888509 - 3-200-37e: Import/Export/Re-Export of biological specimens (CITES/ESA) for scientific research
Attachments: Re: Application for FWS permit- RSA wild dog samples to Cornell University

Follow Up Flag: Flag for follow up
Flag Status: Flagged

Hi Angela.

Please find attached email with the additional information requested. I have also uploaded the attachments in FWS.

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: Galarreta, Angela A <angela_galarreta@fws.gov>
Sent: Wednesday, February 7, 2024 5:44 PM
To: Jen Powers <jhb19@cornell.edu>
Subject: CS3888509 - 3-200-37e: Import/Export/Re-Export of biological specimens (CITES/ESA) for scientific research

Hello,

My name is Angela and I am the biologist assigned to your ESA application, CS3888509. First, I want to apologize for missing your communications through ePermits. It seems that the email notifications were being redirected another folder. However, I have reviewed your application and find that we require additional information to move forward with our review. Please provide the following:

1. Per question 6.f., the description of packaging, please provide an estimate for the size/dimensions of packaging and the estimated amount of sample each package will contain (e.g, 5-mL glass vials containing up to 3 mL whole blood). Also, please note if the number of samples is not consistent with

the amount of packaging. For example, if each vial contains two nasal swabs and you have a total of 32 nasal swabs then the number of vials used for the nasal swab samples would be 16.

2. In response to question 8.d., the name of the individual(s) who collected the animals/samples, the application stated "Sample collection procedure done by Dr. Louis van Schalkwyk and Dr. Mmadi M. Reuben." However, in response to question 12, CV or resume of the researchers and field technicians collecting samples, the application only included a CV for Dr. van Schalkwyk. Additionally, we note that some of the documents provided indicated that Dr. Peter Buss and Dr. Lufuno Netshitavhadulu would also be responsible for collecting biological sample. If any individuals other than Dr. van Schalkwyk collected samples requested for import then we will need their CV or resume including experience with the species.
3. Also in response to 8.d., the application indicated that there were several authorizations which were supposed to attached including the "South African National Parks AUCC approval, University of Pretoria AEC approval, DALRRD Section 20 permit approval, TOPS permit." It is clear which document is the AUCC approval but it's not clear to me based on the documents or their file names which is the AEC approval or Section 20 permit or TOPS permit. Please clarify or provide the documents with file names to indicate which is which.

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, **March 23, 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.*

Thank you,

Angela Galarreta, M.S. (she/her)
Senior Biologist
Division of Management Authority
U.S. Fish and Wildlife Service
5275 Leesburg Pike, MS: IA
Falls Church, Virginia, 22041-3803, USA

https://www.fws.gov/program/international-affairs
https://fwsepermits.servicenowservices.com/fws/

Galarreta, Angela A

From: Galarreta, Angela A
Sent: Tuesday, March 12, 2024 12:55 PM
To: Jen Powers
Subject: Re: [EXTERNAL] RE: CS3888509 - 3-200-37e: Import/Export/Re-Export of biological specimens (CITES/ESA) for scientific research

Hello Jen,

Thank you for the clarification and additional documentation.

After review of the additional information, I just have a few follow up questions. Some of the authorizations have expired (e.g., AEC Approval and DALRRD Section 20 permission) and as the research is ongoing, it would seem that these authorizations need renewal. Can you provide the most recent authorizations as well? Additionally, the SANParks AUCC approval and duration is contingent upon a "approved and signed research contract with SANParks" so can you provide a copy of the signed agreement?

Thank you,

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Division of Management Authority
U.S. Fish and Wildlife Service
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UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

18 January 2024

Approval Certificate
Annual Renewal
(EXT1)

AEC Reference No.:	REC078-22 Line 1
Title:	Can vaccination protect African wild dogs from canine distemper? Addressing a conservation emergency
Researcher:	A/Pr RW Woodroffe
Student's Supervisor:	Dr OL van Schalkwyk

Dear A/Pr RW Woodroffe,

The **Annual Renewal** as supported by documents received between 2023-09-11 and 2023-11-20 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2023-11-20.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	Approved
Wild Dogs - Kruger NP	32
Samples	Approved
Wild Dogs wild dog - blood (samples from live animals)	96
Wild Dogs wild dog - nasal swabs (samples from live animals)	32
Wild Dogs wild dog - rectal swabs (samples from live animals)	32

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2025-01-18.

3. Please remember to use your protocol number (REC078-22) on any documents or correspondence with the AEC regarding your research.

4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

5. All incidents must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.

6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

We wish you the best with your research.

Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

agriculture, land reform
& rural development

Department:
Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Land Reform and Rural Development
Private Bag X138, Pretoria 0001

Enquiries: Ms Marna Laing • Tel: +27 12 319 7442 • Fax: +27 12 319 7470 • E-mail: MarnaL@Dalrrd.gov.za
Reference: 12/11/1/1/6 (6145SldlR)

Responsible person: Dr. Mmadi Mogolodi Reuben

Institution: University of Pretoria - Faculty of Veterinary Science - Department of Veterinary Tropical Diseases

Email: mmadireuben@gmail.com

Dear Dr. Mmadi Mogolodi Reuben

RE: AMENDMENT OF SECTION 20 APPROVAL IN TERMS OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984) – EXTENSION OF THE EXPIRY DATE

Title of research project / study: "Can vaccination protect African wild dogs from canine distemper? Addressing a conservation emergency."

An amendment is hereby granted on the Section 20 approval with reference number 12/11/1/1/6 (2678AC) that was issued for the above-mentioned study on 2022-09-27.

i) The validity of the section 20 approval is extended to 31 January 2026;

All other conditions as specified in the Section 20 approval with reference number 12/11/1/1/6 (2678AC) remain in full effect. This includes the validity of laboratory approvals in terms of SANAS and DALRRD.

Kind regards,

DIRECTOR: ANIMAL HEALTH

Date: 2023 -11- 01

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Attachments: AEC_Approval_Certificate_Renewal.pdf; DALRRD_Section_20_Permit_Renewal.pdf; SANParks Research Agreement 2022_signed.pdf; SANParks_ Annual_Research_Permit_2023.pdf

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https://www.fws.gov/program/international-affairs
https://fwsepermits.servicenowservices.com/fws/

South African
NATIONAL PARKS

RESEARCH AGREEMENT

BETWEEN

SOUTH AFRICAN NATIONAL PARKS
herein represented by **Dr D Govender**
in his/her capacity as **GM: Scientific Services**
(hereinafter referred to as "SANParks")

AND/OR

Rose Woodroffe

Passport no. [redacted]

(hereinafter referred to as "the Researcher")

WHEREAS the Researcher submitted a research application to SANParks to conduct a research on **"Can vaccination protect African wild dogs from canine distemper? Addressing a conservation emergency."** ("Research") and to collect a sample of a biological resource or information ("Material / Data") in the **"Kruger National Park"** ("the Park");

AND WHEREAS SANParks accepted the Researcher's application to conduct Research and obtain the Material in the Park subject to the terms and conditions as stipulated hereunder:

THE PARTIES AGREE AS FOLLOWS

1. DEFINITIONS

1.1 In this Agreement, unless the context clearly indicates a contrary intention, the following terms shall have their meanings assigned to them hereunder, namely:

1.1.1. "**Agreement**" means this Agreement together with all annexures hereto;

1.1.2. "**Annexure A**" means additional information to this Agreement;

1.1.3. "**Annexure B**" means the data and metadata requirements;

1.1.4. "**Annexure C**" means the ranger notification template;

1.1.5. "**Background Intellectual Property**" means all intellectual property rights that existed prior to the Effective Date of this Agreement;

1.1.6. "**Bona Fide Research Assistant**" means an additional research assistant indicated to SANParks in writing as described in clause 4.4 herein;

1.1.7. "**Intellectual Property**" means any and all rights vesting in technical information, any inventions, processes, information and/or know-how, improvements, copyrightable works, designs and trade secrets, including, but not limited to, records of confidential information generated or maintained, data, test results, bibliographies, research findings, organisms, cells, DNA sequences, and other biological materials, whether in a written or electronic form, raw or derived, in the form of text, multimedia, computer programmes, spreadsheets, formatted fields in records, forms within files, databases, graphics, digital images, compositions and/or executions of processes, developed by the Researcher / Recipient within the scope of this Agreement;

1.1.8. "**Material**" means biological resources consisting of –

1.1.8.1. a living or dead animal, plant or other organism of an indigenous species;

1.1.8.2. a derivative of such an animal, plant or other organism, as defined in section 1 of the National Environmental Management: Biodiversity Act No. 10 of 2004; or

1.1.8.3. any genetic material of such animal, plant or other organism, as defined in section 1 of the Biodiversity Act; wherever the term genetic resources is mentioned, it shall be taken as subset of biological resources in a holistic interpretation of all the provisions of the Convention on Biological Diversity as well as to include a reproductive resource, its functional units of heredity or other components which are expressed by such unit(s), excluding commodities marketed as such rather than as a means for developing such units;

1.1.9. "Data" means any information collected by the Researcher within the scope of this Agreement through surveys, interviews, conversations or other correspondence with SANParks, its employees and/or visitors whether in a written or electronic form, raw or derived, in the form of text, multimedia, computer programmes, spreadsheets, formatted fields in records, forms within files, databases, graphics, digital images, compositions and/or executions of processes and includes:

1.1.9.1. Data collected on human subjects;

1.1.9.2. SANParks internal data;

1.1.10 "**Researcher**" means, a person from a public higher education institution listed above and recognised as such in terms of the Higher Education Act 101 of 1997 or a natural or juristic person with ID/passport number with its principal place of business as specified in this document and includes the faculty, staff, and other persons employed or contracted by the Recipient, or the Recipient himself/herself, whether full- or part-time; and/or any other persons, including a

student, a student employee, a graduate student, a post-doctoral fellow, and a non-employee (including visiting faculty, affiliate and adjunct faculty, industrial personnel, fellow, etc.) who participates in the creation or generation of applicable knowledge and/or Intellectual Property in the scope of this Agreement;

1.1.11 **"Effective Date"** means the date of signing of this Agreement;

1.1.12 **"Park"** means the National Park(s) as specified in the Agreement under the management of SANParks in terms of the National Environmental Management: Protected Areas Act 57 of 2003;

1.1.13 **"SANParks"** means South African National Parks, a statutory body established in terms of the National Parks Act No. 57 of 1976 and continuing to exist in terms of section 54(1) of the National Environmental Management: Protected Areas Act 57 of 2003 (as amended), with its principle place of administration at 643 Leyds Street, Muckleneuk, Pretoria, Gauteng;

1.1.14 **"Party/ies"** means SANParks and the Researcher, individually or collectively, as the case may be;

1.1.15 **"Signature Date"** means the date of signature of this Agreement by the last signing Party;

1.2 References to this Agreement shall include the annexures to this Agreement.

1.3 The headings to the clauses in this Agreement are for reference purposes only and shall not be used in the interpretation of this Agreement.

1.4 Words and phrases defined in this Agreement shall also apply in the interpretation of the same words and phrases in annexures to this Agreement, save where specifically indicated to the contrary in such annexure.

1.5 Unless the context otherwise requires:

1.5.1 the singular shall import and include the plural and vice versa;

1.5.2 words indicating a gender shall import and include other genders;

1.5.3 words indicating natural persons shall include juristic persons.

1.6 This Agreement shall be construed and interpreted in accordance with the Laws of the Republic of South Africa.

2. PERIOD OF AGREEMENT

2.1 This Agreement shall commence on the date of the last signature hereto and shall expire on 31 December 2024.

2.2 Either Party may terminate this Agreement by giving the other Party at least 2 (two) months written notice.

2.3 SANParks reserves the right to terminate this Agreement with immediate effect should the Researcher or any employee or agent of the Researcher assisting with the Research be charged with transgression of any statutory provision relating to any conservation asset or non-compliance with a statutory provision relating to any conservation asset.

2.4 Should credible information arise implicating the Researcher or any employee or agent of the Researcher in activities deemed detrimental to any conservation asset by SANParks, SANParks may terminate this Agreement after consultation with the Researcher and allowing the Researcher to provide reasons why this Agreement should not be terminated.

3. THE RESEARCH

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, with the worst all but wiping out the largest population in the northern hemisphere. Previous 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, recent attempts to implement CDV vaccination in Kruger provoked minimal immune responses in free-ranging wild dogs. Building on a successful evaluation in captivity, this project aims to identify a more effective protocol using a different vaccine, to inform urgent conservation efforts both within Kruger and throughout wild dog range.

Our project will provide (i) a formal evaluation of the safety and likely efficacy of modified-live CDV vaccination in free-ranging wild dogs and hence (ii) CDV management guidelines for this endangered species.

Our project is specifically designed to inform time-sensitive conservation decisions for this endangered species. As CDV has killed at least one wild dog pack in Kruger, our findings will help to inform wild dog management in Kruger. Beyond Kruger, CDV has devastated a wild dog population in Kenya and caused whole-pack deaths in Tanzania and South Africa. Using our data and models, we anticipate developing guidelines for CDV management to be shared with conservationists throughout Africa.

4. THE RESEARCHER'S OBLIGATIONS

4.1 The Researcher acknowledges that he/she (assistants or team included) will conduct the Research in the Park entirely at its own risk.

4.2 The Researcher shall furnish SANParks with proof of ethical clearance, where necessary, obtained from his/her institution for each research project prior to the commencement of fieldwork;

4.3 The Researcher shall comply with the SANParks Statement of Ethical Practice for Social Science Research available (available from SANParks at request);

4.4 The Researcher will obtain the prior written permission from SANParks, which consent will not be unreasonably withheld, to take out of the Park any Material and restricted to the total number that will give sufficient results of the Research. Furthermore, the Researcher shall obtain any other necessary permits from relevant authorities for the possession and transportation of such Material.

4.5 The Researcher shall sign both this Agreement and SANParks' standard indemnity form before Research can begin and shall ensure that all co-workers and bona fide research assistants sign the indemnity form before commencing the Research in the Park.`

4.6 The Researcher shall indicate in writing whether he/she will need any bona fide research assistants over and above any registered co-workers for field work and is required to submit the names, contact details and Identity numbers of these assistants as well as their roles in the Research and the duration that they will be assisting in the Research.

4.7 The Researcher shall carry a signed copy of the research authorization when working in the Park.

4.8 The Researcher shall notify SANParks to arrange their visit to the Park at least 10 (ten) working days in advance using the ranger notification template (Annexure C).

4.9 The Researcher shall adhere to *Protected Areas Act* 57 of 2003 and the regulations under that Act as well as the tourist traveling times and park rules and regulations when doing fieldwork in the Park. Where sampling has to be done at night, the Researcher shall obtain relevant permission from SANParks, which permission will not be unreasonably withheld.

4.10 Where necessary and reasonable, the Researcher shall be accompanied by a game guard during their fieldwork within the Park, and they will pay for the use of a game guard (including a daily fee, overtime and subsistence and travel costs) in accordance with SANParks' standard tariffs.

4.11 The Researcher shall submit an annual report to SANParks in accordance with SANParks' standard format.

4.12 Where biological Material was collected, the Researcher shall submit duplicate samples to SANParks' Scientific Services Biological Reference Museum unless specified otherwise by SANParks in writing, which specification will not be unreasonably withheld.

4.13 The Researcher will provide a well-organized documented electronic copy of data sets generated from this Research on an annual basis to SANParks, with the prescribed metadata files (Annexure B). For social Research it is required that all raw anonymous data be provided. All data will be lead time protected until 2 (two) years after expiry or termination of this Agreement as set out in clause 2 unless otherwise agreed upon by both Parties in writing and it is signed by both Parties.

4.14 It is agreed between the Parties that issues relating to benefit sharing of the proceeds of the Intellectual Property developed from the Research will be discussed as they arise, and appropriate sharing proportions will be formalized in a separate benefit-sharing Agreement.

4.15 The Researcher shall make available copies of publications, reports or theses arising from this Research to SANParks. Failure to do so will be taken into consideration in respect of future research applications to SANParks.

4.16 The Researcher shall acknowledge SANParks as the source of the Material / Data in any publication ensuing from such data and due consideration to co-authorship should be given.

4.17 The Researcher shall not share any aspects of the Research to the media (including social media on any platform), until it has been approved by SANParks. SANParks shall provide comment on any proposed release within 10 (ten) working days of receipt. However, SANParks shall not have the right to prohibit academic publications.

4.18 SANParks' name or branding or other intellectual property shall not be used for advertising or sponsorship material regarding the Research without the prior written permission, which permission will not be unreasonably withheld, to be obtained from the SANParks marketing division.

4.19 The Researcher will comply with the Standard Operation Procedure document on Research Application Process, available on the SANParks website.

5. OBLIGATIONS OF SANParks

5.1 SANParks shall afford the Researcher (and his/her registered co-workers and bona fide research assistants) free park entry, if in accordance with the policy of that particular park

5.2 SANParks shall provide discounted accommodation to the Researcher (and his/her registered co-workers and bona fide research assistants) where or when research accommodation is available.

5.3 Where research accommodation is not available the Researcher will have to book tourist accommodation and pay SANParks' standard tourist rates.

5.4 Where deemed necessary by SANParks, SANParks shall provide a game guard to accompany the Researcher and his/her co-workers and bona fide research assistants during field work, provided SANParks is notified well in advance and subject to availability.

5.5 Where required, SANParks will supply the Researcher with a SANParks vehicle decals (at a refundable cost of R100.00 (one hundred Rand) per pair after approval) if fieldwork will be in view of tourists.

5.6 Where available, SANParks shall provide basic laboratory facilities which shall not be exclusive to the Researcher.

5.7 With effect from the date of this agreement, any funds contributed from the researcher to SANParks shall not be utilized for purposes other than directly related to meeting the objectives of this research project. On termination of the research agreement and any valid extensions, SANParks will return any and all funds that have not been utilized in fulfillment of this research to the researcher or project funder

5.8 Where no conflict of interest arises, SANParks shall make available existing datasets (including GIS data layers) subject to the Researcher signing a data user Agreement form. These datasets should not be distributed to other Parties. Some datasets including lead-time and copyright protected datasets will not be available to the Researcher.

6. PERMIT TO COLLECT NATURAL RESOURCES MATERIAL

6.1 In compliance with regulation 4(1) of the regulations under the *National Environmental Management: Protected Areas Act* No. 57 of 2003, permission is hereby granted to the Researcher to collect the following natural resources Material in the Park:

6.1.1 The Material to be collected: (type, quantity, source): On each capture, 20ml of blood will be collected, from the cephalic or jugular vein of African Wild Dogs. A 25kg wild dog would be expected to have approximately 2 litres of blood (80ml/kg), so this sample volume is equivalent to 1% of total blood volume.

6.1.2 The reason for collection permit: Research

7. OBLIGATIONS PERTAINING TO COLLECTED MATERIAL

7.1. The Researcher agrees that the Material will be used for research purposes only and not for commercial, industrial or bio-prospecting purposes.

7.2. The Researcher shall take every reasonable precaution that the Material is not in the possession of any unauthorized third Party. Should there be a need to transfer the Material to a third Party a prior written consent must be obtained from SANParks, which consent is not to be unreasonably withheld.

7.3. The Researcher agrees that all information disclosed by SANParks will remain confidential and should not be divulged without the prior written consent of SANParks.

7.4. The Researcher shall ensure that the importation, transport, use, maintenance and disposition of the Material will be conducted in strict accordance with all appropriate local, national and international laws as well as guidelines and regulations.

7.5. Once the Material has been used for the agreed purpose, or at the termination of this Agreement the Researcher agrees to return the Material to SANParks or dispose of the Material in the manner agreed with SANParks and will provide SANParks with the necessary proof.

7.6. SANParks undertakes to make Material available to the Researcher in accordance with the terms and conditions of this Agreement.

7.7. SANParks shall provide the necessary written consent after a reasonable request by the Researcher to provide the Material or to disclose information to a third Party has been assessed and it was concluded that it poses no danger or disadvantage to SANParks.

8. OBLIGATIONS PERTAINING TO COLLECTED MATERIAL/DATA

8.1. The Researcher agrees that the Data will be used for research purposes only and not for commercial, or industrial or bio-prospecting purposes.

8.2. The Researcher shall take every reasonable precaution that the Data is not in the possession of any unauthorized third Party. Should there be a need to transfer the Data to a third Party a prior written consent must be obtained from SANParks.

8.3. The Researcher agrees that all information disclosed by SANParks or obtained under this Agreement will remain confidential and should not be divulged without the prior written consent of SANParks.

8.4. The Researcher agrees to keep the identities and personal information of individuals participating in this project, particularly those being interviewed or

9.4. Notwithstanding the provisions of this clause, any Party shall be entitled to institute any proceedings for urgent interim relief arising out of or in connection with this Agreement in a court of the Republic of South Africa having jurisdiction over the Parties.

10. BREACH OF AGREEMENT

10.1. Should any Party commit a breach of any of the provisions of this Agreement and fail to remedy the breach within a period of 7 (seven) business days after receipt of the notice by the injured Party to remedy the breach, the injured Party shall at its reasonable discretion and without prejudice to any other rights be entitled to terminate the Agreement but will provide reasonable notice to that Party of its intentions.

11. INDEMNITY

11.1. SANParks, its Board, directors, employees and agents are not liable for any loss or damage:

11.1.1. to the property or possession of the Researcher or its bona fide research assistant / team, whether such damage is caused by the negligent act or omission of SANParks; and

11.1.2. arising from death or any bodily injuries of whatsoever nature sustained by the Researcher whether such injuries are caused by the negligent act or omission by SANParks, and/or by the defective functioning of any apparatus.

11.2. The Researcher and its bona fide research assistants / team will conduct the Research in the Park at their own risk and the Researcher hereby indemnifies

SANParks against any damage, loss, injury or death suffered by any person resulting from the Research in the Park.

11.3. The Researcher shall be liable for any loss or damage suffered by SANParks as a result of a negligent act or omission by the Researcher while conducting the Research in the Park and/or as a result of the willful breach of the terms of this Agreement.

12. AMENDMENT

12.1. This document constitutes the entire Agreement between two (2) Parties and no amendment thereof shall have any effect unless reduced to writing and signed by both Parties.

12.2. No indulgence on the part of either Party shall constitute a waiver of rights in terms of this Agreement.

12.3. The Researcher shall not be entitled to cede or assign this Agreement, nor in any other way transfer any of its rights or obligations under this Agreement.

13. DOMICILIUM CITANDI ET EXECUTANDI

13.1. The Parties choose as their *domicilium citandi et executandi* for all purposes under this Agreement the following addresses:

SANParks	**The Researcher**
Manager: Legal Services	Prof. Rosie Woodroffe
643 Lleyds Street	Institute of Zoology, Zoological Society of London
MUCKLENEUK	Regent#39 Park
PRETORIA	London

0001
Tel: (012) 426-5000
Fax: (012) 343-0155

NW1 4RY
Tel: +44 7964 905775
Cell: +44 7964 905775
Email: rosie.woodroffe@ioz.ac.uk

13.2. Any notice or communication required or permitted to be given in terms of this Agreement shall be valid and effective only if in writing.

13.3. Either Party may by written notice to the other Party change the physical address chosen as its *domicilium citandi et executandi* to another physical address where postal delivery occurs, provided that the change shall become effective on the seventh (7th) business day from the deemed receipt of the notice by the other Party.

13.4. Any notice to a Party :–

13.4.1. Sent by prepaid registered post (by airmail if appropriate) in a correctly addressed envelope to it at the address chosen as its *domicilium citandi et executandi* to which post is delivered shall be deemed to have been received on the fifth (5th) business day after posting (unless the contrary is proved);

13.4.2. Delivered by hand to a responsible person during ordinary business hours at the physical address at is *domicilium citandi et executandi* shall be deemed to have been received on the day of the delivery.

13.5. Notwithstanding anything to the contrary herein contained a written notice of communication actually received by a Party shall be adequate written notice of communication to it notwithstanding that it was not sent to or delivered at its chosen *domicilium citandi et executandi*.

SIGNED at Skukuza on the 13th day of October 2022

WITNESSES

1. _____

2. _____

_____
SANPARKS

SIGNED at PORKELLIS on the 21st day of SEPTEMBER 2022

WITNESSES:

1. _____

2. _____

_____
RESEARCHER

Annexure A – Additional Information about the Research

Refer to SANParks AUCC Ethics certificate Reference No 03-22

Annexure B - Data and Metadata requirements

General metadata required:

1. The final report needs to be completed as requested.
2. Abstract for the dataset.
3. Geographic coverage. Area of the study needs to be stipulated e.g. Entire KNP or where you are working with transects the beginning and end point coordinates need to be given. If points are used then a GPS point for each should be given.
4. Temporal coverage. The dates that the data was collected
5. Keywords
6. Taxonomic coverage of the dataset. Please provide the genus and specie name of the individuals that were sampled in your dataset. This can be provided in a table format.
7. Data Usage rights. Enter a paragraph that describes the intended usage rights of the data. Specifically include any restrictions (scientific, technical, and/or ethical) to sharing your data within the public scientific domain. If your dataset is lead time protected please include the length of this period.
8. Access control .If you do want to restrict the dataset but have certain people that you would like to be able to access this data they should be mentioned here
9. Methods. The methods of the study should be discussed here. If you already have them in your project proposal please just copy and paste them.
10. People and organizations. Please supply the contact details of the people that you would like to be associated with the dataset and also the role that they played on the dataset e.g. metadata provider, principal investigator.

The metadata needed for each dataset is as follows

1. **GIS data and Imagery**
   Each shape file needs to be submitted with a FGDC xml metadata document that can be made via the metadata tool of Arc catalogue.
   Any imagery needs to be accompanied by a text file that indicates the level of processing of the image.

2. **Spreadsheet or column data**
   Excel spreadsheet and any other column data (e.g. Access tables) need to be exported as text files. For each column in the text file the following information is needed.
   1. Column heading
   2. Column description
   3. Type of variable i.e. numeric, date/time, enumerated (i.e. if you have codes you need to describe all the codes used. This description may be in another text file then just indicate that here.
   4. Measurement unit e.g. mm, parts per million (ppm) etc.
   5. Precision of the measurement i.e. if your measurements are in meters and your precision is 1 it means that your measurement is accurate to the nearest meter.
   6. Bounds if the variable that you measured can only take on certain values stipulate them e.g. if a value can only be between 0 1 and 1 say min =0 max = 1.

This data and metadata need to be submitted the applicable Science Liaison officer. If your data does not fit in any of the above categories please contact judith.botha@sanparks.org for help.

Annexure C: Ranger Notification

South African
NATIONAL PARKS

RANGER NOTIFICATION

- Name of the researcher and co-workers
- Name of Park and section of the park where they will be working
- Dates on which they will be in the field
- Researcher's activities in the area
- Car registration, colour and type of the car
- Will they be accompanied by a game guard (where necessary)?
- Researchers contact details (preferably mobile phone)

South African NATIONAL PARKS

Research Permit Kruger National Park
Reference SS418
Issue Date 2023-06-07

Scientific Services
Kruger National Park, Private Bag X402, Skukuza, 1350

Senior Research Rosie Woodroffe
Title Can vaccination protect African wild dogs from canine distemper? Addressing a conservation emergency.
Co-Workers Mmadi Reuben, Grant Beverley, Louis van Schalkwyk

Herewith the permit for your research project valid from 07 June 2023 until 30 June 2024. The approval is subject to the following conditions. The Park Management staff must be contacted prior to entry into the park (see website for contact details).

Standard Conditions

PLEASE CONTACT THE PARK MANAGEMENT STAFF IF RESTRICTED AREAS NEED TO BE ACCESSED.

No damage shall be permitted to any natural vegetation, environment or property.

Uncontrolled vehicle access and parking could cause damage to vegetation and soil erosion and therefore only the use of approved vehicles routes and parking areas is allowed.

Fires can cause loss of vegetation, soil erosion and life and therefore fires, and braai's are not permitted unless in dedicated braai areas.

Other visitors to the area and or neighbours may not be hindered in any way.

No pollution or excessive noise is permitted.

Your permit must be retained and kept on your person at all times, and produced on request.

The areas under the control of SANParks are used entirely at your own risk. South African National Parks, its Board, directors, employees and agents are not liable for any loss or damage to the property or possession of any guest or participant (or accompanying minor) whether such damage is caused by the negligent act or omission of South African National Parks; arising from death or any bodily injuries of whatsoever nature sustained by a guest or participant (or accompanying minor) whether such injuries are caused by the negligent act or omission by South African National Parks, and/or by the defective functioning of any apparatus. The guest or participant and/or his/her/their estate hereby indemnifies South African National Parks against any claim, action, judgment, costs and/or expenses which may be made against South African National Parks and as may in any way be related to the above. The onus lies with the company or applicant to ensure that they are adequately insured.

addo elephant
agulhas
augrabies
bontebok
camdeboo
golden gate highlands
karoo
kglalagadi transfrontier
knysna lake area
kruger
mapungubwe
marakele
mokala
mountain zebra
namaqua
table mountain
tankwa karoo
tsitsikamma
richtersveld
west coast
wilderness

South African NATIONAL PARKS

Please note that you (your delegates, staff etc) are subject to the conditions set in terms of Section 86(1) of the National Environmental Management Act (107 of 1998) and the National Environmental Act: Protected Areas Act (Act 57 of 2003) for the duration of your stay in the National Park. Your attention is specifically drawn to sections 64(1) (a), (b) & (c) which refers to penalties in terms of the Act.

SANParks staff's instructions, notices, regulations and signs must be complied with.

The activity shall be restricted to the area applied for.

Gate and operating times to be complied with.

NO PETS ALLOWED

Special Conditions:
Research is entitled to 90 Accommodation days annually at all Research Camps

Any contraventions of the above will result in your permit being revoked

S.N. Mabuza

Samantha Mabuza, Science Liaison Officer, Savanna Research Unit, Skukuza, 1350

addo elephant
agulhas
augrabies
bontebok
camdeboo
golden gate highlands
karoo
kglalagadi transfrontier
knysna lake area
kruger
mapungubwe
marakele
mokala
mountain zebra
namaqua
table mountain
tankwa karoo
tsitsikamma
richtersveld
west coast
wilderness

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reservations@parks.co.za
www.parks-sa.co.za