February 14, 2020

To Whom It May Concern,

Attached, please find the permit application for the Maryland Zoo in Baltimore to import blood and tissue samples from ESA-listed maned wolves (*Chrysocyon brachyurus*) from Bolivia. These samples are to be used for conservation research purposes only and have no commercial value. In addition to our 3-200-37 application for the import of animal samples, please also find:

- A detailed list of the tissue samples to be imported
- Documentation of agreements with the Bolivian government for the collection of the samples
- Research papers produced by the applicant concerning the samples in question
- The curricula vitarum of Dr. Ellen Bronson and , DVM and Dr. Louise Emmons, PHD, who will be conducting the research.

Please feel free to reach out to me with any questions concerning the importation of these specimens and the use to which they will be put.

Thank you,

Ian Shelley
Registrar, Maryland Zoo in Baltimore
ian.shelley@marylandzoo.org
443-552-3351

Form 3-200-37

OMB Control No. 1018-0093
Expires 08/31/2020

RCVD FEB 20 2020

Department of Interior
U.S. Fish and Wildlife Service
Federal Fish and Wildlife Permit Application Form

U.S. Fish and Wildlife Service
Division of Management Authority
Branch of Permits, MS: IA
5275 Leesburg Pike
Falls Church, VA 22041-3803
1-800-358-2104 or 703-358-2104

Type of Activity

EXPORT/RE-EXPORT/IMPORT/INTERSTATE AND FOREIGN COMMERCE/TAKE OF ANIMALS (LIVE/ SAMPLES/PARTS/PRODUCTS) under the Convention on International Trade in Endangered Species (CITES) and/or the U.S. Endangered Species Act (ESA)

Complete Sections A or B, and C, D, and E of this application. U.S. address may be required in Section C, see instructions for details. **Instructions on how to make your application complete and help avoid unnecessary delays are attached.**

Section A: Complete if applying as an individual

1.a. Last Name		1.b. First Name	1.c. Middle Name/Initial	1.d. Suffix
2. Date of Birth (mm/dd/yyyy)	3. Telephone Number	3.a. Alternate Telephone Number	4. E-mail address	

Section 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)	
Maryland Zoo in Baltimore			
2. Tax identification no.		3. Description of business, agency, Tribe, or institution	
52-0996352		Zoological Park	
4.a. Principal officer Last name	4.b. Principal officer First Name	4.c. Principal officer Middle name/initial	4.d. Suffix
Hutchinson	Donald		
5. Principal officer title		6. Primary contact name	
President/CEO		Ian Shelley (Registrar)	
7.a. Business telephone number	7.b. Alternate telephone number	7.c. Business fax number	7.d. Business e-mail address
443-552-3351			ian.shelley@marylandzoo.org

Section C: All applicants complete address information

1.a. Physical address (Street address; Apartment #, Suite #, or Room #; no P.O. Boxes)				
1876 Mansion House Drive				
1.b. City	1.c. State	1.d. Zip code/Postal code	1.e. County/Province	1.f. Country
Baltimore	Maryland	21217	Baltimore City	United States
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

Section D: All applicants MUST complete

1. Attach the **nonrefundable application processing fee** in the form of a check or money order payable to the U.S. FISH AND WILDLIFE SERVICE **in the amount of $100.** 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. 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.

Signature of applicant/Principal Officer for permit (No photocopied or stamped signatures) Date of signature (mm/dd/yyyy)

Donald P. Hutchinson 2/12/2020

Please continue to next page

E. EXPORT/RE-EXPORT/IMPORT/INTERSTATE AND FOREIGN COMMERCE/TAKE OF ANIMALS (Live/samples/parts/products) (CITES and/or ESA)

Allow at least 90 days for the application to be processed. Applications for endangered species permits must be published in the Federal Register for a 30-day public comment period.

Complete all questions on the application. Mark questions that are not applicable with "N/A". If needed, use separate sheets of paper. On all attachments or separate sheets you submit, indicate the application question number you are addressing. If you are applying for multiple specimens, be sure to indicate which specimen you are addressing in each response.

NOTE: The import of live southern white rhinoceros from South Africa and Swaziland must meet specific CITES criteria for an import permit to be issued. If you are requesting authorization for the import of these species, please ensure that you respond to question 14 below.

Electronic submission of inventories, photographs, and receipts: Some applications contain extensive inventories and /or a large number of photographs or receipts. You may provide electronic versions of the documents. Such a submission will assist the processing of your application since it may reduce data entry by the U.S. Fish and Wildlife Service. If you wish to provide information electronically, once you have received an application number via the e-mailed acknowledgment letter, e-mail your information to Permits@fws.gov. Be sure to include the application number provided in the acknowledgment e-mail that will be sent to you when we receive your application.

☑ I will be submitting documents electronically.

1. Name and address where you wish the permit to be mailed, **if different from page 1**. 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.

   Please mail to the same address listed on Page 1

2. Who should we contact if we have questions about the application (name, phone number, and e-mail)?

   Ian Shelley. 443-552-3351, ian.shelley@marylandzoo.org
   Dr. Ellen Bronson, DVM. 443-552-3389, ellen.bronson@marylandzoo.org

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.

4. What activity are you requesting authorization to carry out (Indicate appropriate activities):

☐ EXPORT ☐ RE-EXPORT ☑ IMPORT ☐ TAKE (e.g., cull, lethal harvest)
☐ INTERSTATE COMMERCE ☐ FOREIGN COMMERCE

Note: Interstate Commerce permits authorize the sale of endangered and threatened species across State lines, but only for activities that will contribute to enhancing the propagation or survival of that species. Interstate commerce activities with wildlife require the buyer to obtain a permit prior to the sale or offer for sale.

5. For **EACH** animal/specimen involved in the proposed activity provide:

a. Scientific name (genus, species, and, if applicable, subspecies)	**b. Common name**	**c. Birth/ Hatch Date** *(mm/dd/yyyy)* (approximate of actual unknown)	**d. Wild or captive-born**	**e. Quantity**	**f. Gender** (male or female, if known), if	**g. Permanent markings**, if alive (e.g., tattoo, ID #, microchip #, scars)	**h. Type of sample or product** (e.g., blood, tissue, DNA)
EXAMPLE: *Pan troglodytes*	Chimpanzee						
Chrysocyon brachyurus	Maned Wolf	Unknown/ Multiple	Wild	Several Samples	Multiple Animals	N/A	Blood (Serum, Plasma, Whole)
Chrysocyon brachyurus	Maned Wolf	Unknown/ Multiple	Wild	Several Samples	Multiple Animals	N/A	Urine Samples
Chrysocyon brachyurus	Maned Wolf	Unknown/ Multiple	Wild	Several Samples	Multiple Animals	N/A	Fecal Samples
Chrysocyon brachyurus	Maned Wolf	Unknown/ Multiple	Wild	Several Samples	Multiple Animals	N/A	Vaginal Swabs

6. The current location of the specimen(s) (address and country):

Name:
Address: Museo de Historia Natural, Noel Kempff Mercado
City: Av. Irala 565
State/Province: Santa Cruz de la Sierra
County, Postal Code: Bolivia

7. Recipient/Sender:

- If **export**, provide name and address of the recipient in the foreign country.
- If **re-export**, provide the name and address of the recipient in the foreign country.
- If **import**, provide name and address of the exporter in the foreign country.
- If **interstate or foreign commerce**, provide name and address of the proposed seller/supplier.

Name:
Address: Museo de Historia Natural, Noel Kempff Mercado
City: Av. Irala 565
State/Province: Santa Cruz de la Sierra
County, Postal Code: Bolivia

SOURCE OF SPECIMEN (answer question 8 or 9 for **EACH** animal/specimen involved, as appropriate).

8. For captive-bred animals or animal(s) from which the specimen(s) are/were obtained, provide a signed and dated statement from the breeder that includes the following:

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

    b. Name and address of the facility where the animal was bred and born;

    c. Birth/hatch date (mm/dd/yyyy), and, if applicable, identification information;

    d. Location (name of facility, address, city, State, postal code) of parental stock;

    e. A statement that the animal was bred at the above facility;

    f. Documentation demonstrating the history of transactions (e.g., chain of custody or ownership of the animal).

9. For **EACH** animal/specimen **taken from the wild**, provide the following:

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

Maned Wolf (Chrysocyon brachyurus)

    b. Specific location of where, when, and by whom (name and address) the specimen was removed from the wild;

Bood, urine, feces, and vaginal swabs were taken from maned wolves residing in Noel Kempff Mercado National Park in Bolivia. Samples were collected in the field; animals were not removed from the wild.

    c. Purpose of removal and length or approximate length of time held in captivity. Discuss issues such as the method of collection, was the collection done as part of a larger study, were animals returned to the wild after sampling, and did any mortalities or injuries occur due to collection or holding;

Maned wolves involved in this study were not maintained in captivity. They were sampled in the field while immobilized only for the time needed to immobilize them and collect biological samples for research. The capture and immobilization are carried out by veterinary professionals experienced in their chemical restraint. The animals are released following the sample extraction.

    d. If and how any remuneration, either financial or in-kind, was provided for taking or capturing animals or for the collection of samples.

These samples were collected for scientific purposes and have no commercial value

    e. Your efforts to use captive specimens (e.g., captive-born, captive-held), or parts thereof, in lieu of taking animals from the wild.

In order to monitor the health of the wild population, samples were required from wild individuals. The collection process does not remove individuals from the wild population, as samples were collected in the field and the animals were released after collection was complete

    f. Copies of your foreign or domestic collecting permit, license, contract or agreement;

    g. Documentation showing that the specimen(s) was/were legally obtained by the applicant; and

    h. Copies of any applicable State, Tribal, Federal, or Foreign government permits or licenses that authorized the removal of this animal from the wild.

JUSTIFICATION FOR REQUESTED ACTIVITY.

10. Provide a detailed statement justifying the proposed activity, particularly the following:

a. Describe the purpose of your proposed activity. For example, if the purpose is scientific research, attach a copy of your research proposal outlining the purpose, objectives, methods (e.g., specific information on survey/collection methods, sampling regime, equipment to be used), and whether similar work has already been done or is currently being done. If the purpose includes conservation education, provide copies of educational materials (e.g., handouts, text of signage or public presentations), and include the purpose and objectives of the proposed activity. If the purpose is for propagation for conservation purposes (including culling as part of herd management), provide a description of how the species will be propagated and the disposition of progeny, as well as long-term goals of the breeding program, how the breeding program is managed to maintain genetic vitality, and information on any cooperative breeding programs or agreements that are/will be established, including any future plans for re-introduction.

Blood samples were collected in order to monitor the wild maned wolf in Noel Kempff Mercado National Park for diseases and parasites, allowing biologists to study the health of the population. This permit application seeks permission to export these already-collected samples to the United States for further research. This ongoing research will help inform future decisions about the health of the population and determine what the primary risk factors are that threaten the population. Attached, please find copies of research papers that the applicants have published concerning this project.

b. Description of the technical expertise of each person (please also include CV or resume), as it relates to the proposed activities. If the proposed activity involves live animals, include the experience of each animal caretaker working with the species.

Please see the attached CV for Dr. Ellen Bronson, Senior Director of Animal Health, Conservation, and Research at the Maryland Zoo in Baltimore, and Dr. Louise Emmons, Research Associate for Vertebrate Zoology at the Smithsonian Institution.

c. Copies of contracts, agreements or other documents that identify persons involved and dates of activities for which authorization is being requested.

11. A statement on how the activities will **enhance or benefit the wild population** (e.g., in-situ and ex-situ projects).

This ongoing research project utilizes blood samples taken from wild maned wolves to monitor the population for diseases which may threaten the long-term survival of the species. Introduced diseases, particularly those carried by and transmitted to maned wolves by domestic dogs and other canids are one of the leading threats to the survival of this species. Data collected from this research project will help biologists determine how to best protect wild maned wolves from these diseases.

12. If live specimens are to be held in captivity as part of the proposed activity:

a. Provide a detailed description (e.g., size, construction materials, protection from the elements) and photographs or diagrams (no blueprints, please) clearly depicting the existing facilities **where the wildlife will be maintained**. If the specimens will be housed at multiple facilities, either immediately or within the next year, provide a full description of each facility. If you are unsure of which facilities may be receiving specimens (e.g., final decisions on placement have not been made), please indicate likely candidates and the mechanism that will be used to determine recipient facilities;

Live specimens are not to be maintained in captivity as part of the proposed activity

b. A statement of the specific technical experience of CV or resume available to the recipient(s) for maintaining and propagating live specimens of the same or similar species;

Live specimens are not to be maintained in captivity as part of the proposed activity

c. The number of years each species has been maintained at the facility;

Live specimens are not to be maintained in captivity as part of the proposed activity

d. The number of births by year for each species for the last 5 years; and

Live specimens are not to be maintained in captivity as part of the proposed activity

e. Mortalities at the facility with these or similar species in the last 5 years, causes of such mortalities, and steps taken to avoid or decrease such mortalities.

Live specimens are not to be maintained in captivity as part of the proposed activity

IMPORTS, EXPORTS, OR RE-EXPORTS.

13. For shipment of LIVE specimens, the transport conditions for animals must comply with the CITES Guidelines for Transport of Live Animals or, in the case of air transport, with the International Air Transport Association (IATA) live animal regulations (contact airline for information). As such, describe:

a. The type, size, and construction of any shipping container; and

Live specimens will not be transported as part of the proposed activity

b. The arrangements for watering or otherwise caring for the wildlife during transport.

Live specimens will not be transported as part of the proposed activity

14. For import of live southern white rhinoceroses from South Africa and Swaziland, a determination that the importing facility meets the CITES "appropriate and acceptable destination" annotation must be made. Therefore, provide written documentation demonstrating that the proposed activity would promote *in situ* conservation of the species. **Note:** ***For any permit authorizing trade of live rhinoceroses under an "appropriate and acceptable destination" annotation, the rhinoceros horn from these animals may not enter commercial trade and the animal may not be sport hunted.***

15. **For import of LIVE CITES Appendix-I listed marine mammal species**, provide a copy of your FWS or NOAA Fisheries permit or authorization.

16. 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 .

17. For export of 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.

18. If the specimen is 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 (if applicable); and

    b. A cleared copy of Form 3-177, wildlife Declaration for Import (hard copy or electronic release); **OR**

    c. If you did not make the original import, provide a copy of the importer's documents outlined above and the invoice or other documentation that shows you acquired the wildlife from the original importer or history of transactions which demonstrate chain of ownership.

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).

Samples for exportation to USA to Maryland Zoo in Baltimore

As of November 2018

Maned wolf/Borochi/Lobo de crin (*Chrysocyon brachurus*):

CB13 9 Sept 2014

Serum: 5 vials
Plasma: 1 vial
Filter paper with whole blood: 1
Blood slides: 4
Whole blood cells: 1 vial
Frozen whole blood: 1 vial
Frozen urine: 1 vial
Urine in formalin: 1 vial
Filter paper with urine: 1
Frozen feces: 1 vial
Feces in formalin: 1 vial
Vaginal slides: 2
Whole blood in buffer: 1 vial

Bolivian river dolphin/Bufeo/Delfín boliviano (*Inia boliviensis*)

Serum: 14 vials
Plasma: 14 vials
Whole blood cells: 12 vials
Whole blood in Queen's buffer: 6 vials
Tissue from dorsal fin in ethanol: 3 vials

Recibido
21/08/14

sernap

FORMULARIO
AUTORIZACIÓN DE INGRESO

ESTADO PLURINACIONAL DE BOLIVIA

Solicitante: Lic. Kathia Rivero Guzmán.....**En Fecha.** 20/08/2014

Motivo del Ingreso: Continuación del estudio de ecología de carnívoros (Proyecto Borochi)

Duración: 01/09/14 -20/10/14.....**Fecha de Ingreso:** 01/09/13

Localización de la Expedición: El Refugio y Pampa de Los Fierros **Nº de Personas:** 4 (José Miguel Castro, José Luis Poma, Jean Carla Zabala, Mariely Negrette, Ellen Bronson y Louise Emmons).

Responsable de la expedición: Dra. Louise Emmons

Nombre de la Expedición o Proyecto: Ecología del borochi (*Chrysocyon brachyurus)* y otros mamíferos en el Parque Nacional Noel Kempff Mercado y alrededores.

Equipo a Emplear: Equipo de radiotelemetría, trampas cámara, trampas Sherman, trampas tomahawk y redes de neblina.

Objetivo: Entender la ecología y comportamiento del borochi (salud, dieta, diversidad genética, uso de hábitats, reproducción) y otros carnívoros asociados en el mosaico de bosques, sabanas secas e inundadas del Parque y estudiar las consecuencias de los incendios naturales recientes (2009, 2012) en la ecología de los borochis y de sus recursos alimenticos (roedores y frutos).

Descripción Resumida de los Trabajos a Realizarse:

- Captura y liberación de borochis (en trampas tipo jaula para colocado de collares GPS).
- Toma de muestras para estudios sanitarios de los individuos capturados
- Colecta de heces de borochi para estudios de su dieta
- Monitoreo de borochis y de mamíferos grandes con trampas cámaras en el área de estudio.
- Monitoreo de la fenología de frutos que forman parte de la dieta de los borochis
- Grabación de los gritos de borochis.
- Inventario de murciélagos mediante redes de neblina.
- Trampeo de roedores con trampas Sherman en parcelas para monitoreo de las poblaciones en la pampa (alimento principal de los borochis).

El SERNAP autoriza la realización de los trabajos de campo arriba descritos, debiendo el solicitante coordinar con el Director del Área Protegida el acceso a la zona, asimismo se compromete a entregar copia de los resultados del trabajo realizado al SERNAP y a la Dirección del Área Protegida.

Firma del Solicitante
Nº de CI 3832941 SC

Lic. Kathia Rivero G.
Jefa Area de Zoología
Museo de Historia Natural N.K.M.
U.A.R.G.M.

Lic. Sandro Añez P.
DIRECTOR
PN Noel Kempff Mercado
Firma Autorizada
Director de Área Protegida

Estado Plurinacional de Bolivia

MMAyA
Ministerio de Medio Ambiente y Agua

La Paz, 01 SET. 2010

MMAyA-VMA-DGBAP Nº 1111

Señora
Ing. Patricia Herrera
Directora del Museo de Historia Natural Noel Kempff Mercado
Santa Cruz.-

Ref.: Aprobación del proyecto "Ecología del Borochi (*Chrysocyon brachyurus*) y otros mamíferos en el Parque Nacional Noel Kempff Mercado y alrededores"

De mi mayor consideración:

Mediante la presente, cumplo en informarle que el proyecto remitido a esta instancia y que será ejecutado por las investigadoras Lic. Kathia Rivero y Dra. Louise H. Emmons del Smithsonian Institution, National Zoological Park –USA, que se realizará en el Parque Nacional Noel Kempff Mercado (PNNKM) y alrededores, ha sido aprobado por esta Dirección en el marco de la Resolución Administrativa VMABCC Nº 026/09 y el informe técnico MMAyA-VMA-DGBAP Nº 1104/10, con las siguientes consideraciones a tomar en cuenta:

- Se autoriza el proyecto "Ecología del Borochi *(Chrysocyon brachyurus*) y otros mamíferos en el Parque Nacional Noel Kempff Mercado y alrededores", con el objeto de estudiar la ecología, comportamiento, estado sanitario, recursos alimenticios, desarrollar un plan de monitoreo de manejo e inventariar las comunidades de pequeños mamíferos en hábitats diferentes del PNNKM.
- Se autoriza la colecta de las siguientes familias: Canidae, Muridae, Caviidae, Sciuridae, Didelphidae, Phyllostomidae, Vespertillionidae, Molossidae, Natalidae, Thyropteridae, Emballonuridae, Mormoopidae, Noctilionidae, Sciuridae, Echimyidae y Erethizontidae, tal como se detalla en el formulario de proyecto.
- Se informa a la ICA y a los investigadores operativos, que deben cumplir con lo determinado en el formulario de proyecto y cumplir con los objetivos y cronograma de actividades planteadas, iniciando del 1 de septiembre de 2010 hasta el 30 de septiembre de 2015.
- Se informa a la ICA que es responsable de precautelar el material colectado y su destino final, cumpliendo con lo determinado en la R. A. VMABCC Nº 026/09 art. 17 numerales I y II. También se deberá presentar el formulario de exportación en caso de traslado de muestras al exterior, de acuerdo al art. 20, inciso e) anexo III y la colecta y exportación de especímenes que se encuentran en CITES se regirá a través de norma específica.
- La ICA debe cumplir con las responsabilidades establecidas en el Art. 15 del Reglamento de Investigación, asimismo, deberá coordinar con las Autoridades locales del área de estudio, informando los alcances y aportes del proyecto en la conservación de la biodiversidad del país.
- Se informa a la entidad ejecutora que se encuentra totalmente prohibida la comercialización de especies colectadas o sus derivados y partes a través del presente proyecto, quedando como responsable el Museo en calidad de Institución Científica Autorizada.

Sin otro particular, me despido atentamente.

Lic. Omar Rocha Olivio
DIRECTOR GENERAL DE BIODIVERSIDAD
Y AREAS PROTEGIDAS
VMA - MMAyA

ORO/LAB/oca/mrt
Adj. Formularios legalizado
C. c. Archivo
DGBAP-UVSAP
HR. DGB 15311

UNIVERSIDAD AUTONOMA GABRIEL RENE MORENO
348
MUSEO DE HISTORIA NATURAL "NKM"
02 AGO. 2010
RECIBIDA

ESTADO PLURINACIONAL DE BOLIVIA
MINISTERIO DE MEDIO AMBIENTE Y AGUA
VICEMINISTERIO DE MEDIO AMBIENTE, BIODIVERSIDAD Y CAMBIOS CLIMÁTICOS

FORMULARIO DE PRESENTACIÓN DE PROYECTOS CIENTÍFICOS

Título del Proyecto: *Ecología del Borochi (Chrysocyon brachyurus) y otros mamíferos en el Parque Nacional Noel Kempff Mercado y alrededores*

INSTITUCIONES CIENTÍFICAS PARTICIPANTES

INSTITUCIÓN CIENTÍFICA AUTORIZADA

Nombre de la Institución: *Museo de Historia Natural Noel Kempff Mercado*

Responsable Institucional: *Ing. Patricia Herrera*

Responsable Operativo del Proyecto: *Lic. Kathia Rivero*

Domicilio Legal de la Institución: *Santa Cruz de la Sierra*
Dirección: Av. Irala 565
Teléfono: *336-6574; 337-1216*
Fax: 336-3710
E-mail: *pherrera@museonoelkempff.org; krivero@museonoelkempff.org*

INSTITUCIÓN CONTRAPARTE (SI CORRESPONDE):

Nombre de la Institución: *Smithsonian Institution, National Zoological Park*

Responsable Institucional: *Dra. Suzan Murray*

Responsable Operativo del Convenio *Dra. Louise H. Emmons*

Domicilio Legal de la Institución:
Dirección: *3001 Connecticut Ave., NW*
Teléfono: *202-633-1249*
Fax:
E-mail: *emmonsl@si.edu*
País: *USA*
Ciudad: *Washington, DC*

Especialistas: *Dra. Louise H. Emmons*

Institución Financiera: *Varios donantes.*

Fecha de inicio (*): *Septiembre 2010*	**Fecha de conclusión (*):** *Septiembre 2015*

Ámbito de ejecución: *Parque Nacional Noel Kempff Mercado (PNNKM) y alrededores.*
Departamento: *Santa Cruz*
Provincia: *Velasco*
Localidad:
Propiedad privada: El Refugio Huanchaca (14°33 S'; 60°55' O)
Estancia Caparú (14°54' S; 61°05' O)
Área Protegida:

Parque Nacional NKM-Campamento Los Fierros (14°33' S; 60°55' O)

Indicar si el proyecto se desarrolla en área protegida	**SI**
Indicar si el proyecto implica colecta de especimenes	**SI**
Indicar si el proyecto implica el acceso a recursos genéticos	**NO**

CONDICIONES DE LA AUTORIDAD AMBIENTAL COMPETENTE NACIONAL

Las instituciones que son parte del convenio de cooperación para estudios científicos deben sujetarse a las siguientes condiciones para la ejecución de la solicitud:

1. En marcar sus actividades en las normas legales vigentes en el país
2. La suscripción de este formulario no significa cesión alguna de derechos sobre material genético ni de bioprospección, ya sea el componente intangible de este recurso o el material en si o productos derivados de sus uso en el presente o en el fututo. Asimismo no constituye permiso de exportación de material genético o especies provenientes de vida silvestre. Todo autorización de exportación de carácter comercial o no comercial deberá, ser requisito indispensable para obtener los permisos de exportación de especimenes destinadas a la investigación científica.
3. El presente permiso podrá ser suspendido temporalmente o revocado por la Autoridad Ambiental Nacional Competente cuando esta lo instruya o cuando el desarrollo del proyecto no se ajuste a las condiciones establecidas en la presente autorización emergentes que vayan en contra de los objetivos del proyecto aprobado a la normativa legal vigente.
4. Toda investigación que involucre la colecta de muestras de especimenes vivos de fauna, debe utilizar métodos que reduzcan al mínimo el estrés causado sobre los especimenes. En caso de proceder a su sacrificio, el mismo deberá ser rápido e indoloro.

Descripción resumida del Proyecto
(Esta información debe ser un resumen ejecutivo del proyecto inextenso)

Objetivos del Proyecto (*):

1) Estudiar la ecología y comportamiento de los borochis, su estado sanitario y sus recursos alimenticios;

2) Evaluar el potencial de su conservación en PNNKM;

3) Desarrollar un plan de monitoreo y manejo para el borochi y el mantenimiento de su hábitat de sabanas;

4) Inventariar y monitorear las comunidades de pequeños mamíferos en hábitats diferentes del PNNKM;

5) Estudiar los efectos de inundaciones, incendios y cambios climáticos sobre la ecología de las sabanas (recursos de fauna y flora claves para borochis y otros carnívoros de la zona)

Metodologías (*):

- *Captura e inmovilización de borochis u otros canidos silvestres a través de la utilización de trampas jaula y dardos anestésicos; para la colocación o reemplazo de collares GPS,*
- *Seguimiento por radiotelemetría de los individuos capturados,*
- Colecta y análisis de muestras biológicas (sangre, orina, ectoparásitos), de los individuos capturados,
- *Colecta de muestras de heces, sangre y parásitos de borochis u otros canidos para evaluar incidencia de enfermedades,*
- *Monitoreo de borochis y otros animales con trampas cámara para identificación de especies e individuos,*
- *Captura de micromamíferos con trampas jaulas y redes de neblina. Se realizaran colecta de muestras (pieles y esqueletos, tejidos, y/o muestras en alcohol) solo en caso que no puedan ser identificados en campo,*
- *Colecta de muestras de herbario de plantas que forman parte de la dieta de los borochis.*

Resultados esperados (*): *Comprender la ecología de los borochis y otros mamíferos en las comunidades que cambian en las sabanas de PNNKM, con énfasis en los efectos de cambios de clima y de incendios. Aprender como manejar o mejorar las condiciones ecológicas para los borochis en áreas protegidas y mantener sus recursos en forma sostenible.*
Taxa(s) estudiados (Nombre común, Especie, Género, Familia, Orden): • *Borochi, Chrysocyon brachyurus, Canidae, (Carnivora)* • *Zorro, Cerdocyon thous, Canidae* • *Perro de monte, Speothos venaticus, Canidae* • *Ratones y roedores: unas 30 o más spp. de 18 géneros de Muridae, Caviidae, Sciuridae, Echimyidae, Erethizontidae; (Rodentia)* • *Marsupiales: unas 18 o más spp. de 12 géneros, de Didelphidae (Marsupialia)* • *Murciélagos: 50 o mas spp de Phyllostomidae, Vespertillionidae, Molossidae, Natalidae, Thyropteridae, Emballonuridae, Mormoopidae, Noctilionidae (Chiroptera)*

Nombre de la Institución Autorizada: *Museo de Historia Natural Noel Kempff Mercado*	**Detalle de especimenes depositados (*):** *Todos los especímenes colectados en el marco de esta investigación serán depositados en la colección científica seca o húmeda, según corresponda, del Museo NKM.*
Nombre de la Institución Contraparte: *Smithsonian Institution*	**Detalle de especimenes depositados (*):** • *Las muestras biológicas (sangre, orina y otras), heces y ectoparásitos serán exportadas para ser analizadas en laboratorios del. National Zoological Park.* • *En caso de ser necesario se exportaran especimenes (pieles y cráneos o en alcohol) para identificación taxonómica en calidad de préstamo al National Museum of Natural History..*

Para que este formulario tenga validez, debe contar con las rubricas originales de la Autoridad Ambiental Competente Nacional y la Institución Científica Autorizada.

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DECLARACIÓN JURADA Y FIRMA

Yo Patricia Herrera con C.I. Nº 3274579 SC en calidad de Representante legal de la institución científica autorizada (Museo de Historia Natural Noel Kempff Mercado) juro la exactitud y veracidad de la información detallada en el presente formulario, y me comprometo a no realizar actividades diferentes a las señaladas en el presente formulario, a cumplir con las normas consignadas en la Ley 1333 sus reglamentos, disposiciones conexas y normas técnicas aplicables a mi actividad y reparar los daños que pudieran producirse como resultado de mi actividad.

Ing. Patricia Herrera
Institución Científica Autorizada

Louise H. Emmons
Responsable Operativo Contraparte

Lugar y fecha: *Santa Cruz de la Sierra, Julio 7 de 2010.*

Handling Protocol for free-ranging Maned Wolves (*Chrysocyon brachyurus*)

Proyecto Borochi

Parque Nacional Noel Kempff Mercado

Drafted 9/10/10 Ellen Bronson, DVM

I. Methods of capture of maned wolves (MW)

1. Trapping in wood or hardware cloth/mesh guillotine traps with odor attractants (NZP captive urine), food attractants (canned sardines in oil, charque dried salted beef, etc) and water in bowels is the traditional form of capture. Traps are checked daily each morning before temperatures increase and to keep time in trap to a minimum. Generally maned wolves remain calm in the traps until approached. Anesthesia is induced in traps by darting with a Telinject pistol and pipe with 1.5 ml or 3 ml (or 5 ml if using ketamine 50 mg/ml and xylazine combination) Telinject darts with 1.5 x 30 mm uncollared needles, which is generally easy to accomplish by darting in the thigh musculature. Animals can also be hand-injected if their thigh region is pressed against the side by distracting the animal in the trap from the opposite side.

2. Free-ranging darting with pistol/pipe or dart rifle (Daninject or Telinject pipe in rifle stock). This is generally accomplished from a blind at a watering hole at night or free-ranging when following a radiocollared maned wolf when the location is known. The same Telinject or Daninject darts are used (Daninject darts should be used if possible in the Daninject rifle, some darts are not compatible between systems). The rifle allows for darting accurately from further distances, but should be tested/calibrated in the field at current temperatures for accuracy before use. The high end of the anesthetic doses is recommended for this type of capture to ensure that the animal is recumbent quickly and can be located, especially at night. If darting at night, place reflective tape near the tail of the dart. After darting, 10 minutes or appropriate time should be waited before searching for animal, and then a rapid search should be made to find the animal. NOTE: Filled darts will swell within a few hours, even if decompressed, and no longer fit into the rifle barrel. Drugs should be removed from the darts with a syringe and the darts prepared immediately before use.

II. Anesthetic protocols

1. **Telazol®/Zoletil® (Tiletamine/Zolazepam)**

This lyophilized drug is a combination of a dissociative anesthetic (tiletamine related to Ketamine) and a benzodiazepine (zolazepam related to diazepam/Valium®). There is no readily available and reliable reversal for either of the components, with the exception of flumazenil, which can be given at 1 mg per 20 mg zolazepam IM or IV, and reverses just this portion of the drug. In some European countries tilatemine/zolazepam is marketed as Zoletil® and contains different amounts of drug, so always be sure to double check drug concentrations.

The advantages of Telazol ®include a smooth, relatively quick (10-15 min) induction, very safe and stable anesthetic plane, and a smooth but often long recovery. The main disadvantage is the recovery time, although animals that have time to recover in a trap are typically fully recovered when released 4-6 hr later in the afternoon/at dusk. In rare cases in other carnivores (tigers, possibly other felids such as jaguars and lions), telazol® can anecdotally cause greatly prolonged recoveries over several days, but animals typically do not resedate once recovered. Telazol® should NEVER be supplemented after the initial dart with telazol ®itself, unless it is thought that all or most of the initial dart did not inject/take effect. Ketamine should be used as a supplement. Telazol® is a controlled substance (CIII) in the USA and is not available for sale in Bolivia as of 2010.

Telazol® must be reconstituted with sterile water or sterile saline. Typically, 5 ml of sterile water or saline are added to the powder in the bottle for an end concentration of 100 mg/ml.

Dosing:

Telazol® is typically dosed at 4-5 mg/kg for free-ranging canids/carnivores.

MW size	Body weight (kg)	Total amount of telazol® (mg)	Volume of telazol® (ml) **at 100 mg/ml dilution**
Small/Juvenile MW	18-22	80-100	**0.8-1.0**
Medium/Large (Adult) MW	22-28	100-140	**1.0-1.4**

Supplementation: ketamine at 50 mg (1 ml of 50 mg/ml or 0.5 ml of 100 mg/ml) intervals either IM or IV.

2. **Ketamine/Xylazine**

This combination consists of ketamine, a dissociative anesthetic, and xylazine (Rompun®), an alpha-2-agonist. Xylazine can be reversed with yohimbine, which is the direct antagonist of xylazine. The advantages of this protocol include fast induction time (5-15 min), stable anesthetic plane if dosed high enough, and partial reversibility (xylazine is reversed, ketamine cannot be reversed) for a quicker recovery than telazol®. The disadvantages of this protocol include hypotension (decrease in blood pressure, difficult to detect in the field, but most likely present), peripheral vasoconstriction (leading to pale mucous membranes, low pulse oximetry

readings for SaO2, and difficulty visualizing vessels during blood draw), and respiratory and/or cardiac depression (drop in respiratory rate, and at times heart rate). These side effects are typically minor and of minimal consequence for brief procedures such as for those we perform on MW in the field, since they are reversed with yohimbine. Supplementation with this protocol can be accomplished with ketamine IM or IV. Additional xylazine should NOT be given. The reversal with yohimbine should not be done until at least 30 minutes after the initial dose of xylazine in order to prevent rough recoveries (so that most of the ketamine has already been metabolized since it should only have an effect for 30-40 min.). However, if an animal is showing decreased respiratory rate (below 6 breaths/minute) or decreased heart rate (below 60 beats/minute), yohimbine should be given immediately IM before giving other emergency drugs and will likely remedy the problem. In rare cases, resedation has been anecdotally reported in other carnivores (typically in felids), and can cause the animal to become lethargic and drowsy several hours to 1 day later, after a normal recovery. In this case, yohimbine should be given again if possible.

Ketamine is available over-the-counter in Bolivia and is not controlled, but the maximum available concentration is 50 mg/ml, which will necessitate using a 5 ml Telinject dart. Ketamine is a controlled drug (CIII) in USA and available in 100 mg/ml or 200 mg/ml. Xylazine is not a controlled drug in USA. Yohimbine is also not controlled.

Dosing:

Ketamine/Xylazine is typically dosed at 5-10 mg/kg for ketamine and 1-2 mg/kg for xylazine for free-ranging canids/carnivores. The following is recommended per EB, different doses may be used at other veterinarians' discretions based on each case.

MW size	Body weight (kg)	Total amount of ketamine (mg)	Volume of ketamine (ml) **at 100 mg/ml dilution**	Volume of ketamine (ml) **at 50 mg/ml dilution**
Small/Juvenile MW	18-22	175	**1.75**	3.5
Medium/Large (Adult) MW	22-28	250	**2.5**	5.0

MW size	Body weight (kg)	Total amount of xylazine (mg)	Volume of xylazine (ml) **at 100 mg/ml dilution**	Volume of xylazine (ml) **at 20 mg/ml dilution**	Total amount of yohimbine (mg)	Volume of yohimbine **at 2 mg/ml dilution**
Small/Juvenile MW	18-22	25	**0.25**	1.25	2.7	**1.35**
Medium/Large (Adult) MW	22-28	30	**0.3**	1.5	3.5	**1.75**

Supplementation: ketamine at 50 mg invervals either IM or IV.

Yohimbine as a reversal should be given IM (can be given IV in case of emergency). If a weight is available, it is more accurate (better) to calculate at 0.125 mg/kg. Yohimbine should always be given, if possible, even if animal is already stirring or quite awake to prevent cardiovascular side effects post-anesthesia.

III. Emergency procedures for anesthetic and capture complications

Note in text	Problem	Drug	Dose for 25 kg	Volume of drug	Route of delivery
1	Respiratory depression (Resp rate < 6/min) or arrest (not breathing >30 seconds)	Doxapram (Dopram ®)	120 mg	**6 ml** (20 mg/ml)	IV best, can also be injected into the underside of the tongue[a]
2	Cardiac depression (heart rate <40/min) or arrest (no heartbeat)	Atropine	10 mg	**18 ml** (0.54 mg/ml) **CHECK CONCENTRATION**[b]	IV
2	Cardiac and respiratory arrest (no heartbeat or breathing)	Epinephrine	2.5 mg	**2.5 ml** (1 mg/ml) **CHECK CONCENTRATION**[b]	IV (do NOT give into tongue)
3	Shock of any form (cardiovascular, anaphylactic)	Dexamethasone	100 mg	**25 ml** (4 mg/ml)	IV best, could give IM
4	Seizure	Diazepam	12.5 mg	**2.5 ml** (5 mg/ml)	IV or rectal
5	Lack of recovery from ketamine/xylazine	Yohimbine	3 mg	**1.5 ml** (2 mg/ml) **CHECK CONCENTRATION**[b]	IM, either hand-injected or darted
6	Signs or concerns of infection	Penicillin G	750,000 IU	**2.5 ml** (300,000 IU/ml)	SQ
		Excede® (Ceftiofur Long-acting)	200	**1 ml** **(200 mg/ml)**	SQ (NOT IM/IV)

[a] Needle injection into the tongue can result in mild to moderate hemorrhage, which can be halted with manual pressure. Injections are best given on the underside of the tongue laterally and avoiding the central vessels.

[b] These drugs are available in various concentrations. Like with all drugs be sure to double check the concentration before administering to avoid serious consequences!

1. **Respiratory depression or arrest**

Definitions:
Respiratory depression =respiratory rate < 6 breaths/minute and/or very shallow
Respiratory arrest = no visible or audible respirations within 30-60 seconds

If breathing becomes decreased, the first step should be to vigorously stimulate the animal by slapping the chest, moving the head, pulling the tongue out of the mouth, pinching the nose, and closely watching for shallow breathing.

If breathing becomes substantially decreased in rate or depth, doxapram can be given at the listed dose IV (best) or as an alternative, injected into the underside of the tongue into the large muscle mass. This drug has recently been shown to have possible negative side effects in brain function, but anecdotally in EB and SLD's hands, it is effective in many cases of mild to severe respiratory depression in wildlife and zoo cases, including in one geriatric and compromised maned wolf, CB2, under telazol® anesthesia. If using ketamine/xylazine anesthesia and breathing becomes seriously compromised, the first action should be to reverse the xylazine by giving yohimbine immediately, if possible IV. Often the animal will rouse after reversal enough that the procedure will need to be aborted.

Often full respiratory arrest precedes or coincides with cardiac arrest, and if this is the case, atropine and epinephrine should also be given immediately.

2. **Cardiac depression or arrest**

Definitions:
Cardiac depression =heart rate < 40 breaths/minute and faint
Cardiac arrest = no audible heart beat or palpable pulse

This is the most serious situation and is accompanied by respiratory arrest if cardiac arrest is present.

- If using ketamine/xylazine anesthesia, the first action should be to reverse the xylazine by giving yohimbine immediately.
- For cardiac depression with faint or slow heartbeats, the next step should be the administration of atropine. This drug should be avoided if possible in conjunction with xylazine, so yohimbine should always be given first. This drug

should ideally be given IV, however it is not harmful if it ends up around the vein, so any attempt at IV is helpful. If necessary it can also be given IM, however, if true cardiac arrest is occurring, this route of administration will not be rapid enough to be helpful.

- The next step for full cardiac arrest should be the administration of epinephrine IV. This drug CANNOT be given into the tongue and should not be given into the muscle.
- If cardiac and respiratory arrest are occurring, they are often accompanied by cardiovascular shock (see next section), so administration of dexamethasone is indicated.

3. **Shock** (cardiovascular, anaphylactic)

Cardiac and respiratory arrest are usually accompanied by cardiovascular shock, so administration of dexamethasone (steroid) is indicated, and should be given IV, but can also be given IM or into the bottom of the tongue.

Dexamethasone can also be given via any of these routes in the rare case of anaphylactic shock from insect bite or reaction to an anesthetic drug. In this case, one may see swelling of the back of the mouth and pharynx (occluding airway), swollen face, shallow respirations, etc.

4. **Seizure**

Ketamine and tiletamine (dissociative anesthetics) decrease the seizure threshold and not uncommonly can produce seizures, either grand-mal, or more commonly, mild twitching or more serious paddling of limbs under anesthesia. This is one reason that both of these drugs are used in combination with another drug in current protocols. Seizures can still be seen, and are most likely to occur after xylazine reversal if the last injection of ketamine was less than 20-30 min before reversal and the ketamine is then remaining.

A seizure that is severe or lasts more than 60 seconds (actually observe with a watch, since seizures always appear to last longer than they actually do) should be treated. Diazepam is a safe and relatively short-acting drug that can be most easily given into the rectum with a syringe (without a needle). For fastest effect, it should be given IV, but this is often difficult with an animal that is paddling or shaking. Diazepam can be given IM, but has slow and poor absorption from muscle and is considered much more effective when delivered IV.

5. **Lack of recovery or resedation**

In rare circumstances, for example if reversal is not given fully IM, yohimbine may not reverse xylazine sufficiently. If an animal is not rousing within 30 minutes of administration of yohimbine, a second dose of yohimbine should be given at the original dose (0.125 mg/kg). Patience is required for observation of recovery, if the animal is

quiet and breathing well, it is often better to wait, however if no progress is seen after initial reversal (no increase in respiratory rate or depth, no movements at all, even when stimulated), a second dose can be given. In gray wolves, excitation has been reported with higher doses of yohimbine, but the animals recovered fine.

In rare cases in felids and some other carnivores in zoos and possibly free-ranging (EB and SLD have noted this in tigers, lions, polar bears), animals can "resedate" after reversal with yohimbine, which can occur many hours to even days later. The physiology of this process is not understood completely, but it is suspected that the yohimbine is metabolized and detaches from the receptors, and remaining xylazine is able to cause renewed signs of anesthesia. In such rare cases, administering a second dose of the yohimbine at the original dose (0.125 mg/kg) should be helpful and show improvement in lethargy and reduced responsiveness within 20 minutes. This yohimbine can be given either by hand-injection or via dart.

If atipamezole is available, this can be given at 0.2 mg/kg (5 mg for average MW, or 1 ml) as an alternative for the repeat dose of reversal and is often in zoo settings preferred after yohimbine appears to have lost effect.

6. **Infection**

If an infection is suspected at the time of anesthesia or as a result of darting or procedure, several antibiotic choices exist, depending on what is available. Penicillin G will last for 2-3 days if given SQ and is a good general antibiotic, especially for skin infections and wounds. Penicillin should ideally be kept cooled, which limits its usefulness in the field.

Excede® (ceftiofur)is a newer long-acting depot product that lasts for 2 wks in cattle, 10 days in horses, and is not yet tested in other exotic animals. However, it has been used extensively by EB and others in the zoo setting for a huge array of animals, including carnivores, hoofstock, and even birds and reptiles, and so far it appears to anecdotally last at least 1 wk in those species. It has a broad spectrum of activity (3rd generation cephalosporin), more so than penicillin, and can be kept at room temperature. Not yet available commercially in Bolivia, the maned wolf project veterinarians will try to bring Excede® to Bolivia on each trip.

IV. Biomedical sample collection during procedures

1. Blood

a. Blood collection sites

Blood can be collected from a number of veins in maned wolves, including the following:

- Medial saphenous vein: inside of rear leg, have someone hold up upper leg and hold off vein on medial aspect of leg on the ground with the back of the hand with pressure to make vein stand out. Vein should be palpable, although xylazine can make this vein difficult to feel. Keep small amount only of vacuum on the syringe, vein often collapses, but a fair amount of blood can be collected from this site.
- Lateral saphenous vein: outside of rear leg, best palpable around the hock joint. Have someone put a hand around the leg distal to the knee to hold off this vein. Use a small amount of vacuum only on the syringe.
- Cephalic vein: this vein traverses the dorsal surface of each front leg. Have someone hold off vein by putting thumb and first finger around the front leg above the elbow.
- Jugular vein: in jugular groove of lateral neck region.

b. Samples needed

- Purple top tube (EDTA anticoagulant): 1 small tube needed
    - This is used for cell counts, PCV and total protein (TP) counts, blood smears, whole blood cells can be saved for genetic testing
- Red top tiger or serum separator tube: several (at least 1, ideally 4-5)
    - This is spun or allowed to clot and serum is separated off for serology and biochemistry panels, and the remainder is banked
- Blood buffer tube for genetics (at least 1, ideally 2)
    - Add 2- ml fresh blood to pre-filled cryotube (ideally 2 tubes). Freeze when possible.

2. Urine

a. Methods of collection

Urine is best collected via cystocentesis with a 6-12 ml syringe and a 1.5 inch 22 ga (or similar) needle with manual palpation in the field. This procedure should only be performed by a veterinarian accustomed and skilled in the procedure in dogs and cats. If the bladder is not palpable, the procedure should not be performed. If cycstocentesis is not possible (e.g. small bladder or no experienced person present), attempts can be made to manually compress the bladder with a hand on the caudal part of the abdomen, especially in males, with a clean nalgene or cryotube over the end of the penis or near the vulva. The bladder should not be compressed after an attempt at cystocentesis, since the bladder could leak with the administered pressure if previously punctured by a needle.

3. Ectoparasites: collect any found (mites, fleas) in ethanol

4. Feces: collect from the rectum if possible and place in both formalin 10% for endoparasite ID. Save some feces if available in nalgene or other vial without preservative for freezing.

5. Physical examination

a. Components, at a minimum:

- Heart rate, ideally every 5 min
- Respiratory rate, ideally every 5 min
- Temperature, rectal, ideally every 5-10 min
- Dental exam, be specific and include photographs of all teeth
- Palpation of abdomen, verify presence or absence of right kidney

6. Physiologic parameters under anesthesia

- Heart rate: 100-150/minute (range we have seen in the field: 70-190)
- Respiratory rate: 15-30/minute (range we have seen in the field: 15- 100, at times panting if the animal is light and weather is warm)
- Temperature: 99-102° F (37-39° C); typically temperatures run at the high end due to the warm temperatures. If temperatures climb above 103° F (39.5° C), start to cool the animal by moving to shade, applying water or alcohol to foot pads, and if severe, recover animal

V. Laboratory field procedures

1. Blood

a. Red top serum separator tubes:

- The tubes should be allowed to clot, which typically takes 15-30 minutes.
- Red top serum separator tubes should be placed in the centrifuge for 20 min (or less desirable, allow to clot at room temperature for several hours). Remember to balance the centrifuge adequately.
- After 20 min centrifugation, decant the clear serum into small cryotubes, can also be pipetted if desired. Note if serum is slightly/moderately pink or red (= hemolysis).
- Label each cryotube and consider stickers as well, markers often smear or run when samples are frozen (consider placing all samples from each animal in own bag and label bag as well).
- Store serum as soon as possible at freezing temperatures, as constant as possible under field conditions.
- Can also attempt to keep the blood cells, but if using tubes with separator gel, this is often difficult. If possible, collect into cryotubes and freeze as well.

b. Purple top EDTA tube:

- Make 3-4 good quality slides per animal. Allow to dry at room temperature for several hours. Fix with methanol and allow to dry. Blood smears should be made as soon as possible once arriving at the station.

- Fill 2 microhematocrit tubes (non-heparinized), stick into clay, and place into a red top or other tube and place in centrifuge (be sure to balance with another like tube). These tubes can be centrifuged with the serum tubes if ready. Centrifuge for 5-10 minutes or until cells obviously separated from plasma.
    i. Once spun, read PCV/hematocrit on sliding scale
    ii. break one or both of the glass tubes near the border between cells and plasma, and blow onto glass of refractometer. Read the total protein (g/dl) scale (not specific gravity).
- Filter paper: Place 8-12 drops around edge (1 large or 1-2 small is sufficient) and label in the middle of the filter paper. Let air dry and put each sample into a separate bag with silicone pack.
- If available, perform Unopette or Natt-Herrick WBC count:
    i. Unopette: draw up blood in provided small pipette for accurate amount, mix in provided solution vial. Let sit for 5-10 minutes, then inject small amount slowly without air bubbles into the side of each chamber of the hemocytometer between the glass slide and the glass hemocytometer (keep both parts moist during 5-10 minute wait). Allow to settle for 3-5 min and then read ALL 9 large chambers, counting stained WBC only. Place into formula (x110). It is reported that Unopette is no longer commercially available in 2010, but may be available again in the future.
    ii. Natt-Herrick stain: draw up exactly 20 µl whole well-mixed blood into pipetter, mix thoroughly. Let sit for no more than 5 minutes, then place into moist hemocytometer as described above. Let settle for 3-5 min and read as above. Natt-Herrick solution is prepared before coming to the field in vials ready to be mixed.
- If blood is leftover, either place 1 ml in DNA buffer or spin in centrifuge for 10-20 min and pipette off the plasma and place into cryotube and freeze. The cell portion can also be saved, frozen in a cryovial for future studies.

2. Urine

a. Place one drop of unspun urine on the refractometer, measure specific gravity
b. Place few drops of urine onto a Dipstick stick and let sit according to package (30-60 seconds).
c. Filter paper: Make drops around edge as with blood, label in the middle of the filter paper, let air dry, put each in a separate bag with silicone pack.
d. Microscopic exam: Make 3 unstained slides and examine under 10x and 40x for ova and calculi.
e. Spin 0.5-1.0 ml of urine in centrifuge for 15 minutes. Discard supernatant, remix sediment and make 3 unstained slides and examine under 10x and 40x for ova and calculi.
f. Place some of the unspun urine in formalin.

g. Place remainder of unspun urine in cryovial and freeze.

3. Ectoparasites: should be placed in ethanol and labeled.

4. Feces: If enough sample available, place 1-2 grams each in: formalin and freeze a portion

VI. Other species

Species	Approx. wt (kg)	Telazol® (**mg**)	Xylazine (**mg**) 1-2 mg/kg	Ketamine (**mg**) 5-10 mg/kg	Yohimbine (approx. **mg**) 0.125 mg/kg
Crab-eating fox/Zorro (*Cerdocyon thous*)	4-6	20-30	5-10	40-60	0.6
Bush dog (Speothos)	5-7	25-35	6-10	50-70	0.75
Oncilla	2-3	10-15	2-4	20-30	0.4
Jagarundi	3-9	20-40	5-10	40-70	0.75
Ocelot	8-16	50-75	10-20	80-150	1.5
Puma	35-60 (F) 65-100 (M)	100-300 (F) 200-400 (M)	40-60 (F) 80-100 (M)	200-300 (F) 400-500 (M)	6.0 (F) 10.0 (M)
Jaguar	35 (F) 55 (M)	100-300 (F) 200-400 (M)	40-60 (F) 80-100 (M)	200-300 (F) 400-500 (M)	6.0 (F) 10.0 (M)

*Please note in above table all dosages are **given in mg and NOT ml.**

EXPOSURE OF FREE-RANGING MANED WOLVES (*CHRYSOCYON BRACHYURUS*) TO INFECTIOUS AND PARASITIC DISEASE AGENTS IN THE NOËL KEMPFF MERCADO NATIONAL PARK, BOLIVIA

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EXPOSURE OF FREE-RANGING MANED WOLVES (*CHRYSOCYON BRACHYURUS*) TO INFECTIOUS AND PARASITIC DISEASE AGENTS IN THE NOËL KEMPFF MERCADO NATIONAL PARK, BOLIVIA

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Abstract: Maned wolves (*Chrysocyon brachyurus*) are neotropic mammals, listed as a CITES Appendix II species, with a distribution south of the Amazon forest from Bolivia, through northern Argentina and Paraguay and into eastern Brazil and northern Uruguay. Primary threats to the survival of free-ranging maned wolves include habitat loss, road kills, and shooting by farmers. An additional threat to the conservation of maned wolves is the risk of morbidity and mortality due to infectious and parasitic diseases. Captive maned wolves are susceptible to, and die from, common infectious diseases of domestic dogs (*Canis familiaris*) including canine distemper virus (CDV), canine parvovirus (CPV), rabies virus, and canine adenovirus (CAV). Results from this study show that free-ranging maned wolves in a remote area of Bolivia have been exposed to multiple infectious and parasitic agents of domestic carnivores, including CAV, CDV, CPV, canine coronavirus, rabies virus, *Leptospira interrogans* spp., *Toxoplasma gondii*, and *Dirofilaria immitis*, and may be at increased risk for disease due to these agents.

Key words: Bolivia, *Chrysocyon brachyurus*, infectious diseases, maned wolf, parasitic diseases.

INTRODUCTION

Maned wolves (*Chrysocyon brachyurus*) are neotropic mammals with a distribution south of the Amazon forest from Bolivia, through northern Argentina and Paraguay and into eastern Brazil and northern Uruguay. The maned wolf is listed as a CITES Appendix II species (http://www.cites.org), the United States Fish and Wildlife Service considers it "endangered" (http://endangered.fws.gov), and the IUCN lists the species as "vulnerable" (http://www.redlist.org). There is no global population estimate, but maned wolves are absent from much of their former geographic range. The primary threat to the survival of the maned wolf is considered to be habitat loss.[31,33] Road kills are the major source of mortality near small parks in Brazil, and farmers also shoot maned wolves that they believe hunt chickens.[32]

The risk of morbidity and mortality due to infectious diseases is a significant concern in the conservation of maned wolves and wildlife in general.[10] Domestic dogs (*Canis familiaris*) are known or suspected reservoirs for agents of infectious diseases, which have devastated populations of wild carnivores in many areas of the world.[1,18,26,33] As human populations expand and come into closer proximity with free-ranging maned wolves, there is increased risk of disease transmission from domestic carnivores to maned wolves.

Although studies of exposure to disease agents of free-ranging maned wolves are lacking, captive maned wolves are known to be susceptible to important infectious agents of domestic dogs including canine distemper virus (CDV), canine parvovirus (CPV), rabies virus, and canine adenovirus (CAV). Morbidity and mortality have been reported for CDV,[7,36] CPV,[2,13,16,27] rabies virus,[35] and CAV.[3] Maned wolves may be susceptible to all the known infectious agents of domestic dogs.

Studies on the parasites of free-ranging or recently captive maned wolves have documented the presence of the giant kidney worm, *Dioctophyme renale*, which is known to destroy the right kidney in infected maned wolves.[6] Parasites of the urinary tract, in addition to *D. renale*,[5] gastrointestinal parasites,[12] and ectoparasites[30] have also been documented in free-ranging maned wolves. In captivity, maned wolves are also known to be susceptible to *Dirofilaria immitis*, the causative agent of heartworm disease, *Echinococcus granulosus*, and gastrointestinal parasites.[28]

The objective of this study was to determine the prevalence of exposure to select infectious and parasitic diseases of maned wolves immobilized as part of an ecologic study in the Noël Kempff Mercado National Park, Bolivia (NKMNP).

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MATERIALS AND METHODS

Study period and site description

From February 2000 to October 2003, four maned wolves were immobilized in the NKMNP as part of a radiotelemetry study. NKMNP includes the Serranía de Huanchaca escarpment and the adjacent lowlands between the Río Itenez (Guaporé) and the Río Paraguá and lies between 13°31′–15°05′S and 60°14′–61°49′W. The park is at the interface of Amazonian forest with grassland ecosystems and includes a number of distinct ecosystems including broadleaf semievergreen forest, dry forest, inundated forest, dry savanna (Cerrado), and inundated savanna.[24]

Sample and data collection and analyses

All maned wolves were immobilized using 100 mg tiletamine plus zolazepam (Telazol®, Fort Dodge Laboratories, Fort Dodge, Iowa 50501, USA; 3.5–4.5 mg/kg, i.m.) delivered through Telinject® (Telinject USA Inc., Agua Dulce, California 91390, USA) plastic darts, using a Telinject® pistol. When necessary, anesthesia supplementation was provided with ketamine (Ketaset®, Fort Dodge; 25–50 mg increments, i.v.). Blood was collected by venipuncture of the jugular vein or the lateral saphenous vein. Blood was immediately placed in serum separator tubes (Corvac Sherwood Medical, St. Louis, Missouri 63103, USA) for all the maned wolves. For two of the maned wolves (CYB 1 and CYB 2), the sample tubes were placed in a cool place until clot formation and then sera were separated by centrifugation (Mobilespin, Vulcan Technologies, Grandview, Missouri 64040, USA) at 3,000 *g* for 15 min and stored in liquid nitrogen. Blood of the other two maned wolves (CYB 3 and CYB 4) was allowed to clot at ambient temperature, and the serum was then decanted and kept cool for 48 hr before storage in a −20°C freezer.

Fecal samples were collected manually from the rectum and preserved in 10% formalin. Ectoparasites were collected and stored in 70% isopropanol from all four maned wolves. Urine was collected from CYB 1 and CYB 2 by cystocentesis using a 22 g, 1.5 inch needle and 12 cc syringe. Urine samples were divided into aliquots for freezing and formalin fixation. The remaining urine was immediately centrifuged at 3,000 *g* for 5 min. Urine sediment was immediately examined by direct microscopic examination in the field.

Samples were transported to the United States of America for laboratory testing. Blood and urine samples were transported on dry or wet ice. Fecal and urine samples were transported in 10% buffered formalin. Ticks were transported in 70% isopropanol. Formalin and alcohol were removed before air travel and refilled on arrival in the United States. All appropriate export and import permits accompanied the samples during transport.

Serologic testing for antibodies to CAV, *Brucella canis*, canine coronavirus, CDV, canine herpesvirus (CHV), CPV, *D. immitis*, *Toxoplasma gondii*, and leptospirosis antibody testing was conducted at the New York State Veterinary Diagnostic Laboratory (Cornell University, Ithaca, New York 14853, USA). The 18 *Leptospira interrogans* serovars tested included *L. ballum*, *L. wolffi*, *L. autumnalis*, *L. tarassovi*, *L. pomona*, *L. hardjo*, *L. grippophytosa*, *L. bataviae*, *L. canicola*, *L. ictero/COP*, *L. australis*, *L. pyrogenes*, *L. bratislava*, *L. sejroe*, *L. icterohaemorrhagia/icterohaemorrhagia*, *L. javanica*, *L. szwajizak*, *L. saxkoebing*. Serologic testing for rabies virus was performed at Kansas State Veterinary Diagnostic Laboratory (Kansas State University, Manhattan, Kansas 66506, USA) using the rapid fluorescent focus inhibition test. Three of the maned wolves, CYB 1, CYB 2, and CYB 3, were tested for all the infectious agents listed above, whereas CYB 4 was only tested for the presence of antibodies to CAV, CDV, and CPV.

Fecal samples were examined by direct microscopic examination, sodium nitrate flotation, and sedimentation methods at the New York State Veterinary Diagnostic Laboratory. Adult ticks were identified on the basis of external morphology, using the keys of Jones et al.[22].

Frozen and formalin-fixed urine samples were analyzed at the School of Veterinary Medicine (University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA); these results will be reported elsewhere.

RESULTS

Table 1 summarizes the infectious disease agent serologic tests performed, methods used, level of titers defined as positive, and results for each test. All four maned wolves tested had positive antibody results to CAV and CPV and two had positive antibody results to CDV. Of the infectious diseases for which CYB 1, CYB 2, and CYB 3 were tested, none of them had antibodies to *B. canis* and CHV. All three of these maned wolves were antibody positive for one or more *L. interrogans* serovars including *L. ballum*, *L. grippophyfosa*, *L. icterohaemorrhagica/icterohaemorrhagica*, and *L. szwajizak*. One maned wolf (CYB 1) was antigen positive for *D. immitis* and one (CYB 3) had antibodies to coronavirus, rabies virus, and *T. gondii*.

Gastrointestinal parasites were identified in the

Table 1. Disease agent serologic tests performed, methods used, level of titers defined as positive, and results in the study for select infectious and parasitic disease agents in four free-ranging maned wolves (*Chrysocyon brachyurus*) in Noël Kempff Mercado National Park, Bolivia.

Disease agent (method used)[a]	Positive titer	CYB 1	CYB 2	CYB 3	CYB 4
Canine adenovirus (SN)	1:4	Positive 1:512[b]	Positive 1:512	Positive 1:384	Positive 1:512
Brucella canis (SlideAGG/AGID II)	NA	Negative	Negative	Negative	NA
Canine distemper virus (SN)	1:8	Negative	Positive 1:12	Negative	Positive 1:12
Canine herpesvirus (SN)	1:8	Negative	Negative	Negative	NA
Canine parvovirus (HAI)	1:10	Positive 1:10	Positive 1:10	Positive 1:10	Positive 1:20
Coronavirus (SN)	1:8	Negative	Negative	Positive 1:32	NA
Dirofilaria immitis (occult)	NA	Positive	Negative	Negative	NA
Rabies virus (RFFIT)	1:5	Negative	Negative	Positive 1:13	NA
Toxoplasma gondii (IHA)	1:64	Negative	Negative	Positive 1:128	NA
Leptospira interrogans 18 serovars (microagglutination)	1:100	*L. szwajizak* Positive 1:100	*L. ballum* and *L. icterohemorrhagia/icterohemorrhagia* Positive 1:200	*L. grippo* Positive 1:400; *L. icterohemorrhagia/icterohemorrhagia* Positive 1:100	NA

[a] SN, serum neutralization; Slide AGG/AGID II, slide agglutination/agar gel immunodiffusion test II; HAI, hemagglutination inhibition; RFFIT, rapid fluorescent focus inhibition test; IHA, indirect hemagglutination; NA, not applicable.
[b] Positive titer.

feces of all four maned wolves and included *Ancylostoma* sp., *A. caninum*, *Capillaria* sp., *C. aerophilia*, *Gnathostoma* sp., *Isospora* sp., *Physaloptera* sp., *Strongyles* spp., *Toxocara canis*, *Trichuris* sp., and *Uncinaria* sp.

Ticks collected from all four maned wolves were *Ambylomma* spp. The most prevalent species, *A. tigrinum*, was present on all four maned wolves. A few *A. ovale* ticks were found on CYB 1 and CYB 2. Of the two maned wolf urine samples evaluated in the field, one (CYB 1) was observed to have *D. renale* ova on urine sedimentation. The other (CYB 2) was negative for this parasite.

DISCUSSION

The serologic tests used in this study document exposure but not disease by the detection of antibodies to infectious agents and not the agents themselves. The occult heartworm test is an exception because the test detects the causative agent, *D. immitis*. Therefore, the serologic portion of this study was used to determine whether these maned wolves were exposed to a select number of infectious and parasitic agents known to be of concern for wild carnivore conservation.[17] Disadvantages to serology are the possibility of false positives, because of cross-reaction with other agents, and false negatives. Moreover, the tests used in this study have not been validated for use in maned wolves because there is possible inaccuracy or cross-reacting substances in the host.[21] Currently, there are no serologic tests validated for maned wolves, but the tests developed for domestic dogs, which were used in this study, are widely used in the testing of captive maned wolves.

Results suggest that free-ranging maned wolves in the NKMNP have been exposed to pathogens, which are known to cause high morbidity and mortality in captive maned wolves and other carnivores. This is of particular interest because the NKMNP is located in a relatively isolated area of northeastern Bolivia, far from urban centers and large populations of domestic dogs. We believe that the most likely route of pathogen exposure for these maned wolves stems from domestic dogs, which live in villages and on ranches surrounding the park. Alternatively, these disease agents may be self-sustaining in free-ranging maned wolves and other carnivores in the NKMNP.

Evidence suggests two possible disease epidemics in the wild carnivore populations of the NKMNP during the years we have been working in the park. The first maned wolf (CYB 1) died 8 mo after it was radiocollared. Its remains were found at a time when fox activity was appreciably diminished in the park. In 2003, three pups and an unmarked mother maned wolf disappeared between July and September 2003. Based on reports from

park guards, road kill, shooting, and habitat changes were ruled out as causes of their disappearance. Disease is the most likely cause of the disappearance of CYB 1 and the other four maned wolves that disappeared. It is noteworthy that maned wolf CYB 3, antibody positive for CAV, CPV, coronavirus, rabies virus, *T. gondii*, and *L. interrogans* spp., was sampled in September 2003 after the disappearance of this family group, with which it was known to be in contact. Genetic evaluation of the maned wolf population in the NKMNP has yet to be finished, but it is known that CYB 1 was unrelated to the other maned wolves in this study and that CYB 2–CYB 4 were all related and in documented close contact with each other.

The evidence of exposure in these maned wolves to CDV (two of four) and CPV (four of four) is of greatest concern. CDV has caused serious epidemics and population declines in wild carnivores[11] and is known to cause mortality in captive maned wolves.[7,36] It is most commonly spread by close contact with infectious carnivores through aerosolized respiratory secretions but can remain viable in the environment for weeks under the proper conditions.[19] Parvovirus has been reported as a cause of morbidity and mortality in a number of free-ranging canid species[4] and has caused mortality in captive maned wolves.[13,16,27] Transmitted by the fecal–oral route, CPV can survive for months in the environment and does not require close contact for transmission.

All four of the maned wolves tested were positive for CAV. CAV likewise has been shown to cause mortality in captive neonatal maned wolves,[3] although it is probably of less pathogenic significance than CDV and CPV.

Only one of the maned wolves was antibody positive to coronavirus, rabies virus, and *T. gondii*. All these agents can cause morbidity and mortality in free-ranging canids and have been implicated as a threat to the conservation of a number of carnivore populations.[17] Rabies virus has caused disease in captive maned wolves.[36] Although not a significant pathogen alone, coronavirus infections concurrent with other viral or bacterial agents are likely to increase morbidity and mortality.[14,29] Exposure to *T. gondii* in this maned wolf was most likely through the ingestion of raw meat (i.e., eating small mammals), and thus, probably does not suggest transmission from domestic dogs or other carnivores in the region.

Leptospirosis is a zoonotic bacterial disease commonly associated with fever, sepsis, kidney failure, and reproductive abnormalities in a number of animal species and humans.[20] Many free-ranging canids are seropositive to various *L. interrogans* serovars without showing illness or functioning as important reservoirs.[23] The significance of the findings of positive antibodies to a few *L. interrogans* serovars in the maned wolves is not known.

One of the three maned wolves tested was positive for *D. immitis*, the causative agent of canine heartworm. This is a potentially fatal, mosquito-borne disease of domestic and wild carnivores. In captivity, maned wolves are often maintained on a heartworm prophylactic because of the devastating effect of this parasite. The role of *D. immitis* in morbidity and mortality of free-ranging maned wolves is not known.

The finding of *D. renale* ova was not surprising because this parasite is cited as a common pathogen of recently captive maned wolves.[25] *Dioctophyme renale* is often associated with a hypoplastic right kidney in infested maned wolves and could contribute to mortality of wolves especially with concurrent disease.[25,28]

Free-ranging canid species usually harbor enteric parasites, including those found in the maned wolves in this study.[23] These parasites are not often present in high numbers and do not cause a clinical problem in adult free-ranging canids. However, in animals immunocompromised because of factors such as concurrent disease or physiologic stress related to habitat or population modifications, enteric pathogens may result in disease. The lungworm *Capillaria aerophila*, detected in the feces of two maned wolves in the study, can cause clinical signs associated with bronchitis and pneumonia, but these animals had no overt respiratory signs. *Amblyomma* spp. ticks have been collected from free-ranging maned wolves before this study;[12] however, this was the first documented *A. ovale* record from maned wolves, as previously reported.[30]

Recently, disease has become a recognized threat to the long-term conservation of free-ranging wildlife.[8–10,38] An increased prevalence of disease is most likely associated with global scale anthropogenic changes, which include human population growth, habitat fragmentation and degradation, the isolation of populations of species, and an increased proximity of humans (and their domestic animals) to wildlife;[10] all these appear to be present in Bolivia. Studies to determine pathogen exposure and disease prevalence in both the threatened wildlife and the domestic animals, which share the habitats with these animals, are imperative to minimize the effect of disease on the conservation of wildlife species. For example, a study on the border of the Madidi National Park in northwestern Boliva showed a high prevalence of exposure of domestic

dogs and cats to pathogens, which may infect wild carnivores in that park.[15]

The data presented in this study have been the basis for formulating a large-scale study of the disease ecology of domestic dogs, sympatric crab–eating foxes (*Cerdocyon thous*), and maned wolves in and on the perimeter of the NKMNP. The population of maned wolves in the park has been estimated at 120 breeding pairs.[34] If so, NKMNP is one of the most important remaining protected areas for free-ranging maned wolves. Determining the risk of infectious disease exposure to the whole population will be important to help park managers as they develop policies to protect maned wolves and other carnivores in the park.

Control of disease in domestic and feral domestic animals is likely to become an increasingly important part of protected area management. Although already followed by many National Park systems, we recommend that two rules be universally followed in protected areas: 1) prohibit the release of individuals of wild species from captivity into wild populations, unless the wild populations are threatened and in need of augmentation for survival, and strict health and genetic evaluations have been performed before release[37] and 2) exclude all pets and other domestic animals from parks. If parks include human settlements with domestic animals, we recommend that these are vaccinated and monitored for disease. These measures have been instituted in the NKMNP, elsewhere in Bolivia, and at many other sites around the world.

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Monitoreo Sanitario del Borochi (*Chrysocyon brachyurus*) en el Parque Nacional Noel Kempff Mercado, Bolivia

Health monitoring of Maned wolves (*Chrysocyon brachyurus*) in Noel Kempff Mercado National Park, Bolivia

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RESUMEN

Se examinó la exposición a enfermedades, tanto de origen infeccioso como parasitario en 11 borochis (Chrysocyon brachyurus) capturados en 17 ocasiones como parte de los esfuerzos para los estudios de radiotelemetría en el Parque Nacional Noel Kempff Mercado. Los objetivos fueron evaluar la salud y amenazas por enfermedades en una población en libertad y protegida a fin de sugerir opciones de gestión para reducir la exposición de la población a las enfermedades infecciosas y parasitarias. Todos los borochis fueron seropositivos a algún agente infeccioso (virus y protozoos). La mayoría de los borochis se encontraban infestados con el gusano del corazón (*Dirofilaria immitis*), un nematodo potencialmente letal, y del gusano gigante del riñón (*Dioctophyme renale*), otro nematodo que destruye el riñón derecho, ambos endoparásitos se encuentran en individuos adultos domésticos y silvestres. Además se registraron otros endoparásitos y ectoparásitos. La mayoría de las enfermedades evidenciadas en los borochis estudiados son causas de altas morbilidades y mortalidades en las crías. Todos los patógenos identificados también pueden afectar a los perros domésticos, de donde es posible que originalmente se hayan transmitido por algún tipo de contacto. Para proteger la salud de las personas, sus animales domésticos y los carnívoros silvestres, sugerimos que los planes de gestión de las Áreas Protegidas incluyan programas de vacunaciones nacionales para perros y gatos que viven cerca de parques, priorizando contra la rabia y el distemper o moquillo canino.

Palabras Clave: *Chrysocyon brachyurus*, rabia, moquillo, *Dirofilaria immitis,* Bolivia, enfermedades, vida silvestre

ABSTRACT

Exposure to disease and presence of parasites was examined in 11 maned wolves (*Chrysocyon brachyurus*) captured 17 times for telemetry studies in Parque Nacional Noel Kempff Mercado. Our goals were to evaluate the health of a protected population; judge the threats to the population from selected diseases; and suggest management options to reduce the exposure of the population to infectious diseases or parasites. All maned wolves were seropositive to several of 10 infectious micro-organisms (viruses and protozoans). The majority were also infected with heartworm, a potentially lethal nematode, and giant kidney worm, which destroys one kidney. In addition we recorded a number of intestinal or other parasites. Many of the diseases to which maned wolves showed evidence of exposure cause morbidity and mortality of pups, while a few, such as heartworm, are likely to effect adults. All of the pathogens also can infect domestic dogs, from which some pathogens may originally have been transmitted. To protect the health of people, their domestic animals and wild carnivores, we suggest that protected-area management plans include vaccination programs for dogs and catsat least against rabies and distemper virus.

Key words: *Chrysocyon brachyurus*, rabies, distemper, *Dirofilaria immitis*, Bolivia, diseases, wildlife

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INTRODUCCIÓN

Los borochis (*Chrysocyon brachyurus*) son cánidos de gran tamaño con una distribución al sur del Bosque amazónico de Bolivia, abarcando el norte de Argentina y Paraguay, el este de Brasil y el norte de Uruguay. En su estatus de conservación se encuentra enlistada en el apéndice II de CITES. (), en peligro de extinción en la lista del Servicio de Pesca y Vida Silvestre de los Estados Unidos (http://endangered.fws.gov), y como especie vulnerable en la Lista de la UICN (http://www.redlist.org). Actualmente no se cuenta con ninguna estimación de la población mundial, pero se evidencia que los borochis están ausentes en gran parte de su antigua área de distribución geográfica. Se considera que la principal amenaza para la supervivencia del borochi es la pérdida de hábitat y el influjo humano relacionados con la mortalidad, incluida la muerte en carreteras cercanas a parques en Brasil (Rodden *et al.*, 2004) y la matanza de borochis para el uso de partes del cuerpo en la medicina popular o por los productores agropecuarios que ven al borochi como un predador de su ganadería (Rodrigues, 2002). El riesgo de morbilidad y mortalidad debido a enfermedades infecciosas se está convirtiendo en una importante preocupación en la conservación del borochi y la vida silvestre en general (Deem *et al.*, 2001; Deem y Emmons, 2005). Perros domésticos (*Canis familiaris*) son consabidos o presuntos reservorios de los agentes de enfermedades infecciosas que han devastado las poblaciones de carnívoros silvestres en muchas zonas del mundo (Alexander y Appel, 1994; Gascoyne *et al.*, 1993; Laurenson *et al.*, 1998; Roelke-Parker *et al.*, 1996). Dos estudios recientes confirman que los perros domésticos que viven en la frontera de los parques nacionales en Bolivia han estado expuestos a una serie de enfermedades infecciosas y parasitarias, agentes de interés para la conservación de carnívoros silvestres (Fiorello *et al.*, 2004; Bronson *et al.*, 2008). Como cada vez las poblaciones humanas amplian su área de uso, propiciando una mayor cercanía con sitios de vida libre de los borochis, hay cada vez mayor riesgo de transmisión de las enfermedades de carnívoros domésticos a estos.

El Parque Nacional Noel Kempff Mercado (PNNKM), se encuentra ubicado en el Departamento de Santa Cruz, Bolivia, abarcando 1,5 millones de hectáreas de el Bioma del Cerrado. Aproximadamente el 30% de la diversidad de hábitats del parque son las sabanas inundables y las sabanas de tierras altas que están seccionadas en seis fragmentos que comprenden un total de 3.600 km^2 de un hábitat adecuado para el borochi (Killeen y Schulenberg 1998; Rumiz y Sainz, 2002). El PNNKM es uno de los sitios protegidos con la población más grande de borochis, con una estimación de 120 parejas reproductoras (Rumiz y Sainz, 2002), aunque estudios más recientes sobre la base de telemetría han demostrado que los rangos de uso de hábitat son mucho mayores (alrededor de 80 km^2) que los que se había estimado (Dietz, 1984), que disminuye la población estimada a unos 30-40 parejas (Emmons *et al.*, datos no publicados), o aproximadamente el mismo número que se estima para el Parque Emas en Brasil (Silveira *et al.*, en prensa). Estas muy pequeñas poblaciones reproductoras que están protegidas por grandes parques con hábitats del cerrado, no están libres de una potencial susceptibilidad para la declinación de la población de borochis por sucesos catastróficos, tales como una epidemia de enfermedades infecciosas.

Se ha estudiado la salud del borochi en el PNNKM, en la sabana de Los Fierros desde el año 2000 (Deem y Emmons, 2005). Siendo el objetivo estudiar la prevalencia de la exposición para identificar las enfermedades infecciosas y parasitarias de borochis, capturados e inmovilizados químicamente, como parte de un estudio con telemetría. Nuestro objetivo final es proporcionar información sobre el estado general de salud de la población reproductora en el PNNKM, el establecimiento de una base desde la cual evaluar los cambios futuros, y sugerir políticas de gestión para mantener la salud a largo plazo de las poblaciones en el parque.

MÉTODOS

Los borochis fueron estudiados en PNNKM, principalmente en el área de Los Fierros y la pampa de la Estación Biológica "El Refugio Huanchaca", en partes contiguas de la misma sabana, centrado en UTM 20S 8375654, 723779, a una elevación de 195 m. Un animal fue capturado en un breve estudio preliminar en Mangabalito (20 S 764855, 8474991) en el año 2000. De febrero del 2000 a febrero de 2007, 11 borochis fueron inmovilizados un total de 17 capturas en el PNNKM. Las muestras recogidas después de mayo de 2007 no han sido analizados ni introducidas en los datos que figuran a continuación. Para la inmovilización química de los borochis, el protocolo anestésico utilizado consistió en 90 - 130 mg de tiletamina y zolazepam y cuando fue necesaria la administración de suplementos de la anestesia, se le administró ketamina. La sangre se obtuvo por punción venosa de la yugular o vena safena

lateral. Los tubos con muestras fueron colocados en un lugar fresco hasta la formación del coágulo de 6 - 48 horas, y los sueros fueron separados por centrifugación y se almacenaron en un congelador. Las muestras de heces se recolectaron manualmente desde el recto y fueron conservadas en formol al 10%. Los ectoparásitos fueron recogidos y conservados en etanol al 70%. Las pruebas serológicas para detección de anticuerpos contra el adenovirus canino (AC), *Brucella canis*, coronavirus canino (CC), el virus de moquillo o "distemper" canino (VDC), herpesvirus canino (HC), parvovirus canino (PC), *Toxoplasma gondii* y leptospirosis, así como para la detección del antígeno de *Dirofilaria immitis* se llevó a cabo en el Estado de Nueva York, en el Laboratorio de Diagnóstico Veterinario (Universidad de Cornell, Ithaca, Nueva York 14853, EE.UU.). La prueba para los 18 serovariantes de *Leptospira interrogans* incluyeron *L. ballum, L. wolffi, L. autumnalis, L. tarassovi, L. pomona, L. hardjo, L. grippophytosa, L. bataviae, L. canicola, L. icterohaemorrhagiae / copenhageni, L. australis, L. pyrogenes, L. bratislava, L. sejroe, L. icterohaemorrhagiae / icterohaemorrhagiae, L. javanica, L. szwajizak*, y *L. saxkoebing*. La prueba serológica para el virus de la rabia se llevó a cabo en el Laboratorio de Diagnóstico Veterinario del Estado de Kansas (Kansas State University, Manhattan, Kansas, EE.UU.), utilizando la prueba rápida de inhibición fluorescente focal. Las pruebas serológicas para la detección de anticuerpos contra *Ehrlichia canis*, *Borrelia burgdorferi*, y *Rickettsia rickettsii* se llevó a cabo en el Laboratorio de Diagnóstico Médico Veterinario de Texas, mediante la prueba de immunofluorescencia. Más detalles sobre los métodos se pueden encontrar en Deem y Emmons (2005), y Bronson *et al.* (2008).

No a todos los borochis se le realizaron las pruebas para la detección de todos los agentes infecciosos (véase la tabla 1), debido a la limitada cantidad de sueros y / o modificaciones al diseño del estudio de los agentes considerados motivo de preocupación durante los 8 años de la investigación. Las muestras de heces fueron examinadas por examen coprológico microscópico directo, flotación con nitrato de sodio, y el método de sedimentación, realizadas en el Laboratorio de Diagnóstico Veterinario del Estado de Nueva York. Las garrapatas adultas fueron identificados por R. Robbins sobre la base de la morfología externa, utilizando las claves taxonómicas de Jones *et al.* (1972).

RESULTADOS

Se capturaron 11 individuos de borochi (6 machos y 5 hembras) en un total de 17 capturas con inmovilización anestésica (Tabla 1). En la tabla 2 se resumen los agentes de enfermedades infecciosas, pruebas serológicas realizadas, y si los resultados se consideraron seropositivos o seronegativos. Ningún borochi fue seropositivo a la brucelosis canina (*Brucella canis*) y, por tanto, los resultados no se muestran en la tabla 2. En cuatro borochis se realizaron las pruebas para 18 serovares de *Leptospira interrogans* y en otros cuatro sólo 5 serovares consideradas las más importantes. Cuatro de los 11 borochis fueron positivos para uno o más serovares de *L. interrogans*. Entre estas serovares, se encontraron *L. grippotyphosa, L. icterohaemorrhagiaeni / copenhageni, L. icterohaemorrhagiae / icterohaemorrhagiae*, y *L. szwajizak*. Sin embargo, todos los títulos son bajos en 1:100 - 1:200.

Tabla 1. Detalle de captura de los once borochis (*Chrysocyon brachyurus*) estudiados, su edad estimada, peso corporal, el volumen celular y los sólidos totales del suero medidos con un refractómetro.

Borochi	Año	Sexo	Edad	Peso (kg)	Volumen Celular	Sólidos Totales
1	2000	H	2-3 años	21	41	8.4
2	2001	M	> 10 años	28.5	42	7.2
3	2003	H	2 años	29		
3	2004	H	3 años	27.5		
3	2005	H	4 años	26.5	38	6.9
3	2006	H	5 años	25	35	7.3
4	2002	M	7 meses	23.5		
5	2004	M	3 años	29	39	6.4
5	2005	M	4 años	27.8	40	7.1
5	2007	M	5 años		38	6.8

Cont. Tabla 1

Borochi	Año	Sexo	Edad	Peso (kg)	Volumen Celular	Sólidos Totales
6	2005	M	10-12 años	23	40	7.4
7	2005	H	8 meses	21		
8	2005	M	3 años	27.8		
8	2006	M	4 años	26	36	7.6
9	2006	H	< 6 meses	17	33	7.0
10	2006	M	8 meses	21		
11	2007	H	7-8 años	21	31	7.9

Aunque varias muestras fecales y de ectoparásitos todavía deben ser evaluadas, fueron encontradas en las heces: *Isospora sp., Ancylostoma caninum, Physaloptera sp., Capillaria sp. Capillaria aerophilia* (número elevado), *Gnathostoma sp., Isospora sp., Physaloptera sp. Strongyles spp., Toxocara canis, Trichuris sp.*, y *Uncinaria sp.*. Las especies de garrapatas identificadas incluyen *Amblyomma ovale, A. triste, A. tigrinum* y *Boophilus microplus*. Los huevos del gusano gigante del riñón (*Dioctophyme renale*) se encontraron en tres de los cinco animales a partir de los cuales se recogió la orina.

Tabla 2. Pruebas serológicas realizadas (N/A: no aplicable) y título positivo (+), negativo (-) o inconcluso (*) obtenidos para cada agente patógeno en once borochis (*Chrysocyon brachyurus*) del Parque Nacional Noel Kempff Mercado, Bolivia

ID	Año	DI[1]	TG[2]	Rabia	AC	HC	VDC	PC	CC	EC[3]	BB[4]	RR[5]
1	00	+	-	-	+	-	-	+	-	N/A	N/A	N/A
2	01	-	-	-	+	-	+	+	-	N/A	N/A	N/A
3	03	-	+	+	+	-	-	+	+	N/A	N/A	N/A
3	04	-	+	+	+	+	+	+	-	N/A	N/A	N/A
3	05	-	+	-	+	-	+	+	+	+	-	+
3	06	+	+	-	+	-	-	-	-	-	-	+
4	02	N/A	N/A	N/A	+	N/A	+	+	N/A	N/A	N/A	N/A
5	04	+	+	+	+	-	+	+	-	N/A	N/A	N/A
5	05	+	+	-	+	+	-	-	-	+	-	+
5	07	+	+	-	+	-	-	-	-	-	-	-
6	05	+	+	-	+	-	+	-	-	+	-	+
7	05	-	+	-	+	-	+	-	-	+	-	+
8	05	+	+	-	+	-	-	-	-	+	-	+
8	06	+	+	-	+	-	-	-	-	-	*	+
9	06	+	+	N/A	+	-	-	-	-	-	+	+
10	06	-	-	N/A	+	-	-	-	-	-	+	+
11	07	+	+	N/A	+	-	-	-	-	+	+	-
11[6]		10/16	13/16	3/13	17/17	1/16	7/17	7/17	2/16	6/11	3/11	9/11
		(63)	(81)	(23)	(100)	(6)	(41)	(41)	(13)	(55)	(27)	(82)
TA[7]		7/10	8/11	2/7	11/11	1/10	6/11	5/11	1/10	6/8	3/8	7/8
		(70)	(73)	(29)	(100)	(10)	(55)	(45)	(10)	(75)	(38)	(88)

[1] ***Dirofilaria immitis***; [2] ***Toxoplasma gondii***; [3] ***Ehrlichia canis***; [4] ***Borrelia burgdorferi***; [5] ***Rickettsia rickettsii***; [6] **Total positivos a la prueba/total muestras analizadas (%);** [7] **Total de los animales positivos/total de animales analizados (%).**

DISCUSIÓN

La prueba utilizada para identificar la ocurrencia de la enfermedad parasitaria del gusano del corazón detecta la presencia del agente causal, *D. immitis*. Todas las demás pruebas utilizadas en este estudio se basan en la detección de anticuerpos frente a agentes infecciosos, pero no así a los propios agentes, y, por tanto, lo que se determina es la exposición, no la enfermedad. Las pruebas serológicas realizadas en el presente estudio fueron, utilizadas para determinar si estos borochis habían estado expuestos a una serie de enfermedades infecciosas y parasitarias, agentes que se sabe son de interés para la conservación de carnívoros silvestres (Funk et al., 2001). Una desventaja de las pruebas serológicas es la posibilidad de falsos negativos. La presencia de anticuerpos depende de muchos factores, como el estado inmune del huésped, el momento de la infección inicial y el resultado de la infección; así también la detección de anticuerpos depende de la exactitud de la prueba, la presencia de reacción cruzada con sustancias del sitio de colecta, el proceso en la manipulación de la muestra, y la debida ejecución de las pruebas. Por otra parte, las pruebas utilizadas en este estudio no han sido validadas para su uso en borochis y, por lo tanto es posible que hubiese inexactitud o reacción cruzada (Greiner y Gardner, 2000).

Actualmente, no existen pruebas serológicas validadas para borochi, pero las pruebas que se utilizan para los perros domésticos, que se utilizaron en este estudio, se utilizan ampliamente en el monitoreo sanitario de borochis en cautiverio.

Los 11 borochis muestreados fueron positivos a la prueba para adenovirus canino (AC). El AC se transmite a través de contacto con fluidos corporales infectados y es estable durante días o meses en el medio ambiente. Perros infectados pueden excretar el virus en la orina durante al menos seis a nueve meses (Greene, 1998). El AC ha sido reportado como una de las causas de mortalidad en crías de borochi en cautividad (Barbiers y Bush, 1995).

T. gondii es un parásito protozoario de distribución mundial que infecta a una gran variedad de mamíferos silvestres. Los únicos hospederos definitivos conocidos son los miembros de la familia Felidae. *T. gondii* tiene una amplia distribución en las poblaciones humanas, con la prevalencia de anticuerpos tan altos como 90% en algunas regiones. En el presente estudio el 73% de los borochis fueron positivos para *T. gondii* con anticuerpos IgG. Los animales silvestres pueden infectarse por comer inadvertidamente ooquistes fecales de félidos, diseminados en el medio ambiente o por comer los hospederos intermediarios cuyos tejidos contienen al quiste del parásito. Los borochis en el PNNKM se alimentan también de armadillos (Lilienfeld, 2000) y, por tanto, podrían estar infectados a través de esta presa, ya que el 30% de Tatús (*Dasypus novemcinctus*) en el Gran Chaco fueron seropositivos a este agente (Deem et al. en prensa).

D. immitis causa una enfermedad canina potencialmente fatal que afecta al corazón, transmitida por mosquitos, afectando carnívoros domésticos y silvestres. En cautiverio los borochis reciben tratamientos profilácticos por los efectos devastadores que provoca este parásito. No se conoce el papel de *D. immitis* en la morbilidad y la mortalidad de borochis de vida libre, pero la tasa de infestación en el PNNKM fue extremadamente alta, con seis de los once borochis positivos a la prueba. Uno de los borochis (CB 5), con positividad persistente con título de anticuerpos alto a *D. immitis*, así como microfilarias visibles en frotis de sangre, desarrolló soplo cardiaco en grado IV / VI que creemos, pueden estar relacionados con la Dirofilariasis. A dos de los siete borochis se le encontró positividad con bajos títulos de anticuerpos para el virus de la rabia. La rabia procedente de cepas de perros domésticos han causado epidemias que disminuyeron la población de lobos de Etiopía (SILLERO-Zubiri et al., 1996) y perros salvajes africanos (Gascoyne et al., 1993). También se incluye un informe de un *Leopardus tigrinus* de Bolivia con un alto título de anticuerpos contra la rabia. Se cree que estos individuos serían sobrevivientes a la exposición previa a la rabia (Deem et al., 2004).

El virus del moquillo canino o "distemper" es la principal causa de muerte en cachorros no vacunados de los perros domésticos. Los brotes ocurren cíclicamente cuando los perros que sobreviven protegidos por los anticuerpos son sustituidos por animales jóvenes que no han tenido exposición previa al virus. El moquillo se disemina de animal a animal de forma directa a través de las excreciones respiratorias y otros fluidos corporales. Puede infectar a muchas especies de carnívoros y su presencia entre los borochis analizados de Los Fierros sugiere que muchos carnívoros del parque se encuentran en riesgo de contraer esta enfermedad.

El parvovirus canino es una enfermedad reciente, identificada por primera vez en el decenio de 1970, que es ahora conocida en todo el mundo. Curiosamente, todos los individuos de borochi que secuencial o simultáneamente ocupan territorio en Los Fierros (CB2-CB5) fueron seropositivos para PC, mientras que los ocupantes del territorio vecino (CB6-CB11) fueron todos negativos (Tabla 2). Este virus se propaga a través de las heces, donde pueden persistir durante meses. La territorialidad estricta que observamos entre estos grupos (Emmons et al., Datos no publicados) puede en este caso haber ayudado a reducir la propagación de una enfermedad que puede causar mortalidad en los borochis, sobre todo en cachorros (Maia y Gouveia, 2002; Mann et al., 1980).

El coronavirus canino (CC) es otro virus intestinal que puede causar diarrea y se elimina en las heces. En las pruebas para el CC se obtuvo un individuo seropositivo que posteriormente pasó a ser seronegativo. Este virus por lo tanto, parece de baja prevalencia en el área de estudio.

El herpesvirus canino puede causar mortalidad en los cachorros y se propaga por el contacto directo entre los individuos. Cabe señalar que los dos únicos individuos seropositivos pertenecían a una pareja que por datos de telemetría se supo que se habían apareado.

Desde 2005 en adelante se ha analizado la exposición a algunos parásitos patógenos. Muchos perros domésticos urbanos en América del Sur están infectados y se convierten en sintomáticos con *E. canis*, el cual puede causar una mortal anemia. En Brasil el 19,8% de los perros, en su mayoría del área urbana, fueron seropositivos para este agente patógeno (Labarthe et al., 2003). *E. canis* es transmitida principalmente por la garrapata marrón de los perros, *Rhipicephalus sanguineus*, aunque también las garrapatas *Amblyomma* y *Dermacentor spp.* pueden transmitirlo (Anziani, et al., 1990). El 55% de los borochis que se analizaron presentaron seropositividad aunque con bajo título de anticuerpos a este agente patógeno. Del mismo modo, el 86% de 40 perros a los cuales se les tomó muestras en las comunidades vecinas al parque resultaron seropositivos para este agente, presentando muchos de ellos un alto título de anticuerpos (Bronson et al., 2008). La patogenicidad de este protozoario en borochis, así como el riesgo potencial de transmisión por la cercanía de los perros domésticos, es desconocido. La mayoría de los borochis estudiados resultaron seropositivos a *R. rickettsii* (88%), al igual que la mayoría de los perros en las comunidades (86%; Bronson et al., 2008). Este agente causa la fiebre manchada de Brasil en América del Sur y es principalmente transmitida por la garrapata *Amblyomma cajennense*, que en el presente estudio se obtuvieron de tres de los 11 borochis (Robbins y Deem, 2002). Los resultados sugieren que muchos perros y borochis están infectadas con *E. canis* y *R. rickettsii*, a pesar de reacciones cruzadas con agentes apatógenos estrechamente relacionados dentro de cada uno de estos géneros, que puede causar falsos positivos (Bronson et al., 2008).

Los cánidos silvestres de vida libre por lo general albergan parásitos entéricos, incluyendo las que se encontraron en los borochis en este estudio. Estos parásitos no están a menudo presentes en gran número y, por lo general no causan un problema clínico en los cánidos adultos que viven en libertad. Sin embargo, en animales inmunocomprometidos debido a factores como enfermedades persistentes o estrés fisiológico relacionados con el hábitat o modificaciones en la población, propician que determinada carga parasitaria entérica desencadene un estado de enfermedad. El parásito pulmonar *Capillaria aerophila,* que se detectó en las heces de dos borochis en el presente estudio, puede causar bronquitis y neumonía, pero estos animales no presentaban signos de problemas respiratorios.

Todos los borochis en el área de estudio muestran la exposición a múltiples enfermedades que son comunes a los perros domésticos. Sin duda también pueden estar presentes enfermedades propias de animales silvestres que aún no han sido identificadas, pero actualmente no hay manera de encontrar evidencias de exposición para estas enfermedades, tan sólo para aquellas para las que existen pruebas que han sido desarrolladas para cánidos domésticos. La mayoría de las enfermedades identificadas (*T. gondii*, AC, HC, VDC, PC, *E. canis*), causan mortalidad principalmente en los cachorros, mientras que los adultos seropositivos son sobrevivientes a la exposición encontrándose sanos. La mortalidad de crías es en gran medida inadvertida, ya que en vida silvestre las crías son sólo vistas a partir de alrededor de los tres meses de edad, cuando empiezan a moverse y salir de su madriguera, e incluso así se los ve muy rara vez. En el año 2001 evidenciamos que una de las crías, un juvenil, desapareció, y en el 2003 una camada de tres cachorros que fué visto en mayo se redujo a dos hasta julio y ya no se registró ningún cachorro en septiembre. En la población estudiada no se pudo

conocer el tamaño de la camada al nacer, en el caso de los nacimientos sólo se detectaron en dos ocasiones por telemetría. Por tanto las enfermedades de las crías en gran medida son crípticas, así como los efectos del éxito reproductivo. La pérdida de cachorros podría causar una reducción en la diversidad genética en la población de borochis, o en casos extremos, una lenta declinación de la población.

Algunas enfermedades, sobre todo la del gusano del corazón, gusano gigante del riñón, la rabia y la leptospirosis, tienen más probabilidades de afectar o causar la muerte prematura de los animales más viejos que han tenido la oportunidad de reproducirse. Varias de las enfermedades provocan daño a los riñones como ser la del gusano gigante del riñón, y la leptospirosis, y dado que la mayoría de borochis están infectados con el gusano gigante del riñón sujetos a la pérdida de un riñón (Rodden et al. 2004), los efectos negativos de las infecciones de riñón pueden ser exacerbadas. La mortalidad de adultos podría causar cambios en la estructura social así como la disminución de la población.

Las vacunas o tratamientos para la mayoría de las enfermedades caninas reportadas en borochis, en el presente documento (AC, VDC, PC, Rabia, Dirofilariasis, Leptospirosis) están disponibles. Estas vacunas o tratamientos son caros y fuera del alcance de los pobladores de las comunidades bolivianas que viven cerca de los parques, y es tal vez poco realista prever la eliminación de todas las enfermedades prevenibles de perros y gatos a nivel nacional en torno a los parques. Por otra parte, es probable que varias de las enfermedades que pueden haber sido originalmente introducidas por las mascotas domésticas se hayan ya convertido en endémicas en las poblaciones silvestres y sean difíciles o imposibles de erradicar. Entre ellas figuran los parásitos nematodes, *E. canis*, AC, HC, y PC. La exposición repetida causa por lo general, la evolución hacia una mayor resistencia en los carnívoros silvestres en las áreas protegidas. Sin embargo, las dos enfermedades más mortíferas, la rabia y el moquillo, que se producen en brotes cíclicos, pueden ser controlados en gran medida mediante el uso de vacunas. Recomendamos encarecidamente que en todos los planes de gestión para las áreas protegidas en todo el mundo, incluyendo el PNNKM y otros parques de Bolivia, se incluya programas de vacunación de animales domésticos, al menos, contra estas dos enfermedades en las zonas de amortiguación alrededor de los parques. Esta acción va a proteger la vida silvestre, así como mejorar la vida de los animales vacunados y a sus dueños.

Dado que los parques se van transformando cada vez más en islas dentro de paisajes dominados por la actividad humana, es fundamental para la futura supervivencia de especies raras como el borochi, excluir a los animales domésticos de las zonas de potencial contacto, donde podrían transmitirse enfermedades o parásitos a las especies silvestres. Como señalamos anteriormente, su transmisión puede ser a través de la orina, heces, garrapatas, moscas, carroña, o por el movimiento de especies comensales, como zorros, entre zonas con y sin ganado. A través de una gestión cuidadosa, varias enfermedades potencialmente mortales de los animales domésticos y fauna silvestre pueden ser controladas o eliminadas, y los brotes de nuevas enfermedades impedido.

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6

Morbidity and Mortality

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ABSTRACT. The health status of a population of maned wolves (MW), *Chrysocyon brachyurus*, in Noel Kempff Mercado National Park (NKP), Bolivia, was studied from 2000 to 2009 by direct observations, GPS and VHF telemetry, and biomaterial collection. A total of 12 MW were anesthetized for 33 events. Causes of morbidity included severe dental disease, skin lesions, lameness, endoparasites (among them, *Dioctophyme renale* and *Dirofilaria immitis*), ectoparasites, urinary cystine calculi, traumatic injuries, and exposure to infectious disease agents. During this decade, five of the 12 (42%) MW died. Age at time of death varied from 1.5 to >10 years. Pathologic findings identified postmortem included vertebral pathology (n = 2) and severe dental disease (n = 2). The remaining seven MW either emigrated with fate unknown or were alive in 2009 and ranged in age from 8 months to 7 years. Maned wolves in NKP are geriatric by age 8 or 9. We estimated that a total of seven litters were born to three resident adults. Of these seven litters, five included at least one pup raised to 6–8 months subadults and two litters were lost, one at 16 days and the other at 5 months (Chapter 5). Our data support the observation that dental and skeletal diseases are limiting factors for the longevity of both captive and free-living MW.

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INTRODUCTION

One of the main goals of conservation is to evaluate and, if possible, optimize the factors that are most important for maintaining population fitness of a species. Knowing the causes of mortality and morbidity of a free-ranging species is essential to knowing how best to protect it from hazards that pose present or future threats to species survival. During our studies of the ecology of the maned wolves (MW) (*Chrysocyon brachyurus*) in Noel Kempff Mercado National Park (NKP), we took advantage of the trapping and immobilization required to deploy telemetry collars for collecting as much health information as we could under primitive field conditions. This part of our research was a major collaborative effort that over the years involved not only our field crew and four veterinarians but also the specialist knowledge of diagnostic laboratories,

parasitologists, medical entomologists, laboratory technicians, and a dentist.

Few studies have been conducted on the health of free-living MW, however, available reports include baseline hematology and chemistry profiles (Dietz, 1984; May-Júnior et al., 2009) cystinuria and cysteine calculi (Carvalho and Vasconcellos, 1995; Deem and Emmons, 2005; Dietz, 1984), descriptions of endo- and ectoparasites (Beldomenico et al., 2002; Bevilaqua et al., 1993; Carvalho and Vasconcellos, 1995; Deem and Emmons, 2005; Robbins and Deem, 2002), dental trauma (Furtado et al., 2007), and evidence of exposure to a number of infectious agents (Deem and Emmons, 2005; Deem et al., 2008). All diseases reported in free-living MW (except gunshot wounds and trauma caused by vehicle contact) are also commonly reported in captive MW, including some not yet identified in free-living individuals, such as dermatitis, proliferative gingivitis, neoplasia, and spondyloarthropathy (Fletcher et al., 1979; Hammond, 2012; Maia and Gouveia, 2002; Norton, 1990; Reid et al., 2005; Maned Wolf Husbandry Manual, 2007; Rothschild et al., 2001).

Causes of mortality of MW in captivity are similar to those reported above for morbidity. However, euthanasia (commonly elected due to pain and immobility resulting from skeletal problems) and perinatal losses account for many *ex situ* deaths (National Zoological Park, unpublished data). The majority of captive MW die by 15 years with the longest lived captive-born MW in the North American population recorded at 16 years 7 months for males and 17 years 10 months for females (M. Rodden, pers. comm.). Causes of mortality in the wild, except by vehicle road kill and shooting, are largely unknown, as is age at death. A recent study by Sollmann et al. (2009) in Emas National Park, Brazil, found survival rates of approximately 64% annually for both genders and for subadults and adults alike.

We describe what we learned of the causes of morbidity and mortality in a population of free-living MW in NKP, as an integral part of a study of their ecology. We compare our findings to causes of morbidity and mortality in captive MW at the Smithsonian Conservation Biology Institute.

Materials and Methods

Field Site

Noel Kempff Mercado National Park lies between 13°31′–15°05′S and 60°14′–61°49′W at the interface of Amazonian forest with diverse savanna ecosystems, and it includes broadleaf semi-evergreen forest, dry forest, inundated forest, dry savannas, and flood-prone savannas. The habitats and climate are described and illustrated in Chapter 1. The complex habitat mosaic of NKP results in a rich fauna of 604 bird species (B. Hennessy, Armonia, pers. comm.) and 172 mammal species, including 20 Carnivora (Emmons et al., 2006a, 2006b); all with potential to interact with MW: as prey, predators, and vectors or hosts of pathogens and parasites. The territories of the MW in our study population in Los Fierros savanna are isolated from direct contact with human settlements, pets, or livestock, except horses that travel briefly on one road a few times a year (without staying overnight or grazing). However, at least one GPS-collared maned wolf (M6) traveled 30 km to a neighboring estancia that has dogs, cats, poultry, and hoofstock and then returned into Los Fierros savanna (Chapter 3), so isolation is incomplete. There is little vehicle traffic on the single, potholed, dirt road that crosses the savanna, and we found only one maned wolf road kill, in about 1995, when there were logging trucks still speeding on that road.

Sample and Data Collection in the Field

Maned wolves were captured within NKP in wooden box traps or hardware-cloth cage-traps baited at first with live chicks, but later with sardines and fatty dried beef (see Chapter 1 for more detail). From February 2000 to July 2009, 12 MW were captured for a total of 33 anesthetic events. Immobilization was with tiletamine plus zolazepam (Telazol®, Fort Dodge Laboratories, Fort Dodge, Iowa 50501, USA; 3.5–4.5 mg/kg, intramuscular [i.m.]) or a ketamine (Ketaset®, Fort Dodge; 6–8 mg/kg, i.m.)/xylazine (Xylazine: TranquiVed, Vedco, Inc., St. Joseph, Missouri 64507; 1.1 mg/kg, i.m.) combination delivered through Telinject® plastic darts (Telinject USA Inc., Agua Dulce, California 91390, USA) using a Telinject® pistol (Bronson et al., in preparation). If needed, anesthesia was supplemented with ketamine (Ketaset®, Fort Dodge; 25–50 mg increments, intravenous or i.m.). For the ketamine/xylazine combination, the xylazine was reversed with yohimbine (Wildlife Pharmaceuticals, Inc., Fort Collins, Colorado 80522, USA; 0.125 mg/kg, i.m.).

A physical examination was performed on each maned wolf at the time of anesthesia and included temperature, pulse, respiration, detailed oral examination, detection of skin lesions, and abdominal palpation. Ages were estimated by physical characteristics (e.g., dentition, coat appearance, mammae appearance, and body measurements) according to the criteria of Dietz (1984) and in comparison

to known-aged individuals of our study. We documented with photographs the whole body, dentition, and lesions, and various samples were collected from each maned wolf. Blood was collected by venipuncture of the jugular, cephalic, or the lateral saphenous vein and was immediately placed in ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes (Becton Dickinson, Franklin Lakes, New Jersey 07417, USA) and serum separator tubes (Corvac Sherwood Medical, St. Louis, Missouri 63103, USA). The sample tubes were placed in the shade until clot formation, at which point sera were separated by centrifugation (Mobilespin, Vulcan Technologies, Grandview, Missouri 64040, USA) at 3,000 g for 15 min and stored in a freezer. Alternatively, blood was allowed to clot at ambient temperature, and the serum was then decanted and kept in a cool place for 48 hours before storage in a freezer.

Blood in EDTA was used to prepare thin blood smears fixed with 99% methanol. Packed cell volumes (PCV) were determined using a portable 12 V centrifuge, and plasma total solids (TS) were measured with a handheld refractometer (Schulco, Toledo, Ohio 43608, USA) calibrated at the site. White blood cell (WBC) counts were determined manually with a prepackaged dilution system (Unopette Test 5877, Becton-Dickinson Vacutainer Systems, Rutherford, New Jersey 07070, USA). Samples were transported on dry or wet ice to the Department of Animal Health, Smithsonian National Zoological Park, USA, for storage at –70°C and laboratory testing.

Fecal samples were collected manually from the rectum and preserved in 10% formalin. Ectoparasites were collected, stored, and shipped in 70% isopropanol or ethanol. Urine was collected by cystocentesis using a 0.7mm × 25.4 mm needle, and 12 mL syringe, but not all individuals could be sampled. Urine samples were divided into aliquots and frozen in cryotubes as well as fixed in formalin. The remaining urine was evaluated using a refractometer calibrated at the site, and with Multistix-Reagent Strips for Urinalysis (Bayer Corporation, Elkhart, Indiana 46515 USA), followed by immediate centrifugation at 3,000 g for 5 min. Urine sedimentation was examined directly by microscope in the field. Preservative solutions were removed before air travel and supplemented upon arrival in the USA. All appropriate export and import permits accompanied the samples during transport and the studies were approved by the IACUC of Smithsonian National Zoological Park and Smithsonian National Museum of Natural History.

With the exception of subadults weighing <20 kg, GPS or VHF telemetry collars were placed on each maned wolf at the initial anesthetic event (see Chapter 1). On the basis of signal immobility, MW assumed dead were retrieved and any teeth or bones were collected for later evaluation. Salvaged skeletons were deposited in the Museo de Historia Natural Noel Kempff Mercado, Santa Cruz, Bolivia.

Laboratory Diagnostics

Thin blood smears were stained with a modified Wright-Giemsa stain (Hematology Three-Step Stain, Accra Lab, Bridgeport, New Jersey 08014, USA) or a Diff-Quick stain (DipQuick Stain, JorVet, Jorgensen Laboratories, Loveland, Colorado 80538, USA) and examined for blood parasites, blood cell morphology and WBC differentials. Serum biochemistries were processed on a COBAS MIRA Plus chemistry system (Roche Diagnostic Systems, Inc., Branchburg, New Jersey, 08876).

Serologic testing for antibodies was conducted at the New York State Veterinary Diagnostic Laboratory (Cornell University, Ithaca, New York) using serum neutralization for canine adenovirus (CAV-II), canine coronavirus (CCV), canine distemper virus (CDV), and canine herpesvirus (CHV); using hemagglutination inhibition for canine parvovirus (CPV); using slide agglutination/agar gel immunodiffusion for *Brucella canis*; using indirect hemagglutination assay for *Toxoplasma gondii*; and by microagglutination for *Leptospira interrogans* serovars. The same laboratory was used for detecting *Dirofilaria immitis* antigens using an occult antigen test. The five *L. interrogans* serovars tested included *L. pomona*, *L. hardjo*, *L. icterohaemorrhagicae*, *L. grippophytosa*, and *L. canicula*. Serologic testing for rabies virus was performed at Kansas State Veterinary Diagnostic Laboratory (Kansas State University, Manhattan, Kansas) using the rapid fluorescent focus inhibition test. Antibodies to *Ehrlichia canis*, *Borrelia burgdorferi*, and *Rickettsia rickettsii* were tested at the Texas Veterinary Medical Diagnostic Laboratory using an immunofluorescence assay. These serologic diagnostic tests are targeted at canine pathogens known from North America, leaving unexplored the world of native sylvatic pathogens.

Fecal samples were examined by direct microscopic examination, sodium nitrate flotation, and sedimentation methods at the New York State Veterinary Diagnostic Laboratory. One adult worm was collected perirectal and submitted to Dr. Michael Kinsella for identification. Adult ticks were identified on the basis of external morphology, using the keys of Kohls (1956) and Jones et al. (1972). Urine was assayed semiquantitatively for increased cystine concentrations using the cyanide-nitroprusside method (Shih, 1973).

Data Analyses

Results were analyzed using a commercial statistical software package (NCSS, Kaysville, Utah; SPSS, version 13.0, Chicago, Illinois). Numerical data were inspected for normality and t-tests were performed on normal data and Mann-Whitney U-tests were used where normality was rejected (Petrie and Watson, 2006). Statistical significance was determined as $p < 0.05$.

RESULTS

Among the 12 MW, seven were immobilized once, three were anesthetized two, four, and eight times, respectively, and two others were each immobilized six times. Maned wolves were generally immobilized and sampled once yearly, but a few were immobilized twice within a year at intervals of over three months. Six were male and six were female.

Physical Findings

Body weights were recorded for six females (16 measures) with a mean of 23.2 kg ± 3.6, range 17–29 kg and for six males (13 measures) as 26.2 kg ± 2.4, range 21–29 kg. Males were significantly heavier than females (t-test; $P = 0.0076$) when both age groups (i.e., subadult and adult) were combined. Body weight of adult females, based on 10 measures from three animals, was 25 kg ± 2.9, range 22.9–27.1 kg. Body weight of adult males, based on 11 measures from four animals, was 26.96 kg ± 1.64; range 23–29 kg. Adult female body weights were significantly lower than adult male body weights (t-test; $P = 0.03$).

The most striking lesions on physical examination were the dental lesions, such as abnormal conformation, wear and attrition, fractures, missing teeth, gingivitis, and caries (Table 6.1 and Figures 6.1, 6.2). Male M5 had class II brachygnathia (underbite) and several other oral lesions (Table 6.1). Other specific findings included the following: M2 was blind in the left eye due to a shrunken globe at time of anesthesia, female F3 had signs of old ear pinna trauma (probably from myiasis around an ear tag), M6 was thin with mild crepitus in multiple metatarsal joints on palpation, M8 had moderate conjunctivitis and a grade IV/VI heart murmur and was limping on release following anesthesia, F12 had a laceration on the caudal footpad, and two animals (F3, M4) had bald areas on the lateral thigh regions. Uncaptured individuals photographed by camera traps had similar ear lesions (one) and large bald areas on the thigh (one).

Hematology

Males had higher packed blood cell volumes (PCV) (37% ± 3.8) than did females (32% ± 5.3) and lower total

TABLE 6.1. Clinical and pathological dental findings for free-living maned wolves (MW) (*C. brachyurus*) in Noel Kempff Mercado National Park (NKP). Ages are estimates. Here n/a, not present; X, present.

ID	Age, Years	Abnormal conformation	Wear	Fractures	Missing teeth	Gingivitis	Gingival hyperplasia	Caries	Osteomyelitis
F1*	0.7	n/a	X	n/a	n/a	n/a	n/a	n/a	n/a
M2*	10+	n/a	X	X	X	X	n/a	n/a	X
F3	1.5–8	n/a	X	n/a	X	X	X	X	n/a
M4	0.8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
M5	3–7	X	X	X	X	n/a	X	X	n/a
M6*	10	n/a	X	X	X	n/a	n/a	X	X
F7	1	X	X	n/a	n/a	n/a	n/a	n/a	n/a
M8	3–7	n/a	X	n/a	X	n/a	n/a	X	n/a
F9*	0.6–3	n/a	X	n/a	X	n/a	n/a	X	X
M10	1	n/a	X	n/a	n/a	n/a	n/a	n/a	n/a
F11*	8–10	X	X	X	X	n/a	n/a	X	n/a
F12	0.6	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

*Animal died during course of study. Skull was recovered for evaluation.

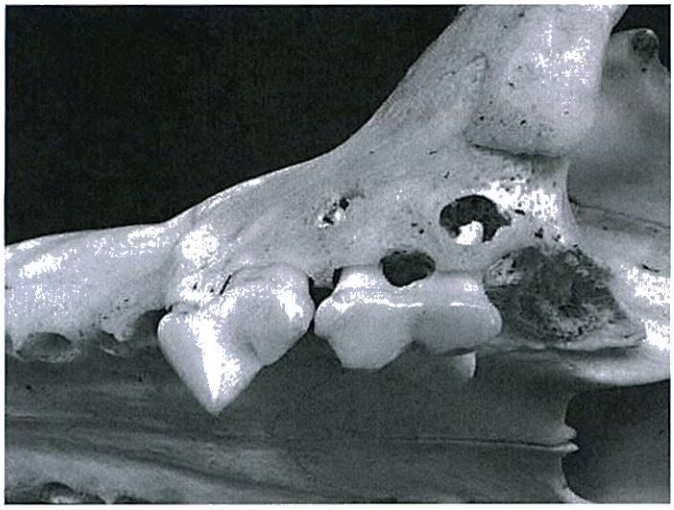

FIGURE 6.1. Upper left teeth P4 and M1 of maned wolf F9, 3 years old at death. Note that M2 was lost before death and the alveolus healed (closed), that the labial cusps of M1 are already worn flat, and that P4 shows almost no wear. P2 and P3 were lost postmortem. Osteomyelitis has eroded the bone at the root of M2. The right M2 was likewise missing. Photograph, L.H. Emmons.

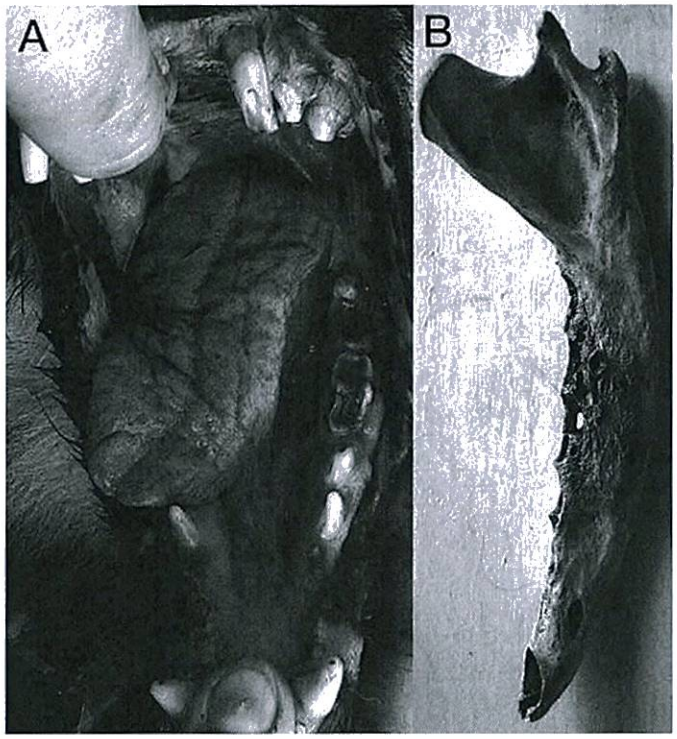

FIGURE 6.2. Teeth of geriatric male M6 with multiple dental lesions. (A) Teeth at capture including slab fracture of upper canine, other canines and premolars worn to root canals or broken off to roots, missing first lower premolars and outer upper incisors, and the broken, worn, and infected lower molars and third premolars. (B) Recovered mandible of M6, who died about 5 months after the photo in A, showing the severe bone infection underlying the cheek teeth shown in A. Photograph, L.H. Emmons.

solids (7.1 mg/dL ± 0.5) than females (7.9 mg/dL ± 0.6) (t-test; $p < 0.05$). One female (F3) had a PCV of 22% in 2007 and 2008 but had values in the normal range in other years (38% in 2005, 35% in 2006, and 31% in 2008). When we removed the two lowest PCV values (22% and both from F3), there was still a significant difference between males and females (34% ± 3.3) (t-test; $p < 0.05$). All other hematological values were not significantly different between the sexes (Table 6.2).

Serum Chemistry

Among all the parameters we measured, only the serum creatinine kinase (CK) activities differed (Mann-Whitney U-test; $p < 0.05$) between genders, with a median of 353 U/L ($n = 10$) for males and of 147 U/L ($n = 12$) for females. This may be due to the high value in one male (M5 1,310 U/L), and that both of the highest CK values were males (Table 6.3).

Serologic Testing

All 11 of the MW had antibodies to CAV-II at every sampling date ($n = 30$) with titers ranging from 512 to 4096 (Table 6.4); 85% of samples were heartworm (*D. immitis*) antigen positive and 56% tested positive for toxoplasma (*T. gondii*) antibodies. A low prevalence of antibodies to CDV, CPV, *Ehrlichia canis*, and the five serovars of Leptospira (*L. interrogens*) was found (Tables 6.4, 6.5).

Endoparasites and Ectoparasites

Eggs of endoparasites were found in all of the 14 fecal samples from the 7 MW evaluated. One adult worm, found perianally on F9, was identified as a dog roundworm (*Toxocara canis*). Eggs of pinworms, mites, and mite eggs from the family Listrophoridae were detected in 4 samples from 3 MWs (F3, M8, and F9). All ticks identified were from the pantropical genus *Amblyomma*. Few

TABLE 6.2. Hematology for free-living MW (*C. brachyurus*) in NKP. Here n/a, not applicable.

Measure	Male *N*	Male Mean (SD) or median	Male Range or 10%–90% quartiles	Female *N*	Female Mean (SD) or median	Female Range or 10%–90% quartiles	*P* value	Both sexes *N*	Both sexes Mean (SD) or median	Both sexes Range or 10%–90% quartiles
PCV (%)*	11	37 (3.8)	31–42	13	32 (5.3)	22–41	0.01*	n/a	n/a	n/a
TS (mg/dL)*	11	7.1 (0.5)	6.4–8.2	12	7.9 (0.6)	6.9–8.8	0.04*	n/a	n/a	n/a
WBC ($X10^3/\mu L$)	9	13,036 (6,130)	4,840–21,780	9	10,872 (5,937)	5,500–23,015	0.46	18	11,954 (5,959)	4,840–23,015
Neutrophils (%)	12	79.5	51.4–91	11	87	53.2–91	0.54	23	81	56.4–91
Bands (%)	12	0	0–2.4	11	0	0–2.6	0.93	23	0	0–2.2
Lymphocytes (%)	12	10	5–33.9	11	8	0–28	0.19	23	10	2–26.4
Monocytes (%)	12	2.5	0.3–12.8	11	3	0.2–10.8	0.58	23	3	0.4–10.6
Eosinophils (%)	12	1	0–8.7	11	1	0–22.6	0.25	23	1	0–12.4
Basophils (%)	12	0	0–0	11	0	0–0.8	0.3	23	0	0–0

*t-test; statistical significance.

individuals had fleas and they were rare on those that did carry them (Table 6.6).

Urinary Findings

Nitroprusside test results were strongly positive for urine from two of four males and for both females tested, indicating that these MW carried the inherited disease cystinuria. Three of five urine sediments that we evaluated microscopically revealed cystine crystals. Curiously, cystine crystals were found in the urine sediment of M6, who tested negative by cyanide-nitroprusside testing. Perhaps dissolved cystine had precipitated, and only the supernatant was examined by nitroprusside testing. In contrast, urine from F3 was negative for cystine crystals by microscopy but positive by nitroprusside testing. Furthermore, ova of the giant kidney worm, *Dioctophyme renale,* were detected in three of five maned wolf samples examined by microscopic evaluation in the field (Table 6.7).

Mortality

Up to September 2009, five of the 12 MW studied died, with four deaths occurring between February and April, and three of these five were over 7 years of age (Table 6.8). Following a major fire that swept the entire area in October 2009 (Figure 1.10), two other adults disappeared (F3, M8) and were presumed dead (Chapter 5, Postscript). Examination of five retrieved skeletons revealed severe vertebral pathologies (Figure 6.3), and tooth loss and osteomyelitis of the skull in two geriatric individuals (Figures 6.1, 6.3). An exception was for elderly F11, who had molar teeth worn down to the gums, but no skeletal and only minor dental lesions: a lost premolar and a small slab fracture of a canine tooth. One female (F9), 3 years old at time of death, had an infected tooth and was positive for *D. renale* and *D. immitis* antemortem and died when the study area was experiencing unusually strong flooding. The other young female that died (F1) was believed to have succumbed to an infectious agent as the fox (*Cerdocyon thous*) population in the same section of NKP was decimated at this time.

We estimated that at least seven litters were born to three females (Chapter 5; Table 5.7). Of these, five included at least one pup raised to ≥6–8 month subadults, and two litters were lost, at 16 days (F3 in 2008) and at 5 months (unmarked mate of M2 in 2003), respectively.

TABLE 6.3. Chemistry profiles for free-living MW (*C. brachyurus*) in NKP. Here n/a, not applicable.

	Male			Female				Both sexes		
Measure	*N*	Mean (SD) or median	Range or 10%–90% quartiles	*N*	Mean (SD) or median,	Range or 10%–90% quartiles	*P* value	*N*	Mean (SD) or median	Range or 10%–90% quartiles
Glucose (mg/dL)	10	70.1 (12.8)	51–88	11	67.1 (16,7)	44–88	0.65	21	68.5 (14.7)	44–98
AST (U/L)	10	57.5	22.7–125.3	12	33.5	18.3–135.3	0.08	22	39.5	19.9–123.9
ALT(U/L)	10	85.5	25.3–350.4	12	58.5	17.9–191	0.21	22	67	24.9–214.4
ALP(U/L)	10	6	1.92–41.7	11	9	1.84	0.20	21	8	1.84–22
TP (g/dL)	10	7.26 (0.8)	5.7–8.2	12	7.66 (0.7)	6.7–8.7	0.21	22	7.5 (0.74)	5.7–8.7
Albumin (g/dL)	10	2.5 (0.5)	1.5–3.5	12	2.3 (0.4)	1.9–2.9	0.32	22	2.4 (0.5)	1.5–3.5
Globulin (g/dL)	10	4.74 (0.5)	3.9–5.5	12	5.35 (1.0)	4.2–7.0	0.96	22	5.07 (0.8)	3.9–7
BUN (mg/dL)	10	29 (14.4)	15–55	12	30.8 (10.2)	16–46	0.84	22	29.6 (12.0)	15–55
Creatinine (mg/dL)	10	1.25 (0.2)	1–1.7	11	1.2 (0.3)	0.9–1.7	0.83	21	1.2 (0.2)	0.9–1.7
Phosphorus (mg/dL)	10	4.2	3.6–6.6	11	4.3	3.3–7.6	0.92	22	4.25	3.53–6.67
Calcium (mg/dL)	10	8.51 (0.97)	6.7–10.2	11	8.54 (0.45)	7.9–9.3	0.94	22	8.5(0.7)	6.7–10.2
Sodium (mmol/L)	10	147.5	127.9–156.8	12	146	140.3–154.4	0.43	22	146.5	140.3–155
Potassium (mmol/L)	11	4.4	4.12–7.12	11	4.3	3.94–4.76	0.13	21	4.4	4.1–4.88
Chloride (mmol/L)	10	121.5	101.4–124.9	12	117	106.9–125.2	0.16	22	118	106.9–124.7
Bilirubin (mg/dL)	10	0.3	0.2–0.49	12	0.3	0.2–0.4	0.68	22	0.3	0.2–0.4
CK (U/L)*	10	352.5	136.6–1310.2	12	146.5	50.9–520.3	0.01*	n/a	n/a	n/a

*Mann-Whitney U-test; statistical significance.

DISCUSSION

Physical Condition

The 12 MW we evaluated in NKP over a 10-year study period included equal numbers of males and females and ranged in age from 0.6 to over 10 years. The sex and age structure suggest juvenile recruitment into the population, although three of seven observed litters had no successful pups reared. Body weights for the MW in our study were less than those for MW in a recent study in Brazil (May-Júnior et al., 2009). The difference in body weight could be related to food availability as individuals lost weight coincident with a decline in rodents (Emmons, 2009; Chapter 4; Figure 4.3).

The most significant physical findings in live-captured MW were associated with dental disease (Figures 6.1, 6.2). Many individuals had lesions consistent with caries, which could be caused by the high quantities of sugars and acids from fruits in the diet (Chapter 4; Bestelmeyer, 2000; Dietz, 1984; Motta-Junior et al., 1996). Many MW had evidence of traumatic injuries to their teeth (slab fractures). This is consistent with findings from free-living MW in Brazil (Furtado et al., 2007) and captive MW (Hammond, 2012). Although we did not see the degree of gingival hyperplasia commonly seen in captive MW in the 1980s and 1990s in North America (Norton, 1990), three MW had some evidence of gingival hyperplasia.

Other clinical findings included probable traumatic injuries (e.g., blind left eye and split lower lip and eyelid in M2 and paw lesion in F12) and possibly infectious agents (e.g., conjunctivitis in M8). Additionally, the IV/VI heart murmur in this individual (M8) may have been associated with heartworm infestation as he consistently tested high positive to *D. immitis* antigen. M8 also demonstrated persistent weight-bearing lameness over 3 years and a slow recovery after one anesthetic event. A diagnosis for the cause of the alopecia in the hindquarters of three youngsters, seen only in 2002–2003, was not determined but most likely was due to parasitic or traumatic events.

One blood sample was removed from the data set for hematology and chemistry analyses as values from this sample were not consistent with life, and thus improper sample handling was suspected. Only PCV and TS differed

TABLE 6.4. Test results for selected parasitic and pathogenic agents of free-living MW (*C. brachyurus*) in NKP. CAV-II, canine adenovirus; CCV, canine coronavirus; CDV, canine distemper virus; CHV, canine herpesvirus; CPV, canine parvovirus; ND, no data.

ID	Date	*Leptospira ictero*	*L. grippophytosa*	CAV-II	CCV	CDV	CHV	CPV	*Dirofilaria immitis*	*Toxoplasma gondii*	Rabies	*Ehrlichia canis*	*Rickettsia rickettsii*	*Borrelia burgdorferi*
F1	15 Feb 00	0	0	512	0	0	0	10	Positive	0	0	ND	ND	ND
M2	20 Oct 01	0	0	512	0	12	0	10	0	0	0	ND	ND	ND
F3	7 Oct 03	0	0	512	0	12	0	10	0	0	0	ND	ND	ND
F3	9 Oct 04	0	0	1536	0	12	0	40	0	256	16	ND	ND	ND
F3	2 Oct 05	0	100	1024	8	16	0	0	0	512	0	80	Positive	0
F3	4 Sep 06	0	0	256	0	0	0	0	Positive	256	0	0	64	0
F3	21 Sep 07	0	0	2048	8	0	0	0	0	ND	0	0	256	ND
F3	15 Jul 08	0	0	4096	0	0	0	0	Suspicious	ND	0	320	0	ND
F3	19 Oct 08	0	0	768	0	0	0	0	Low	180	0	80	64	0
M5	4 Oct 04	0	0	1024	0	24	0	20	High	128	13	ND	ND	ND
M5	28 Sep 05	0	0	512	0	0	8	0	High	128	0	80	Positive	0
M5	29 Jan 07	0	0	384	0	0	0	0	High	256	0	0	0	0
M5	26 Jul 07	200	0	4096	0	0	0	0	High	ND	0	80	0	ND
M5	16 Jul 08	200	0	768	0	0	0	80	0	ND	0	0	0	ND
M6	27 Sep 05	0	0	1024	0	8	0	0	Positive	512	0	80	Positive	0
F7	21 Oct 05	0	0	1024	0	8	0	0	0	128	0	80	Positive	0
M8	25 Oct 05	0	0	512	0	0	0	0	High	128	0	80	Positive	0
M8	5 Sep 06	0	0	512	0	0	0	0	High	128	0	0	64	184
M8	25 Jul 07	0	0	1024	0	0	0	0	High	ND	0	80	0	ND
M8	8 Jul 08	0	0	2048	0	0	0	0	High	ND	0	0	0	ND
M8	22 Oct 08	0	0	768	0	0	0	0	High	180	0	320	64	0
M8	7 Jul 09	0	0	3072	0	0	0	0	High	180	ND	80	64	0
F9	4 Sep 06	ND	ND	512	0	0	0	0	Low	128	ND	0	256	242
F9	18 Jul 07	0	0	4096	0	0	0	0	Positive	ND	0	0	256	ND
F9	7 Jul 08	0	0	768	0	0	0	0	0	ND	0	80	256	ND
F9	12 Oct 08	0	0	2048	0	0	0	0	High	60	ND	80	ND	120
M10	16 Sep 06	ND	ND	512	0	0	0	0	0	64	ND	0	256	25
F11	4 Feb 07	ND	ND	192	0	0	0	0	High	256	ND	80	0	161
F11	18 Jul 07	0	0	1024	0	0	0	0	High	ND	0	0	0	ND
F12	16 Oct 07	0	0	2048	0	0	0	0	0	ND	0	0	256	ND
Total Animals		1/11 (9%)	1/11 (9%)	11/11 (100%)	1/11 (9%)	5/11 (45%)	1/11 (9%)	4/11 (36%)	7/11 (64%)	8/10 (80%)	2/10 (20%)	7/9 (78%)	8/9 (89%)	4/8 (50%)
Total Samples		2/27 (7%)	1/27 (4%)	30/30 (100%)	2/30 (7%)	7/30 (23%)	1/30 (3%)	6/30 (2%)	20/30 (67%)	17/20 (85%)	2/25 (8%)	14/25 (67%)	16/24 (67%)	5/15 (33%)

TABLE 6.5. Comparison of seroprevalence of selected parasitic and pathogenic agents of free-living MW (*C. brachyurus*) in the NKP and domestic dogs on the perimeter.

Animal	*Dirofilaria immitis*	*T. gondii*	Rabies	CAV-II	CHV	CDV	CPV	CCV	*Leptospira*	*E. canis*	*R. rickettsii*	*B. burgdorferi*
MW	7/11	8/10	2/10	11/11	1/11	5/11	4/11	1/11	2/11	8/9	8/9	4/8
(n = 11)	(64%)	(80%)	(20%)	(100%)	(9%)	(45%)	(36%)	(9%)	(18%)	(89%)	(89%)	(50%)
Dog*	13/40	32/40	22/39	7/40	28/40	37/40	34/40	3/40	8/40	19/22	19/22	0/22
(n = 40)	(33%)	(80%)	(56%)	(18%)	(70%)	(93%)	(85%)	(8%)	(20%)	(86%)	(86%)	(0%)
P value	NS	NS	0.04	<0.001	<0.001	<0.001	0.003	NS	NS	NS	NS	0.003

*Data from Bronson et al. (2008). Here NS, not significant.

TABLE 6.6. Ecto- and endoparasites of free-living MW (*C. brachyurus*) in the NKP.

Parasites	Males	Females
Endoparasites (eggs)		
Ancylostoma caninum	1	1
Ancylostoma sp.	3	2
Ascarid-like egg (avian)	0	1
Capillaria aerophilia	3	3
Capillaria sp.	4	2
Coccidia	0	1
Diphyllobothrium sp.	2	1
Fluke-like eggs	1	0
Gnathostoma sp.	0	1
Isospora sp.	1	0
Mite and mite eggs (Family Listrophoridae)	3	2
Physaloptera sp.	2	0
Pinworm eggs	3	2
Strongyle-like egg (avian)	0	2
Trichuris sp.	3	1
Toxocara canis	0	1 adult
Ectoparasites		
Amblyomma sp.	14	6
Amblyomma ovale	4	0
Amblyomma cajennense	6	2
Amblyomma pecarium	0	1
Amblyomma tigrinum	6	5
Amblyomma triste	4	3
Mallophaga (chewing lice)	1	0
Rhopalopsyllus australis spp. (flea)	0	1

TABLE 6.7. Results from urinary testing in free-living MW (*C. brachyurus*) in NKP. Here n/a, not available.

Identification (no. of samples)	Laboratory	Microscope
F1 (1)	n/a	No crystals; *Dioctophyme renale* ova
F3 (1)	Positive	No crystals; *D. renale* ova
M5 (3)	Positive	Crystals (3/3); *D. renale* ova (2/3)
M6 (1)	Negative	Crystals; no ova
M8 (1)	Positive	n/a
M10 (1)	Negative	n/a
F11 (1)	Positive	Crystals; no ova

between our male and female MW. Females had significantly lower PCV and higher TS values than males. One possible explanation for this difference was a chronic underlying infectious process in the females, which may have been responsible for the two low (22%) values for F3. Packed cell volume values in both male and female MW in our study were lower than both those for captive MW in North America, with values of 40.9 % ± 6.5 (sample size of 132 animals and 579 points) (International Species Information System [ISIS], 2002), and for free-ranging MW tested in Brazil (about 40%; May-Júnior et al., 2009).

On chemistry profile, only CK differed between males and females in our study, with males having a higher median value. This may be due to the high value in one male

TABLE 6.8. Findings from five MW (*C. brachyurus*) that died in the NKP during our study period of February 2000–July 2009.

Wolf	Date	Age	Cause(findings)
F1	March–Oct. 2000	Young (8 mo)	Disease epidemic?
M2	Feb. 2004	Old	Date of death during flooding in study area. Changes consistent with geriatric status. Most severe vertebral pathology lesions of all MW we have examined. Maxillary osteomyelitis. Multiple tooth loss.
M6	Jan. or Feb. 2006	Old	Date of death during flooding. Changes consistent with geriatric status. Many teeth missing with lytic bone surrounding, mandibular osteomyelitis, osteoporosis, vertebral pathology.
F9	16 April 2009	3 years	Date of death during flooding. Osteomyelitis of maxillae, zygomatic arch.
F11	22 March 2008	10 years	Date of death during flooding. There were no obvious bony lesions. Molars worn to gums. This female was small in stature.

FIGURE 6.3. Vertebrae of M6 showing severe bridging spondyloarthropathy. Photograph, L.H. Emmons.

(M5) (1,310 U/L) and the fact that both of the highest CK values were from males. Interestingly, in the Brazil study, there was also a difference in CK between males and females, with females having higher values than males (May-Júnior et al., 2009).

A number of differences were evident between chemistry profile results of our NKP MW and those in captivity and free-living MW in Brazil. The glucose mean value (68.5 mg/dL) in Bolivian MW was significantly lower than the ISIS value (114 mg/dL ± 25) and the value for MW in Brazil (106.4 mg/dL ± 5.0) (ISIS, 2002; May-Júnior et al., 2009). The glucose value in the MW of this study was low for a carnivore and warrants further investigation.

Serology and Endoparasites

Maned wolves in NKP were seropositive for parasitic and infectious diseases of concern in carnivore conservation, and known to cause high morbidity and mortality in captive MW. Our original hypothesis was that domestic dogs living at the perimeter of the park were the likely route of exposure for these MW. However, we did not find

support for this, because high seroprevalence was found in MW for a number of pathogens that were at low prevalence in domestic dogs near the park (Table 6.5; Bronson et al., 2008). In 40 domestic dogs around NKP, seroprevalences were high for CDV (93%), CHV (70%), CPV (85%), *T. gondii* (80%), and *E. canis* (86%) (Bronson et al., 2008). In these dogs, CAV-II prevalence was low at 18% as compared to the 100% prevalence in the MW. Because the dogs were short lived and all young, their disease profiles may reflect short exposure times and be highly variable from one year to the next. We recommend further studies that include longer-term sampling and molecular testing for virus typing to determine the relationship between the viruses within the maned wolf and domestic dog populations.

All MW were seropositive for CAV-II with titers waxing and waning during the 10 year study period. Canine adenovirus is a known cause of pup mortality in captive MW (Barbiers and Bush, 1995) and is suspected to have caused hepatitis in one individual (Hammond, 2012). Although we cannot confirm an association based on these data, it is interesting to note that in the years when F3 (2008) and F11 (2007) had high adenovirus titers (4096 and 1024, respectively), neither had successful litters. The mate of F3 (M8) also had a CAV-II titer change from 768 (in 2008) to 3072 (in 2009). This litter died at about 16 days postpartum (August 2008), based on F3's abrupt cessation of movement behavior associated with lactation (Chapter 5).

Seven of the 11 MW tested were positive for the antigen of *D. immitis*, the causative agent of canine heartworm. This is a potentially fatal, mosquito-borne disease of domestic and wild carnivores. In captivity, MW are often maintained on a heartworm prophylactic because of the devastating effect of this parasite. The role of *D. immitis* in morbidity and mortality of free-ranging MW is not known, although one of the MW (M8) had high positive antigen to *D. immitis* and an IV/VI heart murmur during anesthetic events. At the beginning of our study in Los Fierros pampa, geriatric M2 was negative for *D. immitis* (2001), while F3 on the same territory, who was sampled yearly from 2003, was seronegative until she was four. At 5 years she had converted to antigen positive status, 2 years after acquiring a highly-positive mate (M5). Of six animals tested less than 1 year of age, four were antigen negative and two were already positive, one less than 6 months old, born of positive parents. All four adults captured for the first time after 2004 were antigen positive. This suggests an increase during our study to 100% adult prevalence. Thirty-three percent of the domestic dogs tested were also positive to *D. immitis* (Bronson et al., 2008). It is possible that a dog-mosquito-wild carnivore endemic cycle operates in the NKP region of Bolivia, but our data also suggest that currently heartworm may circulate between the MW and mosquitoes.

Many of the MW were positive for *T. gondii*, as in a study of captive MW in Brazil (Vitaliano et al., 2004). This is not surprising for a species that eats positive prey (rodents and other small mammals; Deem et al., 2009) and is sympatric with seven native felid species (the definitive host).

Free-ranging Canidae usually harbor enteric parasites (Kennedy-Stoskopf, 2003), including those found in the MW in this study. These parasites are not often present in high numbers and do not cause a clinical problem in healthy adult free-living canids. However, in animals immunocompromised because of factors such as concurrent disease or physiologic stress related to habitat or population modifications, enteric parasites may result in disease. The lungworm *Capillaria aerophila*, detected in the feces of six MW in this study, can cause clinical signs associated with bronchitis and pneumonia, but these animals had no overt respiratory signs. Eggs of pinworms, mites, and mite eggs from the rodent-specific family Listrophoridae were detected in three MW, probably ingested with their rodent prey (Chapter 4). Likewise, ascarid-like and strongyle-like eggs from bird parasites were found in three MW, indicating ingestion of avian prey. A tapeworm, likely *Spirometra* spp., also associated with rodents, was recovered from maned wolf feces in the savanna (Mike Kinsella, pers. comm.). One adult worm, *Toxocara canis*, was identified in a female maned wolf (F9) approximately 6 months prior to her death around 3.5 years of age. On postmortem evaluation, there was evidence of severe osteomyelitis of the skull, believed to be from a tooth infection (Figure 6.1). Thus F9 possibly succumbed to sepsis, confounded by heartworm and gastrointestinal parasite infestation.

Ectoparasites

Of the five definitively identified tick species collected during this study, only *Amblyomma cajennense*, which we earlier reported from the maned wolf (Robbins and Deem, 2002), is known to be of veterinary importance. This tick has been found infected with *Encephalitozoon*-like microsporidia (Barbosa Ribeiro and Guimarães, 1998) and, together with its congeners, may be a vector of filarial worms, such as *Yatesia hydrochaerus*, which is specific to capybaras (Yates and Lowrie, 1984). *Amblyomma cajennense* also causes paralysis in bovine, ovine, and caprine hosts in Brazil (Serra-Freire, 1983). In the

medical literature, *A. cajennense* is recognized as the principal vector of so-called Rocky Mountain spotted fever in the Neotropics, and, given its vast range and propensity for attacking humans, is a known or suspected vector of arboviruses, Chagas disease, and even leprosy (Guglielmone et al., 2003). We note that eight of nine tested MW were positive for *Rickettsia rickettsii* (Rocky Mountain spotted fever; but the antisera may cross-react with a local variant).

Amblyomma spp. ticks were previously reported from free-living MW (Dietz, 1984); but the first documented *A. ovale* was from our study population (Robbins and Deem, 2002). In Brazil, *A. ovale* has been implicated as a vector of *Rickettsia parkeri*, strain Atlantic rainforest, a novel spotted fever agent pathogenic to humans (Sabatini et al., 2010). *Amblyomma pecarium* is an uncommonly collected parasite of Artiodactyla known from Mexico, Panama, and Bolivia (Fairchild et al., 1966; Robbins et al., 1998). It might occasionally transfer to carnivores that prey on these herbivores or their carrion. *Amblyomma tigrinum* adults are highly specific to wild and domestic carnivores, so its occurrence on a maned wolf is expected. Adult stage *Amblyomma triste* likewise parasitize carnivores. Our records (4♂, 3♀; Table 6.6) from NKP are the first for Bolivia.

Urinary Health

Morbidity from the genetic disease cystinuria, and resulting cystine calculi with urinary blockage, has been a common problem estimated to affect up to 80% of both wild and captive MW and led to nutritional modifications to minimize the occurrence of these calculi among captive MW in the United States (Bovee et al., 1981; Fernandes and Marcolino, 2007; Norton, 1990). Similarly, 71% of MW we tested (n = 7) were cystinuric, but we found no evidence of cystine calculi or urinary blockage. This agrees with another study in a free-living maned wolf population (Dietz, 1984). An ancient mutation and founder effect has been proposed (Bovee et al., 1981; Dietz, 1984; Fernandes and Marcolino, 2007; Norton, 1990), and mutations in the renal basic amino acid transporter have been identified in Newfoundland and Labrador retriever dogs with type I cystinuria and an autosomal recessive trait. However, there are many other breeds with type I and non–type I cystinuria (only males are cystinuric) where the molecular defect has not yet been defined. Initial studies of MW did not identify a mutation in the renal basic amino acid transporter (Kehler et al., 2002). With the recent completion of the full canine and low-density wolf genome sequences, progress can be made in MW. Samples have been preserved for future DNA studies.

Our finding of *D. renale* ova in three MW was not surprising, as this parasite is a common pathogen of recently captive MW (Kumar et al., 1972). *Dioctophyme renale* is often associated with a hypoplastic right kidney in infected MW and could contribute to mortality, especially with concurrent disease (Kumar et al., 1972; Norton, 1990).

Mortality

Forty-two percent (5/12) of the MW in our study died before September 2009, with deaths throughout the study years. This mortality rate is similar to that recorded in Emas National Park, where the survival rate was estimated at 64% per year for all age classes (Sollmann et al., 2009). All deaths at Los Fierros occurred between February and April, the months of flooding when foraging and movement in the study area are limited and could contribute to poor nutrition, debilitation, and death. The fates of the two individuals that vanished after the October 2009 fire are uncertain, as they could have escaped to die elsewhere. One was about 8 years old (F3) and the other 7 years old (M8), the latter in good health but the former geriatric, with severe dental problems. One yearling female survived the fire and was alive in 2011 (Chapter 5, Postscript).

Three of the five (60%) MW that died before September 2009 were estimated at 8 to >10 years old and considered geriatric at the time of death. Two of these three (67%) had severe spondyloarthropathy. This pathologic finding was previously considered a disease of captivity, with an incidence of about 80% of geriatric captive MW (Elizabeth Hammond, pers. comm.; Rothschild et al., 2001). The two affected MW in our study (M2 and M6) had the most severe skeletal lesions among any of three wild-taken and seven zoo skeletons (NZP) evaluated at the National Museum of Natural History (evaluated by LHE). The one geriatric maned wolf (F11) with no vertebral lesions was unusually small (22 kg). We conjecture that the body structure of MW (e.g., long legs and short back) and hunting style, of pouncing on small prey, make MW prone to vertebral pathologies. Further investigation is needed into this pathologic condition of captive and free-living MW. The two animals with severe vertebral lesions had likely lived with the condition for years.

Three of the MW that died had indication of severe osteomyelitis of the maxillae (M2, F9) or mandible (M6; Figure 6.2B) that was most likely associated with a tooth root abscess. M6 was geriatric at the time of death and had evidence that osteomyelitis had spread into the postcranial

skeleton. However, F9 was only 3 years old but had evidence of spread of infection into the adjoining zygomatic arch, above and below the eye (Figure 6.1). This female died when the study area was experiencing unusually prolonged and deep flooding, and we hypothesize that the oral infection and associated pain decreased appetite while flood waters made finding food difficult. Prior to death she traveled from her own flooded territory into the drier adjacent territory, where she may have also experienced social conflict and stress. These two factors, in addition to her positive status for *D. renale* and *D. immitis*, may have led to poor nutrition and possible sepsis as the proximate and definitive cause of death. The other young maned wolf that died (F1) was believed to have succumbed to an infectious agent, as the fox (*Cerdocyon thous*) population in the same section of NKP was also decimated at this time.

Necropsy results of 47 MW housed at the Smithsonian Conservation Biology Institute, Front Royal, Virginia, from 1975 to 2003, list the primary cause of death as follows: 17 perinatal, two urolithiosis (cystine calculi), three infectious agents (e.g., CDV, CPV, and *Escherichia coli*), one trauma, three digestive, one cardiomyopathy, and 19 euthanasias. The reasons stated for euthanasia included four neoplasia, two infectious (one pyometra and one rabies virus), one trauma, four kidney related (two of these were urolithiasis), two spondylosis, one postsurgical, one cull, one arthritis, two old age, and one digestive problems. Thirteen of these 19 (68%) euthanized MW were >10 years old at time of euthanasia, and there were 11 males and 8 females.

CONCLUSIONS

We found a surprising number of significant clinical and pathologic findings in this small population of free-living MW in Bolivia. The NKP maned wolf population was previously estimated at 120 pairs (Rumiz and Sainz, 2002), but we now believe it is closer to 20–30 pairs (see Chapter 3). On the basis of our findings for the 12 MW in our study, the health of the Los Fierros population may be rated as only moderate. The lower body weights, PCV, and glucose values compared to other maned wolf populations, as well as the many dental abnormalities, may indicate a lower plane of nutrition in this population. Recent evidence of rodent population declines indicates that the prey base for this population of MW declined over 94% in biomass during the study (Chapter 4; Emmons, 2009).

We had not anticipated such a high number of dental and bony lesions. The prevalence of dental disease was most likely associated with the dietary intake of acidic, sweet fruits such as *Alibertia edulis* and *Solanum gomphodes* (Chapter 4), and tooth fractures related to chewing fruits with hard stones and perhaps armadillos. The high prevalence of vertebral pathologies in the older MW that died was similar to that found in captive populations and may suggest that this pathology is more a function of body structure and behavior than a disease of captivity as previously thought (Rothschild et al., 2001).

Although there is no definitive diagnosis for the cause of death in the one maned wolf (F1) that died at less than 1.5 years of age; a concurrent large decline in crab-eating foxes implicates an infectious disease epidemic. We therefore recommend continued domestic dog health monitoring, domestic animal vaccination, and enforcement of the prohibition of all domestic animals within the park, especially of carnivores.

Our ability to conserve the growing number of endangered species will necessitate an understanding of the health of both captive and free-living populations. We anticipate that studies similar to this one will provide data necessary for the long-term conservation of animals in their natural habitats and proper captive care and propagation of endangered species in captivity.

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Postgraduate Education

June 2003 – June 2006, National Zoological Park, Washington D.C.

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August 2001 – June 2003, Maryland Zoo in Baltimore, Baltimore, MD

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Annual guest lecturer at University of Pennsylvania School of Veterinary Medicine

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Selected publications:

- Kilburn JJ, **Bronson E**, Shaw GC, Labelle P, Weigt A. Phacoemulsification in an American toad (*Anaxyrus americanus*). J Herp Med Surg. 2019;29:17-20.
- **Bronson E** and CK Vance. Anuran Reproduction. In: Miller RE, N Lamberski, P Calle (eds.). Fowler's Zoo and Wild Animal Medicine, Volume 9. 2019. Elsevier, St. Louis, Missouri. Pp. 371-379.
- Rivas A, MJ Dykstra, K Kranz, **E Bronson**. Environmental fungal loads in an indoor-outdoor African penguin (*Spheniscus demersus*) exhibit. J Zoo Wildl Med. 2018;49:542-555.
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2000-1: Mammalogist, Amazon Conservation Association
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1995-97 Senior Ecologist, Conservation International Rapid Assessment Program, Mammalogist.
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1989-91: Research Fellow, Universiti Kebangsaan Malaysia, Kampus Sabah (honorary).
1985-: Field Research Associate, Duke University Primate Center. ,
1979: Pennsylvania State University School of Forest Resources. Temporary position to curate mammal collection.
1976-1979: Senior Project Associate (appointment for research only) Pennsylvania State University.
1975-1976: Chercheur Associé, Laboratoire de Primatologie et d'Ecologie Equatoriale (Centre National de la Recherche Scientifique, France).
1966-1969: Laboratory Assistant to Dr. D. M. Marcus, Albert Einstein College of Medicine (Immunochemistry; three publications not relevant to current research interests).
1961-1962: Research Assistant to Dr. D. S. Grosch, Woods Hole Marine Biological Laboratory (studies on long term effects of radiation on *Artemia*). Summers.

Field research:

1969: William Beebe Tropical Field Station, Trinidad: social organization of bats (with J. Bradbury). Feb-Sep.
1970: Laboratoire d'Ecologie Equatoriale, Gabon: pilot study of the fruit bat *Epomops* Jun-Sep.
1971-1973: Laboratoire d'Ecologie Equatoriale, Gabon: thesis research on 9 species of squirrels. 25 mo. in field.
1975-1976: Laboratoire d'Ecologie Equatoriale, Gabon: ecology of brush-tailed porcupine, *Atherurus*. Dec-Oct.
1977: Eastern Ecuador, mammalogist on Biological Survey of proposed Yasuni National Park, and other field studies, Sep-Dec.
1978: Cocha Cashu Biological Station, Manu Park, Peru: small mammal fauna Jul-Aug.
1979: Manu National Park, Peru: ecology of spiny rats. Jun-Dec.
1979-1980: Tambopata Reserve, Peru: mammal survey for Biological Survey (for Peruvian Ministry of Agriculture, Peruvian Safaris, USFWS, and WWF -US). Dec-Jan.
1981: Laboratoire d'Ecologie Tropicale, Gabon: synecology of fruit and vertebrate frugivores - a large collaborative study. Jan-Sep.
1982: Manaus, Brazil: mammal survey for Minimum Critical Size of Ecosystems Project (World Wildlife Fund-US; T. Lovejoy, Dir.). Feb-Jul.
1982-1984: Manu National Park, Peru: ecology of felids, with J. Terborgh, 16 mo in field.
1984: River Arataye, French Guiana: collecting for Smithsonian Institution and mammal census. Sep-Oct.
1986: Rio Xingu, Brazil: faunal survey (joint expedition Smithsonian/Museu de Zoologia, São Paulo). Aug-Sep.
1987: Ranomafana Region, Madagascar: preliminary study of the small mammal fauna (with G. K. Creighton). Jul.
1988: French Guiana: field observation and collections of pygmy squirrels. (with V. L. Roth, Duke University). July
1989-1991: Sabah, Malaysia: field study of treeshrews (Tupaiidae). Feb-Aug 89, Aug 90-Sept 91.
1994: Paracou, French Guiana: mammal inventory (with R. Voss). Sep-Oct
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2000: Le Nouragues French Guiana, July-Aug. Studies of Leaf Area Index and mammal inventory methods, in collaboration with ECOFIT program (France)
2000: Los Amigos field station, Peru. Overflight and evaluation . Nov. 10 d.
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2001: Santa Cruz, Bolivia. Mammal communities at the savanna forest interface, El Refugio and Parque Nac. Noel Kempff. Sept-Nov
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2003: Los Amigos, Peru. Studies of mineral licks, bamboo. Feb-Apr.
2003: Pampas del Heath, Bolivia. Survey for location of guard station and research camp. Apr
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Conservation International Rapid Assessment Program (RAP) Surveys:

1990: Rio Madidi region of Northern La Paz, Bolivia, May-June.
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1992: Tambopata/Candamo Region, Peru; and NE Pando, Bolivia. May-Jul.
1993: W. Kanuku Mts. Guyana.
1994: Hans Meyer Range, New Ireland, Papua New Guinea, Feb.
1994: Cordillera del Condor, Peru, Jun.
1994: Santa Cruz, Bolivia, dry forests of the Tucavaca Valley, Oct.
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1998: Lower montane forests of the Cordillera de Vilcabamba, Peru, May.

Field Course teaching, with RAP.

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1970 Cornell University Research Grant
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1990-1991 National Geographic Society, Douroucouli Foundation.
1990 Atherton Seidell Fund of S.I. (for book distribution).
1997 Field work in Bolivia supported by El Refugio Corp and Museo Noel Kempff Mercado, Santa Cruz.
1998 Weedon Foundation; Douroucouli Foundation.
1999 W. Alton Jones Foundation, Douroucouli Foundation, Wildlife Conservation Society.
2000-01 Wildlife Conservation Society, National Geographic Society Research Grants
2002 Amazon Conservation Association support for field research
2003 National Geographic Society CRE grant (maned wolves), Wildlife Conserv. Soc. grants
2004 Wildlife Conservation Society, Sea World Busch Gardens, maned Wolf SSP support for maned wolf project.
2005 Wildlife Conservation Society (small annual awards as part of Bolivia Program (all years to 2007)
2006 Maned Wolf SSP, Friends of the National Zoo.
2012 Weedon Foundation Grant for Maned Wolf Studies

Awards:
AAAS Fellow, elected 1991.
2000 Parker Gentry Award for excellence and innovation in conservation/environmental biology. Field Museum of Natural History
2001 Society of Women Geographers Award for Outstanding Lifetime Achievement
2008 Association for Tropical Biology Honorary Fellow
2009 Sydney Anderson Award, Bolivian Mammal Society

Professional activities:

Board of Editors, *Studies in Neotropical Fauna and Environment* 1998-2016
Board of Editors, *Mammalia* 1993-present.
Board of Directors, Conservation International, 1987-1990.
American Society of Mammalogists, International Relations Committee, 1990-94.
IUCN Rodent Specialist Group (Species Survival Commission).
IUCN Insectivore and Tupaiid Specialist Group
Board of Directors, NCRI Chesapeake (a non-profit foundation) 1994-1999.
Consultant, mammalogy: The National Geographic Society, The National Wildlife Federation, IUCN, Conservation International, BBC, and others.

Professional societies, current memberships:
The Society of Women Geographers, active member.
Washington Biologist's Field Club, active member 1995-present.
American Society of Mammalogists
Association for Tropical Biology and Conservation

American Association for the Advancement of Science
Biological Society of Washington
Asociación Boliviana de Investigadores de Mamíferos

Miscellaneous professional activities:

1997-8 Production of a CD set *Sounds of Neotropical Mammals*, with Cornell Laboratory of Natural Sounds. An audio field guide companion to *Neotropical Rainforest Mammals: A field guide.*
1998. Cornell Laboratory of Natural Sounds workshop on field recording of sounds.

Languages: English, French, Spanish, some Portuguese and German.

Publications:
116 scientific publications including 2 books, listed at:
http://research.si.edu/srb_search_action.cfm?keyword=Emmons&limit=author&date=&department=

Re: [EXTERNAL] Re: FWS Permit App 70028D

Ian Shelley <ian.shelley@marylandzoo.org>

Wed 8/12/2020 10:39 AM

To: Cate, Emily B <emily_cate@fws.gov>

1 attachments (15 KB)

Maned Wolf Samples.docx;

Hi Emily,

Ellen is back at the zoo now and I was able to confirm. Yes, she was using the same protocol in 2014 as she was in 2006. Attached is a more detailed description of exactly what the materials will consist of and how they will be packaged. Thanks!

Ian

On Fri, Aug 7, 2020 at 9:41 AM Cate, Emily B <emily_cate@fws.gov> wrote:

> **Good morning Ian,**
>
> **Thank you for your email and happy Friday. I just have a couple of clarifying questions for you about the application.**
>
> **The protocol submitted appears to be from 2006. Can you please confirm that the same protocol was used when collecting the samples in 2014 that are proposed for import? As soon as I receive confirmation or clarification, I will put this application in line for publication in the Federal Register for the mandatory 30-day public commenting period.**
>
> **Finally, while we do not need the following information to move forward with the FR publication, we will need to know more specifics about the packaging/amount proposed for import because, pending permit approval, it would appear on the face of the permit. Can you please provide more specifics on each of the sample types (just as an example- Serum - a total of 20 mL of serum in five vials in 4 mL aliquots).**
>
> **Please let me know if you have any questions or concerns.**
>
> **Regards,**
> **Emily**
>
> ---
>
> **From:** Ian Shelley <ian.shelley@marylandzoo.org>
> **Sent:** Wednesday, August 5, 2020 8:48 AM
> **To:** Cate, Emily B <emily_cate@fws.gov>
> **Subject:** Re: [EXTERNAL] Re: FWS Permit App 70028D
>
> Hi Emily,
>
> Thank you very much for your reply. Is there any other information that USFWS will need to make a decision on our application? Thanks again,

Ian

On Tue, Aug 4, 2020 at 3:01 PM Cate, Emily B <emily_cate@fws.gov> wrote:

Hi Ian,

Apologies that it took so long to get back to you. After discussing internally, it appears that the Maryland Zoological Society is acting on behalf of the State of Maryland and you are therefore exempt from the fee.

Regards,
Emily

From: Cate, Emily B <emily_cate@fws.gov>
Sent: Tuesday, July 28, 2020 9:53 AM
To: Ian Shelley <ian.shelley@marylandzoo.org>
Subject: Re: [EXTERNAL] Re: FWS Permit App 70028D

Hi Ian,

Apologies, I am still waiting to hear from someone about the fee exemption question. We will extend the due date by another 45 days so that your application will not be administratively closed. Please see below for details.

In accordance with 50 CFR 13.11(e), if the requested information is not received by this office by **September 11, 2020**, your application will be abandoned and administratively closed. Once a file is closed you will need to submit a new application and all required fees for the Service to consider your proposed activity. Please refer to permit number 70028D in your correspondence.

Regards,
Emily

From: Ian Shelley <ian.shelley@marylandzoo.org>
Sent: Tuesday, July 28, 2020 9:35 AM
To: Cate, Emily B <emily_cate@fws.gov>
Subject: Re: [EXTERNAL] Re: FWS Permit App 70028D

Hi Emily,

Thank you very much. I just wanted to check, since we are getting close to August 1 - are we all set while you are getting this information, or does our application expire if you don't have the check by then? Thanks,

Ian

On Mon, Jul 27, 2020 at 9:25 AM Cate, Emily B <emily_cate@fws.gov> wrote:

Hi Ian,

Thank you for providing the additional information and follow-up. I will check internally and get back to you about the fee exempt question Additionally, I will review the information and let you know if I have any follow-up questions about the application.

Regards,
Emily

From: Ian Shelley ian.shelley@marylandzoo.org>
Sent: Monday, July 27, 2020 9:20 AM
To: Cate, Emily B <emily_cate@fws.gov>
Subject: Re: [EXTERNAL] Re: FWS Permit App 70028D

Hi Emily, how are you?

Dr. Bronson wanted me to check to see if it was possible that we are, in fact, exempt. We are a state-aided institution, owned by the State of Maryland on land owned by the City of Baltimore, but run by the Maryland Zoological Society. Would this qualify us as a government institution exempt from the fee? With the economic impact of COVID-19, we just wanted to make sure before we cut the check. Thanks,

Ian

On Fri, Jul 24, 2020 at 3:17 PM Ian Shelley <ian.shelley@marylandzoo.org> wrote:

> I'm sorry, I hit "send" there before I added the attachments. Here they are.
>
> On Fri, Jul 24, 2020 at 3:17 PM Ian Shelley <ian.shelley@marylandzoo.org> wrote:
>
> > Hi Emily, how are you?
> >
> > Below are our answers to your questions regarding our permit:
> >
> > 1. How many maned wolves were sampled for the samples proposed for import?
> >   1. One
> > 2. When were the maned wolves sampled and by whom (name and address)?
> >   1. September 2014 by Dr. Ellen Bronson
> > 3. Did any mortalities or injuries occur due to the collection?
> >   1. No, animal was trapped as part of the research project in Bolivia and released after sampling.
> > 4. Please provide the research proposal for the project.
> > 5. Thank you for providing the research papers, they were very informative. Can you clarify what additional (or supplemental) information you are hoping to yield from these samples?
> >   1. We are hoping to evaluate disease exposure to infectious diseases of carnivores. We have sampled this animal in the past (previously imported samples) and would like to compare the differences over time to exposure.
> > 6. For each type of sample, please provide information on how the samples are packaged. What may be easiest is if you provide the total quantity of the samples (ml, number, grams, etc.), the total number of containers that the samples are going to be in, and the amount of the total samples that are going to be in each container (e.g., a total of 40 ml of serum in 20 vials in 2 ml aliquots).

1. See attached chart of samples

Attached, please find a list of the samples that we seek to import, as well as a copy of the research proposal. It originally had been my thought that the Zoo would be exempt from the fee because of our somewhat unique ownership status (combination of city and state government and private non-profit), but after reading the regulation as it is written I'm no longer so sure. We will have a check for $100 drawn up to cover the application.

Thank you very much, and please let us know if there is anything else you need in order to process this application,

Ian

On Thu, Jun 18, 2020 at 9:38 AM Cate, Emily B <emily_cate@fws.gov> wrote:

> Good morning Ian,
>
> Thank you for your quick reply. At this time, we are not extending permit expiration dates due to COVID-19, though we certainly acknowledge and appreciate the severe disruption in travel plans that many are experiencing. We remain open and available for addressing concerns or answering questions. In this case, pending approval, an import permit would be valid for one year from the issuance date. If the import could not occur during that time (for any reason), you would certainly be welcome to apply for a renewal of the original permit.
>
> I look forward to receiving the information. Please let me know if you have any questions or concerns in the meantime.
>
> Best,
> Emily
>
> ---
>
> **From:** Ian Shelley <ian.shelley@marylandzoo.org>
> **Sent:** Thursday, June 18, 2020 8:30 AM
> **To:** Cate, Emily B <emily_cate@fws.gov>
> **Subject:** [EXTERNAL] Re: FWS Permit App 70028D
>
> Dear Emily,
>
> Thank you very much for your reply. We will get the information to you soon. I was wondering, however, if FWS was extending the expiration dates of permits due to COVID-19 travel concerns. With the current state of the virus in South America, we are not yet sure when our veterinarian will be able to go and retrieve the samples. Thank you,
>
> Ian
>
> On Wed, Jun 17, 2020 at 2:40 PM Cate, Emily B <emily_cate@fws.gov> wrote:
>
> > Dear Ian and Ellen,
> >
> > I have your application dated 02/12/2020, received 02/20/2020, regarding the proposed import of samples derived from maned wolves (*Chrysocyon brachyurus*) at Noel Kempff

Mercado National Park in Bolivia. I apologize for the delay in processing your application.

Please provide the following information so that I may continue to process your application:

1. Please mail a check or money order in the amount of $100 to our office for the processing fee for this application. Please include a cover letter indicating that the check or money order is associated with pending permit number 70028D. Alternatively and if appropriate, please email me back documentation of fee exempt status [50 CFR 13.11(d)].
2. How many maned wolves were sampled for the samples proposed for import?
3. When were the maned wolves sampled and by whom (name and address)?
4. Did any mortalities or injuries occur due to the collection?
5. Please provide the research proposal for the project.
6. Thank you for providing the research papers, they were very informative. Can you clarify what additional (or supplemental) information you are hoping to yield from these samples?
7. For each type of sample, please provide information on how the samples are packaged. What may be easiest is if you provide the total quantity of the samples (ml, number, grams, etc.), the total number of containers that the samples are going to be in, and the amount of the total samples that are going to be in each container (e.g., a total of 40 ml of serum in 20 vials in 2 ml aliquots).

Please let me know if you have any questions or concerns.

In accordance with 50 CFR 13.11(e), if the requested information is not received by this office by **August 1, 2020**, your application will be abandoned and administratively closed. Once a file is closed you will need to submit a new application and all required fees for the Service to consider your proposed activity. Please refer to permit number 70028D in your correspondence.

Regards,
Emily

Emily Cate | Permits Biologist
U.S. Fish and Wildlife Service | International Affairs
Division of Management Authority | Branch of Permits
5275 Leesburg Pike, MS:IA
Falls Church, VA 22041-3803

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Ian Shelley

Registrar
Maryland Zoo in Baltimore
443-552-3351

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Ian Shelley
Registrar
Maryland Zoo in Baltimore
443-552-3351

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Ian Shelley
Registrar
Maryland Zoo in Baltimore
443-552-3351

Samples for exportation to USA to Maryland Zoo in Baltimore

As of November 2018

Maned wolf/Borochi/Lobo de crin (*Chrysocyon brachurus*):

CB13 9 Sept 2014

Serum: 5 vials
Plasma: 1 vial
Filter paper with whole blood: 1
Blood slides: 4
Whole blood cells: 1 vial
Frozen whole blood: 1 vial
Frozen urine: 1 vial
Urine in formalin: 1 vial
Filter paper with urine: 1
Frozen feces: 1 vial
Feces in formalin: 1 vial
Vaginal slides: 2
Whole blood in buffer: 1 vial

National Zoological Park

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

RESEARCH PROPOSAL QUESTIONNAIRE

Please fill out this form if:

You are a NZP staff member proposing to conduct research on living animals on or off NZP premises.

You are a NZP Research Associate, fellow or student proposing to conduct research on living animals on or off NZP premises under the auspices of the National Zoological Park (e.g. you are using your affiliation with NZP to obtain funds, resources or permission for research).

Please fill out all items directly on this form, indicating "N/A" to those items that do not apply. Return this form along with a copy of the full project proposal (or a progress report if this is a continuation or extension of ongoing research), including abstract to:

Dr. Jon Ballou,

Chair, CRC IACUC

National Zoological Park

3001 Connecticut Ave., NW

Washington, D.C. 20008

A. ADMINISTRATIVE DATA:

Principal Investigator: Sharon L. Deem, DVM, PhD, Dipl ACZM

Research Capacity: NZP staff (research veterinarian)

Institutional Affiliation: SNZP

Address: Department of Animal Health

3001 Connecticut Avenue

Smithsonian National Zoological Park

Washington DC 20008

Telephone/FAX: 202 633 3197 tel / 202 673 4733 fax in USA

Telephone: 011 241 07598689 in Gabon

E-Mail Address: deems@si.edu

Name(s) and Affiliations of Co-investigators:

Dr. Louise Emmons – National Museum of Natural History

NZP Staff Collaborators:

Dr. Nucharin Songasen (Co-PI)

Dr. Suzan Murray (CI)

Dr. Ellen Bronson (CI)

Project Title: Ecology and Health Status of Maned Wolves Living in Brazilian and Bolivian National Parks.

Funding Source(s) (prospective and/or actual):
AZA CEF ($25,320) Actual for 2005-2006.
AZA CEF ($24,000) Will request for 2006 - 2007 (due April, 2006)

CEF proposal for 2005 – 2006 is submitted along with this IACUC.

Is Research to be Conducted in Field __X___ Laboratory/Zoo __X____ (check one or both)

Project Starting/Continuation Date: August 2006

Project Completion Date: September 2007

B. ANIMALS

Species: Maned Wolves (*Chrysocyon brachyurus*) and Crab eating foxes (*Cerdocyon thous*)

Number(s) to be Used: < 10 of each species

Source: Free-ranging animals in the Noel Kempff Mercado National Park, Bolivia

Indicate Permits Required or Obtained (Attach copies of permits):

All permits were obtained for the first few years of the project. The IACUC committee should have copies of these permits from the first time we submitted an IACUC for this project. If the committee would like another copy, please let the PI know. We are currently applying for a renewal of these permits, but do not anticipate any problems.

Indicate Where Animals will be Housed: N/A

Animal Care Requirements: N/A

Animal Enclosures: N/A

Social Groupings: N/A

C. DESCRIPTION OF RESEARCH/OBJECTIVES OF STUDY

Briefly explain in non-technical language the objectives of and scientific rationale for this research. (Note this IACUC request is for the Bolivia part of this study.)

The maned wolf (*Chrysocyon brachyurus*) lives in habitats severely compromised by agricultural development. Little is known about the health status of wild populations or how this species is influenced by human activities. Using a cooperative, multidisciplinary approach, we will conduct field studies to understand the ecology and health status of the species living in the Serra da Canastra National Park (Brazil) and Noël Kempff Mercado National Park (NKMNP) (Bolivia), where wolves are abundant. Because the two habitats differ in: (1) ecological characteristics, and (2) anthropogenic factors, we hypothesize that ecology and health statuses of these two populations are different. The health portion of this study (and the primary reason for handling wolves) is to compare risk of pathogen exposure for wolves living in a habitat surrounded by high human activities (i.e., Serra da Canastra) and those in a relatively isolated area (i.e., Noël Kempff Mercado). Additionally, we are looking at the pathogens present in the domestic dogs surrounding the NKMNP and sympatric carnivores (crab eating foxes) within the park to better understand the potential risks and routes of disease transmission for wolf populations in this region. (The domestic dog sampling for NKMNP is finished.) This project will generate new biological information on a magnificent canid that has received little attention. This is the first study to compare the health and conservation status of maned wolves in the two countries that are holding the largest populations of this species. For the ecologic portion of our study, telemetry collars will be applied to maned wolves during the anesthesia procedures.

D. VALUE OF STUDY.

Explain in non-technical language the likely benefits of this study.

The risk of morbidity and mortality due to infectious diseases is a significant concern for the long-term conservation of maned wolves, and wildlife in general. In this study we

will identify those pathogens to which maned wolves, domestic dogs, and other carnivores are exposed to, in and around the NKMNP. These data will help us determine the possible role of infectious diseases for maned wolf conservation in this region of Bolivia. The ecologic monitoring and use of radiotelemetry will provide us with much needed data on habitat utilization and inter maned wolf interactions.

E. JUSTIFICATION OF SPECIES.

State how and why you selected the species to study.

The maned wolf (*Chrysocyon brachyurus*), a Neotropical canid, lives in habitats severely compromised by agricultural development. The species is found chiefly in Brazil, Bolivia, Argentina and Paraguay. Although listed as 'near threatened' on the IUCN Red List, the maned wolf now is extinct in Uruguay with other populations increasingly at risk due to habitat loss to agriculture. Despite its flagship status, the number of maned wolves living in nature is unknown. A survey (conducted almost 40 years ago) estimated that ~1,500 to 2,200 individuals remained in Brazil, the country believed to have the most animals. It is estimated that 120 breeding pairs of maned wolves live in the NKMNP. Few projects have been performed to address disease threats to maned wolf *in situ* even though we know that infectious diseases cause significant morbidity and mortality in captive maned wolves. Thus, this species was chosen for the study. Crab eating foxes share habitat with maned wolves and may serve as one means of transfer of pathogenic agents to maned wolves, possibly serving as a link between the wolves and domestic dogs. Better information on the diseases of maned wolves and the disease epidemiology in neighboring domestic dogs and foxes will help to develop effective conservation management strategies, as well as preventive health programs for domestic animals.

F. JUSTIFICATION OF THE NUMBER OF ANIMALS TO BE USED.

Provide a detailed justification for the number of experimental and control animals to be used. Indicate what steps you have taken to minimize the number of animals to be used.

We would like a total of 15 maned wolves and 10 crab eating foxes, as well as the 40 domestic dogs we have already sampled. We have 8 wolves and 3 foxes thus far. This should provide us with the data necessary to determine the prevalence of exposure in these species to the select pathogenic agents for which we are concerned.

G. ANIMAL PROCEDURES

Describe the study procedures including animal handling requirements and experimental procedures.

Data will be collected as previously described in Deem and Emmons (2005) and briefly described here. Upon capture, individual wolves will be anesthetized, using Telazol® and/or ketamine, and assessed for general health status and body condition. Body weight

and morphometrics, including (1) total body length, (2) tail length, (3) head length, (4) ear length, (5) hind foot length and (6) shoulder height will be consistently assessed using a caliper and flexible tape, measuring the right side for unilateral measurement. Body temperature, heart rate and respiratory rate will be monitored at 5 minute intervals throughout the expected 30 minute anesthesia period. All indications of injuries or other health issues will be recorded. A blood sample (60 ml) will be collected by venipuncture of the jugular or saphenous vein. Cystocentesis will be performed on all wolves in which the urinary bladder is palpated. Each wolf's body will be examined closely and palpated for external parasites, an indication of level of infestation recorded, and all ectoparasites collected and preserved in 70% alcohol until further identification by an entomologist. Fresh fecal samples will be manually removed from the rectum for parasitological evaluation using sedimentation and floatation methods.

Deem, S.L., and Emmons, L.H. 2005. Exposure of free-ranging maned wolves (*Chrysocyon brachyurus*) to infectious and parasitic disease agents in the Noël Kempff Mercado National Park, Bolivia. J. Zoo Wildl. Med. 36: 192-197.

H. SURGICAL PROCEDURES, POSTOPERATIVE CARE AND PAIN MANAGEMENT

Describe any surgical procedures in detail including anesthesia and post-operative care. Indicate levels of pain to be experienced by animals involved in the research and describe how pain will be minimized and controlled. Describe what steps have been taken to find alternatives to painful procedures.

If any skin growths or lesions are noted, we will collect samples (skin scrapes and punch biopsies) using sterile surgical techniques. Surgical sites will be treated topically following any punch biopsy procedure and long acting injectable antibiotics (penicillin) administered if warranted.

I. STRESS.

Describe potential sources of physical (other than surgery- related) and psychological stress to animals (e.g. social isolation, restraint, environmental alteration, etc) involved in this research and what steps have been taken to minimize stress.

Anesthesia of any free-living wildlife species has some degree of stress associated with it. We will minimize this stress by using trained veterinarians to perform the anesthesia procedures and any invasive sample collections (blood collection, cystocentesis). Animals will be handled for the minimal time possible and they will be recovered from anesthesia in enclosed boxes to minimize recovery related injuries. All animals will be kept cool during the procedures, and recovered and released as soon as the veterinarian deems the animal clinically ready.

J. BIO-HAZARDOUS AGENTS.

Describe and provide justifications for the use of any biohazardous agents in this research (e.g. radioisotopes, teratogenic agents, experimental drugs).

N/A

K. DISPOSITION OF ANIMALS AT END OF THE STUDY.

Describe the anticipated disposition of the animals at the end of the study. If animals are to be euthanized as part of the study, describe euthanasia procedures.

N/A

L. PERSONNEL.

List the personnel to be involved in this research, their roles in the research and their qualifications (attach CVs for principle investigators).

Sharon L. Deem, DVM, PhD, Dipl ACZM (Co-PI)

Nucharin Songasen, DVM, PhD (Co-PI) but for Brazil portion of this study and not necessary for this IACUC request.

Louise Emmons, PhD (CI)

Suzan Murray, DVM, Dipl ACZM (CI)

Ellen Bronson, DVM (CI)

Dr. Deem has years of experience working with maned wolves, both in the wild and in captivity. Additionally, Dr. Deem is a member of the American College of Zoological Medicine and has worked with a variety of non-domestic species in captive and free-ranging conditions. Dr. Deem's short CV is attached to this IACUC.

Additional collaborators are laboratory personnel at a number of Institutions and Universities in the US. Names are available upon request.

M. ADDITIONAL IACUC APPROVALS:

If parts of this study are to be conducted with animals under the jurisdiction of another institution/agency, has it been approved by that institution's IACUC? Please indicate if so and provide a copy of that IACUC's certification of this study.

Dr. Songasen has submitted a separate IACUC for the Brazilian portion of our CEF grant.

N. CERTIFICATION OF PRINCIPAL INVESTIGATOR:

Signature certifies that the principal investigator will conduct the project in full accordance with Smithsonian Institution and National Zoological Park policies governing research, the use of its collections and the use of live animals in research. It is understood that Research Council/IACUC approval is valid for a period of three years following the date of initial approval.

Sharon L. Deem March 10, 2006 (signed hard copy to be mailed to NZP at the end of March when a person can hand carry the form to the USA.

Signature of Principle Investigator Date

Samples for exportation to USA to Maryland Zoo in Baltimore

As of November 2018

Maned wolf/Borochi/Lobo de crin (*Chrysocyon brachurus*):

CB13 9 Sept 2014

Serum: 5 vials (total of 22.5 ml in 5 vials in 5 ml aliquots)
Plasma: 1 vial (total of 1 ml)
Filter paper with whole blood: 1
Blood slides: 4
Whole blood cells: 1 vial (total of 0.5 ml)
Frozen whole blood: 1 vial (total of 2 ml)
Frozen urine: 1 vial (total of 4.5 ml)
Urine in formalin: 1 vial (total of 2 ml)
Filter paper with urine: 1
Frozen feces: 1 vial
Feces in formalin: 1 vial
Vaginal slides: 2
Whole blood in buffer: 1 vial (total of 2 ml)