

REISSUANCE, RENEWAL, OR AMENDMENT OF A PERMIT

□New ■Reissue/Renew □Amendment



Complete Sections A or B, and		this application. U.S.	address may be requi	ired in Sec	tion C."	
A. Complete if applying as an ind 1.a. Last name	lividual	1.b. First name		1.c. Middle	name or initial	1.d. Suffix
2 Date of birth 5.a. Telephone num (mm/dd/yyyy)		. Alternate telephone nber	6. E-mail address			-
B. Complete if applying on behall 1.a. Name of business, agency, Tribe International Elephan	, or institution		gency, Tribe, or institut 1.b. Doing business as (
2. Tax identification no. 75-2815706		n of business, agency, 1 and research conservat	Fribe, or institution ion of all species of elept	nants	3.b. Website URL www.elephant	. (if applicable) conservation.org
4.a. Principal officer (P.O.) last name	4.b. P.O. first n Deborah		4.c. P.O. middle initial J		4.b. P.O. Title Executive	Director
5. Primary contact name Deborah Olson			6. Primary e-mail addres		nservation	.org
7.a. Business telephone number 817-597-0956	7.	b. Alternate phone no.			ary contact telepho 597-0956	one no.

C. All applicants complete address	s information								
1.a. Physical address (Street address;									
c/o Fort Worth Zoo, 19	189 Colonial I	Jarkway							
1.b. City	1.c. State	1.d. Zip code/Postal code	1.e. County/Province	1.f. Country					
Fort Worth	ТХ	76110	Tarrant	USA					
2.a. Mailing Address (include if differen P.O. Box 366	2.a. Mailing Address (include if different than physical address; include name of contact person if applicable) P.O. Box 366								
	2.c. State	1 (in the second s	2.e. County/Province	2.f. Country					
Azle	TX	76098	Tarrant	USA					

	A H	
D.	All	applicants MUST complete
	1.	Include a check or money order, payable to the U.S. FISH AND WILDLIFE SERVICE, a nonrefundable processing fee [50 CFR 13.11(d)(4)]. Federal, Tribal, State, and local government agencies, and those acting on behalf of such agencies, are exempt from the processing fee – attach documentation of fee exempt status as outlined in instructions. (50 CFR 13.11(d))
	2.	If you are requesting a reissue/renew/amendment, what is your permit/file number? 21US09806C/9
	3.	Certification: I hereby certify that I have read and am familiar with the regulations contained in Title 50, Part 13 of the Code of Federal Regulations and the other applicable parts in subchapter B of Chapter I of Title 50, and I certify that the information submitted in this application for a permit is complete and accurate to the best of my knowledge and belief. I understand that any false statement herein may subject me to the criminal penalities of 18 U.S.C. 1001.
-		06/08/2022
The	e indi	vidual/principal officer of the business must print and sign the application. (No photocopied or stamped signatures) Date (mm/dd/yyyy)

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

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

E. REISSUANCE, RENEWAL, OR AMENDMENT OF A PERMIT (For this application, all permits, registrations, and certificates are referred to as a permit.)

NOTE 1: If you are renewing your Designated Port Exemption permit, use <u>form 3-200-2</u> and submit to the appropriate Office of Law Enforcement address. If you are renewing your Import/Export license (required for commercial activities), use <u>form 3-200-3a</u> and submit to appropriate Office of Law Enforcement address.

NOTE 2: This form **cannot** be used to request replacement of a lost or damaged permit. If you need to replace a lost or damaged permit, please use form 3-200-66. The application **must** be submitted to the office that issued the initial permit.

NOTE 3: Some activities, such as all master files for multiple shipments, Certificates of Scientific Exchange (COSE), circus/traveling exhibition certificates, and artificially propagated plant permits, can only be re-issued, renewed, or amended by submitting a new application for permits for those activities. Please refer to <u>these</u> <u>application types</u> to determine if another application form would be more appropriate or contact the Division of Management Authority for more information.

1. Name and address where you wish the permit to be mailed, **if different from physical address**. If you would like expedited shipping, please enclose a self-addressed, pre-paid, computer-generated, courier service airway bill. If unspecified, all documents will be mailed via regular mail through the U.S. Postal Service.

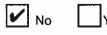
Deborah Olson, Executive Director International Elephant Foundation P.O. Box 366 Azle, TX 76098

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

Deborah Olson 81 7-597-0956 dolson@elephantconservation.org

es/

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?



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.

- 5. Submit the original permit with this application.

FWS Form 3-200-52 (Rev. 01/2020) U.S. Department of the Interior

6. Past activities.

- a. Provide copies of all cleared documents and form 3-177 (FWS declaration of wildlife) associated with this permit.
- b. Provide a summary detailing activities conducted under this permit, as well as a brief statement of why you are seeking reissuance/renewal.
- 7. Annual Report. If required by your permit, and not yet provided for the year, provide an annual report as conditioned.
- 8. **Sport-hunted trophies:** If you did not hunt during the hunting season stated in your original application, you are not eligible for a renewal. Please submit a new application form.
- 9. **Certification -** Complete one of the statements below and supply any additional documentation requested: (original signature is required)
 - a. For NO CHANGES to original application:

I certify that the information submitted in support of my original application for the permit indicated above has not changed and is still currently correct. I hereby request reissuance or renewal of this permit.

Permittee's signature:

Date: 06 08 2022

b. For CHANGES to original application:

On an attached page(s), provide a complete description of any changes (e.g., change in principal officer, personnel, address, location of activities, types of activities). Please sign each attached page. Also note that we may need to request additional information regarding the changes after reviewing your initial request.

I certify that the information submitted in support of my original application for the permit indicated above is still currently correct EXCEPT for the changes noted on the attached, signed page(s). I hereby request re-issuance or renewal of this permit with the indicated changes.

Permittee's signature:

__ Date: ___

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

6. Past Activities:

a) Copies of all cleared documents and form 3-177 associated with this permit included.

b) Summary detailing activities conducted under this permit and brief statement of purpose of seeking renewal of permit

The laboratory of Dr. Ling at Baylor College of Medicine has identified critical mechanisms of immunity by which normal healthy adult elephants appear to control EEHV infections. While many elephants apparently become infected with EEHV without clinical illness, the mechanisms contributing to lethal disease among a subset of young elephants remain a mystery. The opportunity to interrogate immune responses to EEHV in young elephants before, during and after recovery from EEHV-associated illness will provide important insights towards understanding both the vulnerability of some elephants for developing EEHV-associated disease and also the immune mechanisms responsible for control and recovery.

Import 1: To assist important vaccine research, fibroblasts are being derived from umbilical and placental tissue and then reprogramed to induced pluripotent stem cells (iPSCs). This will provide a number of unique research resources - biobanked fibroblasts (a limited resource) and iPSCs (an unlimited resource). Cells will be used for a variety of purposes related to understanding molecular mechanisms of EEHV viruses, vaccine and antibody development, and genome engineering to eradicate the viral genome. This knowledge will contribute greatly towards improving current treatment protocols and vaccine development.

Import 2: Data resulting from the imported samples facilitate research on disease susceptibility and reproductive problems from a genetic perspective to ultimately apply the knowledge gained from the *ex-situ* population to elephants in range countries that are experiencing increased human conflict, habitat loss, increased disease outbreaks and poor reproduction, possibly resulting in genomic variations that could impede the long-term survival of these fragile populations.

Statement of why we are seeking reissuance/renewal

All elephant species in all range countries are becoming increasingly endangered facing habitat loss and growing human elephant conflict. Due to human development, elephants are being isolated into smaller and smaller habitat islands with few opportunities to safely migrate to resources and mates. Based on other similarly restricted animal populations, all these conditions predictably can result in disease outbreaks (is this why we are seeing more EEHV cases in range countries?), poor reproduction, and loss of genetic variation.

Financial resources, research facilities, and researchers in North America are arguably the best in the world. Importing elephant biological samples into the United States will improve our ability to:

- Diagnose diseases and health problems
- Prevent the development or recurrence of disease and reduce the number of elephants who become ill
- Treat illness to improve survival rates or increase the number of elephants who are cured
- Improve the quality of life for elephants in situ and ex situ.

Although IEF is interested in supporting a wide variety of scientific investigation avenues for all elephants, EEHV is and has been the highest research priority currently for the International Elephant Foundations (IEF) as it is the leading cause of death in captive juvenile Asian elephants living in North America and Europe. Moreover, the disease is now recognized as occurring in both captive and wild range country elephants of Asia, captive African elephants in North and Central America and the recent first ever EEHV workshop in Africa (October 2019) identified multiple suspect cases in wild elephants in various countries in Africa. In contrast to Asian elephants, much less is known about the EEHV viruses that are naturally found in African elephants. Significant efforts are being made by research laboratories around the world to understand why this virus is having such a profound effect on the Asian and African elephant (endangered species) and to develop countermeasures for its treatment and prevention (i.e., a vaccine).

Dr. Paul Ling and his staff have spent the last several years developing the tools needed to discover what parts of the EEHV virus might be useful for developing a vaccine that can induce protective immunity for Asian elephants. Long-term, strategies developed for an anti-EEHV vaccine in Asian elephants will be leveraged for a similar vaccine in African elephants. The EEHV vaccine represents a truly innovative endeavor because it is considered an experimental vaccine developed for use in an endangered species. The first vaccine study in mice with a prototype EEHV vaccine has now been completed and it has been submitted for publication. In addition, a second preclinical vaccine trial in mice with a different vaccine platform was initiated with the intention of comparing its efficacy to the first vaccine prototype and to determine whether both vaccines used in combination might be better than either one alone. If promising results are realized, the resulting vaccine will be distributed to institutions throughout North America

FORM 3-201A (1/97) CONVENTION C)N		Page 1 of 4
CTER INTERNATIONA ENDANGERED	L TRADE IN	IMPORT	1. Original Permit/Certificate No. 21US09806C/9
WILD FAUNA AI		PERMIT	2. Valid 2022-05-17
3. Permittee (name and address, country) INTERNATIONAL ELEPHANT FOUNDATION C/O FORT WORTH ZOO 1989 COLONIAL PARKWAY FORT WORTH, TX 76110 U.S.A.	Y	4. Consignor (name and address, cour	try)
5. Special Conditions		5a. Purpose of Transaction	
MUST COMPLY WITH ENCLOSED GENERAL PE	RMIT CONDITIONS.	S	
U.S. ENDANGERED SPECIES [50 CFR 17:22] AN SPECIES [50 CFR 17:40(e)].	D U.S. THREATENED	6. U.S. Management Authority Department of the Interior	
PERMIT MAY BE COPIED FOR MULTIPLE SHIPM RETAINED BY PERMITTEE.		U.S. FISH AND WILDLIFE SERVICE DIVISION OF MANAGEMENT AUT BRANCH OF PERMITS, MS: IA	
PERMITTEE MUST COMPLETE BLOCK 4 (CONS (QUANTITY), AND BLOCK 12 (COUNTRY OF OR) SHIPMENT.		5275 LEESBURG PIKE FALLS CHURCH VA 22041-3803	
THIS RE-ISSUES AND REPLACES 20US09806C/S		2021-05-18 Mana	Generic Authority
-May not be used for commercial purposes. For live an if the transport conditions comply with the CITES Transport of Live Animals or, in the case of air transport Animals Regulations.	Guidelines for		tates Management Authority Act of 1973 (16 USC 1531 et. seg.)
7/8. Common Name and Scientific name (genus and species) of Animal or Plant	9. Description of Part or or numbers (age/sex	r Derivative, including identifying marks	10. Appendix No. and Source
A. Common Name		CAL SPECIMENS INCLUDING	10. 1 W
ASIAN ELEPHANT	DUNG, BIOPSIES, T	ALIVA, TRUNK WASHINGS, SWABS, FISSUES, NUCLEIC ACIDS, S, PARAFFIN BLOCKS, FTA CARDS,	11. Quantity (including units)
Scientific Name ELEPHAS MAXIMUS		EXTRACTED FROM THESE	NO 12. Country of Origin
B. Common Name	1	CAL SPECIMENS INCLUDING ALIVA, TRUNK WASHINGS, SWABS,	^{10.} 1 F
ASIAN ELEPHANT	DUNG, BIOPSIES, T	FISSUES, NUCLEIC ACIDS, S. PARAFFIN BLOCKS, FTA CARDS,	11. Quantity (including units)
Scientific Name		EXTRACTED FROM THESE	12. Country of Origin
ELEPHAS MAXIMUS	SAMPLES.		, ,
C. Common Name	BLOOD, SERUM, SA	CAL SPECIMENS INCLUDING ALIVA, TRUNK WASHINGS, SWABS,	^{10.} 1 W
AFRICAN ELEPHANT	HISTOLOGY SLIDE	FISSUES, NUCLEIC ACIDS, S, PARAFFIN BLOCKS, FTA CARDS,	11. Quantity (including units) NO
Scientific Name	SAMPLES.	EXTRACTED FROM THESE	12. Country of Origin
D. Common Name	BLOOD, SERUM, S/	CAL SPECIMENS INCLUDING ALIVA, TRUNK WASHINGS, SWABS, FISSUES, NUCLEIC ACIDS,	¹⁰ . 1 F
AFRICAN ELEPHANT	HISTOLOGY SLIDE	S, PARAFFIN BLOCKS, FTA CARDS, X EXTRACTED FROM THESE	11. Quantity (including units) NO
LOXODONTA AFRICANA	SAMPLES.		12. Country of Origin
E. Common Name		NTA CYCLOTIS; BIOLOGICAL SCRIBED IN BOX 9 (A, B, C , AND D)	10. 1 W
ALL ELEPHANTS	ABOVE.		11. Quantity (including units)
Scientific Name			NO 12. Country of Origin
ELEPHANTIDAE			

CIES	IMPORT CONTINUATION SHEET	CONTINUATION BRANCH OF PERMITS, MS: IA SHEET 5275 LEESBURG PIKE FALLS CHURCH VA 22041-3803		Page 2 of 4 1. Original Permit/Certificate No. 21US09806C/9
	6. U.S. Managemer FAI	ent Authority LLS CHURCH VA	2021-05-18	Manager
		PLACE	Issuing Date	US8271655
7/8. Common Name and Scientific species) of Animal or Plant	name (genus and		Derivative, including identifying ma	narks 10. Appendix No. and Source
F. Common Name			TA CYCLOTIS, BIOLOGICAL CRIBED IN BOX 9 (A, B, C , AND	DD) ^{10.} 1 F
ALL ELEPHANTS		ABOVE.	,	11. Quantity (including units)
Scientific Name				NO
ELEPHANTIDAE				12. Country of Origin
G. Common Name		9. XXXXXX		^{10.} XXX XXX
XXXXXX				11. Quantity (including units) XXXXXX XXX
Scientific Name				12. Country of Origin XXXXXX
H. Common Name		9. XXXXX		10. XXX XXX
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Scientific Name				12. Country of Origin
I. Common Name		9. XXXXXX		XXXXXX 10. XXX XXX
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Scientific Name				XXXXXX XXX 12. Country of Origin
XXXXXX	,			XXXXXX
J. Common Name		9. XXXXXX		^{10.} xxx xxx
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хххххх	1			12. Country of Origin XXXXXX
K. Common Name		9. XXXXXX		^{10.} xxx xxx
XXXXXX Scientific Name		~		11. Quantity (including units) XXXXXX XXX
XXXXXX				12. Country of Origin XXXXXX
L. Common Name		9. XXXXXX		10. XXX
хххххх				11. Quantity (including units)
Scientific Name				XXXXXX XXX 12. Country of Origin
XXXXXX	!			xxxxxx
M. Common Name		9. XXXXXX		^{10.} xxx
XXXXXX Scientific Name				11. Quantity (including units) XXXXXX XXXX
хххххх				12. Country of Origin XXXXXX



United States Department of the Interior

FISH AND WILDLIFE SERVICE International Affairs 5275 Leesburg Pike, MS: IA Falls Church, VA 22041-3803



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SPECIAL PERMIT CONDITIONS

International Elephant Foundation

1. <u>Samples from confiscated specimens</u>: Samples from confiscated specimens may only be imported with the required chain-of-custody documentation and authorizations from the relevant government(s) and appropriate law enforcement officials, and provided the conditions of paragraphs 9 and 10 below are met.

2. <u>Samples collected from salvage</u>¹: Samples collected from salvaged dead wild animals may be imported provided (a) the collection of the samples occurs in a manner that does not disrupt other animals' movements or behavior during a critical phase of their activities or life cycle, and (b) provided the conditions of paragraph 9 and 10 below are met. (¹ Salvage is defined as the collection of samples from dead animals that were not killed intentionally and that died of causes unrelated either to the collection of samples or the capture of the animal for the purpose of obtaining samples. Samples collected from animals that accidentally died as the results of capture for reasons other than the collection of samples (i.e., capture for translocation or radio collaring) would be considered salvage.)

3. <u>Samples collected from wild-caught animals</u>: Samples collected from wild-caught animals may be imported provided the conditions of paragraphs 5 through 10 below are met.

4. <u>Samples collected from captive-held animals</u>: Samples collected from captive-held animals may be imported provided the conditions of paragraphs 7 through 10 are met.

5. Animals may only be captured in cooperation with local wildlife authorities (e.g., samples may be collected in parks/nature reserves only with the knowledge and consent of the park/reserve manager). No samples may be obtained from animals that are permanently removed from the wild for the sole purpose of collecting samples, or for other than scientific or management purposes approved and carried out by appropriate wildlife authorities.

6. No remuneration, either financial or in-kind, may be offered for the taking of animals from the wild (i.e., killing, trapping) or for the collection of samples from free-ranging wildlife. This condition does not preclude legitimate collection and transportation expenses (e.g., hiring staff, freight costs), but does prohibit the paying of bounties or incentive pay for taking of animals from the wild, or the collection of samples from animals in the wild. Explicit written approval for collection from wild animals must be obtained from appropriate wildlife authorities and retained in the applicant's files for a period of 5 years and must be supplied upon the Division of Scientific Authority's request.



U.S. Management Authority

05/18/2021 Date 7. No animals may be killed intentionally for the purpose of collecting samples.

8. Care must be taken when handling live animals to minimize any possibility of injury. If, for any reason, any wild or captive-held animal dies or incurs a debilitating injury as a result of being restrained for sample collection, or while having the sample collected, further collection of samples must be suspended until methods are evaluated and, if appropriate, modified to prevent further incidences of injury or death. If two or more animals die or incur debilitating injuries within a 6-month period, sample collection must be suspended and the Division of Scientific Authority contacted in writing within 7 days of the death of the second individual. *Before further sampling will be authorized a written account of the details of the event, and recommendations to resolve the situation, must be submitted for a review of sampling procedures (point of contact: Dr. Rosemarie Gnam, Chief, Division of Scientific Authority, MS: IA, 5275 Leesburg Pike, Falls Church, VA 22041-3803; tel. 703-358-1708; fax 703-358-2276).*

9. The applicant *must* maintain a record of all samples imported under this permit which must be made available to the Division of Scientific Authority upon request. This record should include for each import: the species and type(s) of specimens, date(s) collected, the date shipped, the location(s) of collection and name of person who collected the sample(s), conditions under which samples were collected (salvage, captive-held, or wild-caught), authorizing government agency, and any mortalities or debilitating injuries that may have occurred as a result, directly or indirectly, of the collection activities.

10. The applicant must maintain copies of all CITES permits used to obtain and import all specimens which must be made available to the Division of Scientific Authority upon request.

11. No viable gametes or live animals may be imported under this permit.

12. Annual Report: Prior to re-issuance or amendment of this application (or if no re-issuance is needed, at the time all activities under this permit have been completed) the applicant must provide USFWS with a report that contains a record of all samples imported under this permit. The report must include the primary reason for the collection and the name and organizational affiliation of all individuals who conducted the sampling, as well as a copy of all relevant sample collection permits issued by the authorizing government agency, or individual in the case of private landowners. The report must include any mortalities or debilitating injuries that may have occurred as a result, directly or indirectly, of the collection activities. Finally, the report must include for each sample in each import: (a) the species and type of sample collected (e.g., blood, tissue etc.); (b) date of sample collection; (c) date of sample shipment and where the sample was shipped; and (d) the collection location.



U.S. Management Authority

<u>05/18/2021</u> Date ANNUAL REPORT Permit 21US09806C/9 1 June 2022

IMPORT 1

Permit 21US09806C/9

Date of Importation: 6 April 2022

Imported under International Elephant Foundation CITES Permit # 21US09806C/9 and USFWS Form 3-177 and Canada CITES Export Permit # 22CA00651/CWHQ,

22CA00652/CWHQ, 22CA00653/CWHQ, 22CA00653/CWHQ, 22CA00654/CWHQ

The 4 Canada CITES permits are for samples from 4 different elephants. Lily, Natasha, Emily and Opal.

Primary Reason for Collection

The aim of this project is to first derive fibroblasts then reprogram them to induced pluripotent stem cells (iPSCs) from umbilical and placental tissue. This will provide a number of unique resources, biobanked fibroblasts (a limited resource) and iPSCs (an unlimited resource). We aim to generate elephant iPSCs by using novel and published protocols and techniques for mouse, human and rhino iPSCs among others. We will amend these techniques according to the elephant species. This will greatly expand the current tools, techniques, and literature available for conservation efforts in the preservation of non-model organisms.

Derivation of Elephant iPSCs: (Methods adapted from Hysolli, et. al. Stem Cell Reports 2016) Reprogramming experiments will be carried out using the four human transcription factors OCT4, SOX2, KLF4, MYC in pMSCV retrovirus backbone as previously described (Park et al., 2008). Briefly VSV-G peudotyped retrovirus will be generated in 293T cells using GAG-POL, VSV-G and pMIG vector expressing each for four factors. The virus will be collected, filtered in 0.45 um filtering system and centrifuged for 1.5 hours at 23,000 rpm in a Beckman L80 ultracentrifuge. Cells will be passaged into 0.2% gelatin-coated plates containing irradiated or mitomycin-C treated mouse embryonic feeder cells (Millipore, EmbryoMax CF1 strain cat# PMEF-CF). The medium will be switched from 10% FBS in DMEM into embryonic stem cell medium containing KSR and bFGF in DMEM/F12 on day 5, and cells will be maintained in this medium for 3-4 weeks until iPSC colonies formed. Lentivirus will be generated using the packaging vectors MDL, REV and VSV-G. An alternative path to reprogramming will use a piggyback vector whose integration into the genome can be reversed, with the human and elephant OSKM factors mentioned above. We can then use the resulting iPSCs to study diseases in the dish. For example, elephant endotheliotropic herpesvirus-hemorrhagic disease (EEHV-HD) with mortality rates of up to 85%, and yet the disease remains largely unknown will be studied. Cells will be used for a variety of purposes related to understanding molecular mechanisms of EEHV viruses, vaccine and antibody development, and genome engineering to eradicate the viral genome. We will also utilize these cells to understand evolution of the elephant in the dish. iPSCs will provide a powerful tool to study these areas.

Name and Organizational Affiliation of All Individuals Who Conducted Sampling

Charlie Gray Superintendent of Elephants African Lion Safari Cambridge, Ontario, Canada

Dr Jaden Dales Head Veterinarian African Lion Safari Cambridge, Ontario, Canada

Mortalities and/or Debilitating Injuries

There were no mortalities or debilitating injuries incurred directly or indirectly through collection activities

Sample Details:

Species: Asian Elephant, Elephas maximus

Type of Samples: Umbilical and placental tissues expelled after giving No mortalities at the time birth and collection of samples. All calves survived

Captive born at African Lion Safari, Cambridge, Ontario, Canada Opal studbook #637 – Gave birth May 9, 2021 1.8mL microcryovials (10 placenta and 5 umbilical)

Captive born at African Lion Safari, Cambridge, Ontario, Canada Emily studbook #639 – Gave birth April 5, 2021 1.8mL microcryovials (10 placenta and 5 umbilical)

Captive born at African Lion Safari, Cambridge, Ontario, Canada Natasha studbook #356 – Gave birth Feb 23, 2021 1.8mL microcryovials (10 placenta and 5 umbilical)

Captive-born in Ramat-Gan, Israel, Lily studbook #347 – Gave birth Nov 22, 2020 1.8mL microcryovials (10 placenta and 5 umbilical vessel)

After incised from the tissue, each sample was rinsed generously with lactated Ringers solution to remove debris. Then, samples were either placed into a pre-labeled micro cryotube and submerged in liquid nitrogen or were added to a pre-measured and aliquoted FBS/DMSO solution within a pre-labeled cryotube, which was then also submerged into liquid nitrogen.

Date shipped: 6 April 2022

Where samples shipped:	Deborah Olson Executive Director International Elephant Foundation c/o Fort Worth Zoo 1989 Colonial Parkway Fort Worth, TX 76110
Samples transferred to:	Dr Eriona Hysoli Ipsen Innovation Center BioLabs 650 E Kendall St, 2nd Fl Cambridge, MA 02142
Collection location: Africa	n Lion Safari

Collection location: African Lion Safari Cambridge, Ontario, Canada

IMPORT 2

Permit 21US09806C/9

Date of Importation: 12 May 2022

Imported under International Elephant Foundation CITES Permit # 21US09806C/9 and USFWS Form 3-177 and Mexico CITES Export Permit # MX115933

Primary Reason for Collection

Title: Developing Genomic Resources for Elephant Conservation

PI: Dr. Natalia A. Prado, Research Associate, Smithsonian Conservation Biology Institute

Overall Scope: The ability to sequence whole genomes is revolutionizing our ability to manage animal populations, and is providing new approaches for understanding how animals adapt to their environment. These data are important because the success of captive management can be impacted by genetic factors, such as gene diversity and function. Zoo elephants are no exception, and management has been challenging because of poor reproduction and health issues resulting in non-sustaining populations for both species of elephants. This project generated high coverage (30X-60X) genome sequences of African and Asian elephants, which will be used to assess genetic variation, and enable genomics projects in elephants to address fundamental questions about individual and population health and species survival. Genetic markers from these analyses will provide crucial information on the demographic history of our managed populations of elephants and will provide detailed estimates of how individuals are related to each other and eventually, how they are related to wild populations. This will include a detailed family tree of the North American population. We intend to apply genomic tools to address some of the questions that have puzzled elephant managers for decades, such as why do some elephants thrive more in captivity than others and why do some elephants appear more susceptible to EEHV.

A significant amount of data on elephant biology from studies of >300 individuals in U.S. zoos are now available through decades of research efforts. This information includes reproductive rates and ovarian cycle normality, health conditions, disease status, physical characteristics and hormonal profiles, as well as behavioral components related to temperament and sociality. These data are important because zoo elephants exhibit a number of conditions that could be modulated by genetic factors, such as infertility, reproductive tract pathologies (leiomyomas and cysts), foot and joint problems, arthritis, and susceptibility to a number of clinical diseases, such as elephant endothelial herpes virus (EEHV) and tuberculosis. This proposed study will utilize cutting-edge genomic tools to sequence elephant genomes as a first step to evaluating relationships between various genetic markers and physiological processes.

There are very few genomic resources that have been developed to study the genetic basis of phenotypic and disease traits in most species, including elephants. Genetic markers have the potential to be powerful tools that complement traditional studbook-based genetic management of captive populations and to identify markers that could be informative to elephant conservation. We have completed whole genome sequencing and assembly of 196 elephants in the North American captive population to start assessing the genetic variation present in the population, as well as annotation of our reference genome from Kandula. Our objectives over the next year are to: 1) complete collection of whole blood samples from the entire captive population in North America, including Canada and Mexico; 2) to develop a detailed family tree and estimates of relatedness among Asian and African elephants in North America; and 3) identify individuals with particular health problems (e.g., tuberculosis, EEHV shedding, ovarian acyclicity, leiomyomas, arthritis) and look for shared traits in their genome. These data will first aid in the breeding and management of zoo elephants, and facilitate research on disease susceptibility and reproductive problems from a genetic perspective. Ultimately, our aim is to apply the knowledge gained from the *ex-situ* population to elephants in range countries that are experiencing increased human conflict, habitat loss, increased disease outbreaks and poor reproduction, possibly resulting in genomic variations that could impede the long-term survival of these fragile populations.

This study has received endorsement from the elephant North American TAG/SSP, the EEHV Advisory Group, and has NZP IACUC approval (proposal #18-29). It is funded by a Smithsonian Women's Committee Grant, the FONZ Conservation Grant, and a private foundation. Results will also be presented at appropriate conferences such as, Elephant Manager's Association, Smithsonian Research Symposium and the International Elephant Foundation Conference, and a manuscript will be prepared for submission to a peer-reviewed journal.

Collaborators:

- Dr. Janine L. Brown, Smithsonian Center for Species Survival, NZP
- Dr. Jesus E. Maldonado, Smithsonian Center for Conservation Genomics, NZP
- Dr. Michael G. Campana, Smithsonian Center for Conservation Genomics, NZP
- Erin Latimer, Smithsonian National Elephant Herpes Lab, NZP
- Virginia R. Pearson, Visiting Scientist, Rall Laboratory Fox Chase Cancer Center (EEHV)

Name and Organizational Affiliation of All Individuals Who Conducted Sampling

Gerardo Marinez Elephant Manager Africam Safari Carretera Al Oasis No 17302-22 Puebla, Puebla C.P. 72960, Mexico

Mortalities and/or Debilitating Injuries

There were no mortalities or debilitating injuries incurred directly or indirectly through collection activities

Sample Details:

Species: Asian Elephant, *Elephas maximus* African elephant, *Loxodonta Africana*

Name	Lider - African	Timida- African	Bola- African	Independer African	nte- Lunar Africa		Leja- African		nde- ican	Vampi- African	Mediano African
Tail Hair	x	x	x	x	x		x	x		x	x
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Type of Samples: Whole blood, serum, tail hair

8 - 10 ml of blood drawn into 2 EDTA whole blood Vacutainer tubes. Tubes stored frozen and shipped on dry ice to avoid freeze-thaw cycles that could damage the DNA. Whole blood samples that were previously collected and stored frozen were also provided.

Date shipped: 11 May 2022

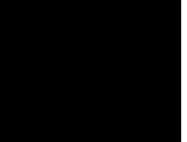
Where samples shipped:	Deborah Olson Executive Director International Elephant Foundation c/o Fort Worth Zoo 1989 Colonial Parkway Fort Worth, TX 76110
Samples transferred to:	Natalia A. Prado, Ph.D. Research Associate Smithsonian Conservation Biology Institute 1500 Remount Road-MRC 5533 Front Royal, VA 22630

Collection location: Africam Safari Puebla, Puebla, Mexico

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15c. Contact Name:

Species Code	16a. Scientific Name 16b. Common Name	17a. Foreign CITES Permit Num, 17b. U.S. CITES Permit Num,	18a. Description Code 18b. Source	19a. Quantity/Units 19b. Total Monetary Value	20, Country of Species Origin Code (ISO Code)	21. Venomous Live Wildlife Indicator
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3. Permittee (name and address, country) INTERNATIONAL ELEPHANT FOUNDATION C/O FORT WORTH ZOO 1989 COLONIAL PARKWAY FORT WORTH, TX 76110 U S.A. Jolson OelephantconServation. 817 - 597 - 0956	05	4. Consignor (name and address. co African Lion Safari c/o 1386 Cooper Road Cambridge, Ontario MIR cgray@lionsafari.com 519 240 2990	Charlie Gray
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Transport of Live Animals or, in the case of air transpo Animals Regulations. 7/8. Common Name and Scientific name (genus and	rt, with IATA Live 9. Description of Part of	AUTHORITY: Endangered Specie r Derivative, including identifying marks	
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United States Department of the Interior

FISH AND WILDLIFE SERVICE International Affairs 5275 Leesburg Pike, MS: IA Falls Church, VA 22041-3803



Page 3 of 4 21US09806C/9

SPECIAL PERMIT CONDITIONS

International Elephant Foundation

1. <u>Samples from confiscated specimens</u>: Samples from confiscated specimens may only be imported with the required chain-of-custody documentation and authorizations from the relevant government(s) and appropriate law enforcement officials, and provided the conditions of paragraphs 9 and 10 below are met.

2. <u>Samples collected from salvage</u>¹: Samples collected from salvaged dead wild animals may be imported provided (a) the collection of the samples occurs in a manner that does not disrupt other animals' movements or behavior during a critical phase of their activities or life cycle, and (b) provided the conditions of paragraph 9 and 10 below are met. (¹ Salvage is defined as the collection of samples from dead animals that were not killed intentionally and that died of causes unrelated either to the collection of samples or the capture of the animal for the purpose of obtaining samples. Samples collected from animals that accidentally died as the results of capture for reasons other than the collection of samples (i.e., capture for translocation or radio collaring) would be considered salvage.)

3. <u>Samples collected from wild-caught animals</u>: Samples collected from wild-caught animals may be imported provided the conditions of paragraphs 5 through 10 below are met.

4. <u>Samples collected from captive-held animals</u>: Samples collected from captive-held animals may be imported provided the conditions of paragraphs 7 through 10 are met.

5. Animals may only be captured in cooperation with local wildlife authorities (e.g., samples may be collected in parks/nature reserves only with the knowledge and consent of the park/reserve manager). No samples may be obtained from animals that are permanently removed from the wild for the sole purpose of collecting samples, or for other than scientific or management purposes approved and carried out by appropriate wildlife authorities.

6. No remuneration, either financial or in-kind, may be offered for the taking of animals from the wild (i.e., killing, trapping) or for the collection of samples from free-ranging wildlife. This condition does not preclude legitimate collection and transportation expenses (e.g., hiring staff, freight costs), but does prohibit the paying of bounties or incentive pay for taking of animals from the wild, or the collection of samples from animals in the wild. Explicit written approval for collection from wild animals must be obtained from appropriate wildlife authorities and retained in the applicant's files for a period of 5 years and must be supplied upon the Division of Scientific Authority's request.

U.S. Management Authority

05/18/2021 Date 7. No animals may be killed intentionally for the purpose of collecting samples.

8. Care must be taken when handling live animals to minimize any possibility of injury. If, for any reason, any wild or captive-held animal dies or incurs a debilitating injury as a result of being restrained for sample collection, or while having the sample collected, further collection of samples must be suspended until methods are evaluated and, if appropriate, modified to prevent further incidences of injury or death. If two or more animals die or incur debilitating injuries within a 6-month period, sample collection must be suspended and the Division of Scientific Authority contacted in writing within 7 days of the death of the second individual. *Before further sampling will be authorized a written account of the details of the event, and recommendations to resolve the situation, must be submitted for a review of sampling procedures (point of contact: Dr. Rosemarie Gnam, Chief, Division of Scientific Authority, MS: IA, 5275 Leesburg Pike, Falls Church, VA 22041-3803; tel. 703-358-1708; fax 703-358-2276).*

9. The applicant *must* maintain a record of all samples imported under this permit which must be made available to the Division of Scientific Authority upon request. This record should include for each import: the species and type(s) of specimens, date(s) collected, the date shipped, the location(s) of collection and name of person who collected the sample(s), conditions under which samples were collected (salvage, captive-held, or wild-caught), authorizing government agency, and any mortalities or debilitating injuries that may have occurred as a result, directly or indirectly, of the collection activities.

10. The applicant must maintain copies of all CITES permits used to obtain and import all specimens which must be made available to the Division of Scientific Authority upon request.

11. No viable gametes or live animals may be imported under this permit.

12. Annual Report: Prior to re-issuance or amendment of this application (or if no re-issuance is needed, at the time all activities under this permit have been completed) the applicant must provide USFWS with a report that contains a record of all samples imported under this permit. The report must include the primary reason for the collection and the name and organizational affiliation of all individuals who conducted the sampling, as well as a copy of all relevant sample collection permits issued by the authorizing government agency, or individual in the case of private landowners. The report must include any mortalities or debilitating injuries that may have occurred as a result, directly or indirectly, of the collection activities. Finally, the report must include for each sample in each import: (a) the species and type of sample collected (e.g., blood, tissue etc.); (b) date of sample collection; (c) date of sample shipment and where the sample was shipped; and (d) the collection location.



U.S. Management Authority

<u>05/18/2021</u> Date

African Lion Safari Canada's Original Safari Adventure™

PRO FORMA COMMERCIAL INVOICE

The samples in this shipment have been obtained from Asian elephants (Elephas maximus) for scientific research purposes only and have \$60.00 USD commercial value.

Charlie Dray

Charlie Gray African Lion Safari 1386 Cooper Road Cambridge, Ontario N1R 5S2 CANADA

RR #1, Cambridge, Ontario, Canada N1R 5S2 👘 lionsafari.com











Cryoport Systems, Inc. 17305 Daimler St. Irvine, California 92614 US

cryoport°

COMMERCIAL INVOICE

	PED F	-		DATE:			4/9/2022	
	Cooper			AIRWAYBILL NO:				
		N N1R 5S	2 CA	SHIPMENT ID:		225397		
		- Oraci tad	an Dalaa	PURCHASE ORDER NO).:			
PHO		e Gray Jad	en Dales	INCOTERMS:			DDP	
EMAI				REASON FOR EXPORT		NC	DT_SOLD	
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PKG	NO. OF	UNIT OF	DESCRIPTION	OF GOODS	COUNTRY	UNIT	TOTAL	
#	UNITS	MEASURE		OF ORIGIN	VALUE	VALUE		
1	15	Each	"E. maximus," "Opal": 1.8mL m	icrocryovials (10 placenta	CA	\$1.00	\$15.00	
			and 5 umbilical), originating in (export permit #22CA00654/CW #21US09806C/9					
1	15	Each	"E. maximus," "Emily": 1.8mL m and 5 umbilical), originating in (export permit #22CA00651/CW #21US09806C/9	Canada - Canadian cites	CA	\$1.00	\$15.00	
1	15	Each	"E. maximus," "Natasha": 1.8m placenta and 5 umbilical), origin Canadian cites export permit # cites permit #21US09806C/9	CA	\$1.00	\$15.00		
1	15	Each	"E. maximus," "Lily": 1.8mL mic and 5 umbilical vessel), origina Canadian cites export permit # cites permit #21US09806C/9	СА	\$1.00	\$15.00		

TOTAL NO. OF PACKAGES:	1	SUBTOTAL:	\$60.00	
TOTAL WEIGHT:	23.0 LBS			
		INSURANCE:	\$0.00	
SPECIAL INSTRUCTIONS:		FREIGHT:	\$0.00	
		PACKING:	\$0.00	
		HANDLING:	\$0.00	
		INVOICE TOTAL:	\$60.00	
		CURRENCY CODE:	USD	
DECLARATION STATEMENT(S):				
These commodities, technology or s	software were exported from Ca	anada in accordance with the export adm	ninistration	
regulations. Diversion contrary to Ca	anada Law is prohibited. I decla	re that all the information contained in this	s invoiceto	
be true and correct.				
SIGNATURE/TITLE		DA	DATE	
		April 9	2022	



United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

National Center for Import and Export

Animal Products

4700 River Road Unit 40 Riverdale, MD 20737

Telephone: (301) 851-3300

FAX: (301) 734-8226 Virginia R. Pearson / Fox Chase Cancer Center Rall Laboratory 333 Cottman Avenue Philadelphia, PA 19111

Tuesday, February 28, 2017

Dear Virginia R. Pearson:

Our office has reviewed your request to renew your Veterinary Services permit 111798 to import Pathological and diagnostic samples from wild and captive African and Asian elephants, including liquid or dried DNA samples extracted from blood, serum, saliva, trunk washings, dung, biopsies, and necropsy tissue. Your request has been assigned application reference number 17027083.

Our office has recently reviewed our requirements for the importation of material derived from elephants, and determined that there is minimal risk of introduction of foot-and-mouth disease (FMD) virus from these materials. As such, an import permit is not required. This change will bring us in line with our requirements for live animals, which currently allow for elephants to be imported with no restrictions related to FMD. Any materials collected from animals infected with or exposed to livestock or poultry pathogens are still subject to permitting by the Organisms and Vectors staff.

Please note that you will be required to show documentation to U.S. Customs and Border Protection CBP) Agricultural Specialists indicating the species from which the material is derived. Additionally, this change does not eliminate the need for permits from other agencies which may have jurisdiction over the material you wish to import, such as the U.S. Department of Interior, Fish and Wildlife Service (FWS).

Please visit the FWS website at www.FWS.gov/permits and/or www.FWS.gov/le/travelers.html to determine if they have additional requirements for this material.

If you have further questions, please contact us at AskNIES.products@aphis.usda.gov or (301) 851-3300, Option 1, or, for questions about infected materials, please contact Organisms and Vectors staff at OV@aphis.usda.gov or (301) 851-3300, Option 3.

Sincerely,



Sufeguarding Animal Health

APHIS is an agency of USDA's Marketing and Regulatory Programs An Equal Opportunity Provider and Employer Federal Relay Service (Voice/TTY/ASCII/Spanish) 1-800-877-8339

Deborah Langford Staff Veterinarian

1. Date of Import/Export (mm/dd/yyyy): 05/11/2022	U.S. FISH AND V	VILDLIFE SERVICE	7. Name of Carrie FEDEX	r:
2. Import/Export License Number:	R		8. Air Waybill or Master: House:	Bill of Lading No.:
3. Indicate One: ✓ import □ export		<u>S ()</u>	9. Transportation	Code: A
4. Port of Clearance: ME			License No. State or Province:	
5. Purpose Code: S	IMPORTATION O	ATION FOR OR EXPORTATION OF R WILDLIFE	10. Bonded Locat ME	ion for Inspection:
6. Customs Document Number(s):	FISH OF	(WIEDER'E	11. Number of Ca	rtons Containing Wildlife
		022ME2939688	1	
	Control#	2022162674	12. Markings on (Wildlife:	Cartons Containing
3. (indicate one)		14. (indicate one)		
U.S. Importer		Foreign Importer		
U.S. Exporter INTERNATIONAL ELEPHANT FOUNDATIO DEBORAH OLSON 1989 COLONIAL PARKWAY FORT WORTH, TX 76110 8175970956 DOLSON@ELEPHANTCONSERVATION.OR		Foreign Exporter AFRICAM SAFARI CAROLINA HOLGUI CARRETERA AL OA PUEBLA, MX cholguin@africamsafat	SIS 17302, INT. 22, CO	LONIA AFRICAM
3b. Identifier Number:	ID Type:	14c. Identifier Number:		ID Type:
15. Customs Broker, Shipping Agent or Fr	eight Forwarder:	15b, Identifier Number:	ID Type:	
Phone Number / Fax Number / Email Address:		15c. Contact Name:		

Species	16a. Scientific Name	17a. Foreign CITES	18a: Description	19a. Quantity/Units	20. Country	21.
Code	16b. Common Name	Permit Num. 17b. U.S. CITES Permit Num.	Code 18b. Source	19b. Total Monetary Value	of Species Origin Code (ISO Code)	Venomous Live Wildlife Indicator
ELEM	ELEPHAS MAXIMUS ASIAN ELEPHANT	MX115933 21US09806C/9	SPE C	18.00 NO \$0	МХ	
LOXA	LOXODONTA AFRICANA AFRICAN ELEPHANT	MX115933 21US09806C/9	SPE W	31.00 NO \$ 0	NA	
LOXA	LOXODONTA AFRICANA AFRICAN ELEPHANT	MX115933 21US09806C/9	SPE C	13.00 NO \$ 0	мх	
			HAL	225.00 NO		
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U.S. FISH AND WILDLIFE SERVICE



Page <u>2</u> of <u>2</u>

13. Name of Importer/Exporter: INTERNATIONAL ELEPHANT
FOUNDATION DEBORAH OLSON
8. Air Waybill or Bill of Lading Number: Master:
House:

2. I/E License Number: *****

DECLARATION FOR IMPORTATION OR EXPORTATION OF FISH OR WILDLIFE 8. Air Wa Master:

CONTINUATION SHEET

19a. Quantity/Units Species 16a. Scientific Name 17a. Foreign CITES 18a. Description 20. Country 21. 19b. Total Code 16b. Common Name Permit Num. Code of Species Venomous 17b. U.S. CITES 18b. Source Monetary Value Origin Code Live Wildlife (ISO Code) Permit Num. Indicator 225.00 NO LOXODONTA AFRICANA MX115933 HAI LOXA NA AFRICAN ELEPHANT 21US09806C/9 W \$ 0 \Box Knowingly making false statement in a Declaration for Importation or Exportation of Fish or Wildlife may subject the declarant to the penalty provided by 18 U.S.C. 1001 21.1 certify under penalty of perjury that the information furnished is true and correct: and 16 U.S.C. 3372(d). Filed Electronically 05/10/2022

U FISH & WILDLIFE SERVICE S



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7/8 Nombre común (género y especie)			cripción parte o derivado, marcas a de identificación (edad/sexo, vin		10. Apéndice y Procedencia	11. Cantidad: número de Ospecimenes y/o Osso nato (kg.)	11a. Total exportado / Cup
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ELEFANTE AS			MUESTRAS BIOLÓGICAS (SANGRE Y SUERO)		IC	18 MUESTRAS	
12. Pais de origen*	Permiso No.	Facha	12a. País de la última reexpo	rtación	No. de certificado	Fecha	12b. No. de la operación **
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Estimado Usuario a efecto de proporcionarie un mejor servicio en la realización de su movimiento transfronterizo autorizado por esta Secretaría, se le sugiere que preferentemente utilice las aduanas siguientes:

Aeropuerto Internacional de la Cludad de México.	Aeropuerto Internacional General Ignacio Pesqueira Garcia,	Cancún, Quintana Roo.				
Cluded Hidaigo, Chispss	Hermosillo, Sonora.	The second s				
Cluded Juárez Chihuphus	Colombia, Huevo León	Ensenada, Baja Celifornia.				
Esteción Sánchez, Nuevo Laredo, Temaulipas	Guadalajara, Tiajomulco de Zúñiga, Jalisco.	Lázaro Cárdenes, Michoacán.				
Manzanillo, Colima.	Metamoros, Ternaulipas.	Nogales, Sonora.				
Nuevo Laredo, Tamaulipas.	Progreso, Yucatán.	Puerto Morelos, Benito Juárez, Quintana Roo.				
Sileo, Guanajuato.	Tijuena, Baja California.	Veracruz, Veracruz				
CONDICIONES						

Una vez realizado el movimiento transfronterizo, deberá dar aviso mediante el formato publicado en el Diario Oficial de la Federación (15-Abril-2011) y al trámite con clave No. SEMARNAT-08-053 "Aviso una vez realizada la Importación, Exportación o Reexportación sujetas a permiso o Certificado CITES" y entregar a la Secretaría en un plazo no mayor a diez días hábiles, la siguiente documentación:

A.- Importación.

- Original del permiso o certificado CITES de exportación o reexportación, debidamente verificado por las autoridades del país de procedencia.
- Copia simple del permiso CITES de importación mexicano, en el que conste la verificación efectuada por la PROFEPA.
- Copia simple del documento donde conste la verificación sanitaria realizada por las autoridades competentes, en su caso.

B.- Exportación o Reexportación.

 Copia simple del permiso o certificado CITES de exportación o reexportación mexicano, en el que conste la verificación efectuada por la PROFEPA.

Este permiso o certificado no lo exime del cumplimiento de otras disposiciones técnico – administrativas y de sanidad exigidas por otras autoridades competentes en la materia, sean Federales, Estatales y Municipales.



Date: May 2nd, 2022

- Consignor: Africam SA de CV Carretera al Oasis 17302, int. 22 Colonia Africam, Puebla, Puebla 72960, Mexico Contact: Carolina Holguín Cellphone: +52 2225358177
- Consignee: International Elephant Foundation, c/o Fort Worth Zoo. 1989 Colonial Parkway, Fort Worth, TX 76110, United States

Consignment: Samples listed below are from <u>Asian Elephants (Elephas maximus)</u> and African Elephants (Loxodonta Africana)

Captive held population residing in _Africam Safari Park, Mexico

The samples are for scientific purposes only and have no commercial value.

18 (eighteen) Number of blood and serum samples from Asian Elephants (Elephas maximus)

62 (Sixty two) Number of blood, serum and tissue (hair) samples from African Elephants (Loxodonta africana)

Purpose: For scientific research purposes only.

Terms: These samples are a gift from Mexico; no monies are involved.

Value for Customs Purposes is US \$___50.00 (Fifty American Dollars)

Ing. Carolina Holguin Africam Safari, México

Km. 16.5 Blvd. Capitén Carlos Camecho E. C.P. 72960 Pubble, Ménico, Tel. (222) 281 7000 africamsafari.com



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United States Department of the Interior

FISH AND WILDLIFE SERVICE International Affairs 5275 Leesburg Pike, MS: IA Falls Church, VA 22041-3803



Page 3 of 4 21US09806C/9

SPECIAL PERMIT CONDITIONS

International Elephant Foundation

1. <u>Samples from confiscated specimens</u>: Samples from confiscated specimens may only be imported with the required chain-of-custody documentation and authorizations from the relevant government(s) and appropriate law enforcement officials, and provided the conditions of paragraphs 9 and 10 below are met.

2. <u>Samples collected from salvage</u>¹: Samples collected from salvaged dead wild animals may be imported provided (a) the collection of the samples occurs in a manner that does not disrupt other animals' movements or behavior during a critical phase of their activities or life cycle, and (b) provided the conditions of paragraph 9 and 10 below are met. (¹ Salvage is defined as the collection of samples from dead animals that were not killed intentionally and that died of causes unrelated either to the collection of samples or the capture of the animal for the purpose of obtaining samples. Samples collected from animals that accidentally died as the results of capture for reasons other than the collection of samples (i.e., capture for translocation or radio collaring) would be considered salvage.)

3. <u>Samples collected from wild-caught animals</u>: Samples collected from wild-caught animals may be imported provided the conditions of paragraphs 5 through 10 below are met.

4. <u>Samples collected from captive-held animals</u>: Samples collected from captive-held animals may be imported provided the conditions of paragraphs 7 through 10 are met.

5. Animals may only be captured in cooperation with local wildlife authorities (e.g., samples may be collected in parks/nature reserves only with the knowledge and consent of the park/reserve manager). No samples may be obtained from animals that are permanently removed from the wild for the sole purpose of collecting samples, or for other than scientific or management purposes approved and carried out by appropriate wildlife authorities.

6. No remuneration, either financial or in-kind, may be offered for the taking of animals from the wild (i.e., killing, trapping) or for the collection of samples from free-ranging wildlife. This condition does not preclude legitimate collection and transportation expenses (e.g., hiring staff, freight costs), but does prohibit the paying of bounties or incentive pay for taking of animals from the wild, or the collection of samples from animals in the wild. Explicit written approval for collection from wild animals must be obtained from appropriate wildlife authorities and retained in the applicant's files for a period of 5 years and must be supplied upon the Division of Scientific Authority's request.



U.S. Management Authority

05/18/2021 Date

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Loxodonta Africana MX115933 SPE 67ml blood/22vials NA African elephant 21US09806C/9 W 0\$USD NA Loxodonta Africana MX115933 SPE 21ml blood/9 vials NA African elephant 21US09806C/9 C 7ml serum/4vials MX 21US09806C/9 C 7ml serum/4vials MX		-		SPE	from 3 E.m	MX	
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All Carl elephant 21US09806C/9 from 5 L.a			MX115933			MX	
		African elephant	21US09806C/9				
					0\$USD		
	25223					-	
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av subject the declarant to the penalty provided by 18 U.S.C. 1001 and 16 U.S.C. 3372(d) Is true and correct				ns.	Signature	2/22/22	_
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Dirección General de Salud Animal Dirección de Importaciones y Exportaciones

Nº de Oficio B00.02.03.02.02.-0090-2022

Ciudad de México a 03 MAY 2022

ING. CAROLINA HOLGUÍN AFRICAM, S.A. DE C.V. PRESENTE

Hago referencia al escrito a través del cual solicita conocer los requisitos sanitarios para la exportación de muestras biológicas de elefantes africanos (*Loxodonta africana*) y elefantes asiáticos (*Elephas maximus*) con fines de investigación a los Estados Unidos de América, como parte del proyecto denominado "*Developing Genomic Tools for Elephant Health and Conservation*".

Al respecto, le comunico que derivado del análisis de su protocolo de investigación, así como de la documentación proporcionada, se determinó que no es requerido un Certificado Zoosanitario de Exportación (CZE) emitido por esta Secretaría para tal fin, por lo que no existe inconveniente para el envío de las referidas muestras, cabe señalar que, lo anterior no lo exime de dar cumplimiento a otras regulaciones y/o permisos requeridos por otras autoridades.

Sin otro particular, reciba un cordial saludo.

Atentamente El Director de Importaciones y Exportaciones AGRICULTURA P SENASIC DIRECCIÓN GENERAL DE SALUD ANIMA 0 3 MAY 2022 MVZ/Fernando Rivera Espinoza C.c.C. GAY OUTIERPEZ - DIRECTOP GENERAL DE SALUD ANIMAL - Presente FRE /) SIGE DIE 0528-2022 annu protes Sur No. 409, P-10, Col. Hipódromo, Cuauhtemoc, CP. 06100, CDMX 1el 55 5895 f600 Ext, 51957 / 51248 gestiondie.dgsa@senasica.gob.mx www.gob.mx/scriasica

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COUNTRY OF ORIGIN	SIMIS	EDTA	#tubes	Tube 1	Tube 2	Tube3	Tube 4	Total volume	LABELS	TOTAL of UNITS
NAMIBIA	8147	13X100mm capacity 6.0ml	4	3ml	3.5 ml	3.5ml	3.5 ml	13.5ml	EDTA587/001, EDTA587/002, EDTA587/003, EDTA587/004	
NAMIBIA	8148	13X100mm capacity 6.0ml	m	3ml	3ml	2.5ml	N/A	8.5ml	EDTA588/001, EDTA588/002, EDTA588/003	
NAMIBIA	8149	13X100mm capacity 6.0ml	e	4ml	4ml	3.5ml	N/A	11.5ml	EDTA589/001, EDTA589/002, EDTA589/003	
NAMIBIA	8150	13X100mm capacity 6.0ml	2	3ml	3ml	N/A	N/A	6ml	EDTA590/001, EDTA590/002	, ,
NAMIBIA	8151	13X100mm capacity 6.0ml	2	2.5ml	3ml	N/A	N/A	5.5ml	EDTA591/001, EDTA591/002	77
NAMIBIA	8152	13X100mm capacity 6.0ml	2	2.5ml	2.5ml	N/A	N/A	Sml	EDTA592/001, EDTA592/002	•
NAMIBIA	8153	13X100mm capacity 6.0ml	2	3ml	3ml .	N/A	N/A	6ml	EDTA593/001, EDTA593/002	
NAMIBIA	8154	13X100mm capacity 6.0ml	2	3.5ml	2.5ml	N/A	N/A	4.5ml	EDTA594/001, EDTA594/002	
NAMIBIA	8155	13X100mm capacity 6.0ml	2	3ml	3ml	N/A	N/A	6ml	EDTA595/001, EDTA595/002	
MEXICO	10090	13X100mm capacity 6.0ml	2	3ml	2ml	N/A	N/A	Sml	EDTA627/001, EDTA627/002	
MEXICO	10162	13X100mm capacity 6.0ml	2	4ml	2.5ml	N/A	N/A	4.5	EDTA628/001, EDTA628/002	
MEXICO	10513	13X100mm capacity 6.0ml	2	2.5ml	2.5ml	N/A	N/A	5ml	EDTA10513/001, EDTA10513/002	6
MEXICO	11162	13X100mm capacity 6.0ml	2	2.5ml	1.5ml	N/A	N/A	4ml	EDTA11162/001, EDTA11162/002	
MEXICO	11472	13X100mm capacity 3.0ml	1	2ml	N/A	N/A	N/A	2ml	EDTA11472/001 (Umbilical cord)	

SERUM

AFRICAN ELEPHANTS (Loxodonta africana)

COUNTRY OF ORIGIN	ZIMS	SERUM	#tubes	Quantity	Labels	TOTAL of UNITS
NAMIBIA	8147	12x40 mm capacity 2ml	1	2ml each tube	Labels SS587/001	
NAMIBIA	8148	12x40 mm capacity 2ml	1	2ml each tube	Labels SS588/001	
NAMIBIA	8149	12x40 mm capacity 2ml	1	2ml each tube	Labels \$\$589/001	
NAMIBIA	8150	12x40 mm capacity 2ml	1	2ml each tube	Labels \$\$590/001	
NAMIBIA	8151	12x40 mm capacity 2ml	1	2ml each tube	Labels SS591/001	9
NAMIBIA	8152	12x40 mm capacity 2ml	1	2ml each tube	Labels \$\$592/001	
NAMIBIA	8153	12x40 mm capacity 2ml	1	2ml each tube	Labels SS593/001	
NAMIBIA	8154	12x40 mm capacity 2ml	1	2ml each tube	Labels SS594/001	
NAMIBIA	8155	12x40 mm capacity 2ml	1	2ml each tube	Labels SS595/001	
MEXICO	10090	12x40 mm capacity 2ml	1	2ml each tube	Labels SS627/001	
MEXICO	10162	12x40 mm capacity 2ml	1	2ml each tube	Labels SS628/001	
MEXICO	10513	12x40 mm capacity 2ml	1	2ml each tube	Labels \$\$10513/001	4
MEXICO	11162	12x40 mm capacity 2ml	1	0.5ml each tube	Labels SS11162/001	

ASIAN ELEPHANTS (Elephas maximus)

COUNTRY OF ORIGIN	ZIMS	SERUM	#tubes	Quantity	Labels	TOTAL OF UNITS
MEXICO	6567	12x40 mm capacity 2ml	3	2ml each tube	Labels SS652/001, Labels SS652/002, Labels SS652/003	
MEXICO	5971	12x40 mm capacity 2ml	2	2ml each tube	Labels SS651/001, Labels SS651/002	7
MEXICO	7055	12x40 mm capacity 2ml	2	2ml each tube	Labels SS729/001, Labels SS729/002	

HAIR

COUNTRY OF ORIGIN	ZIMS	# OF PIECES OF HAIR	# ENVELOPS	DIMENSIONS	LABELS	TOTAL OF ENVELOPES	TOTAL PEICES OF HAIR
NAMIBIA	8147	25	1	9x 16.5 cm	587HAIR		225
NAMIBIA	8148	25	1	9x 16.5 cm	588HAIR		
NAMIBIA	8149	25 .	1	9x 16.5 cm	589HAIR		
NAMIBIA	8150	25	1	9x 16.5 cm	590HAIR		
NAMIBIA	8151	25	1	9x 16.5 cm	591HAIR	9	
NAMIBIA	8152	25	1	9x 16.5 cm	592HAIR		
NAMIBIA	8153	25	1	9x 16.5 cm	593HAIR		
ΝΑΜΙΒΙΑ	8154	25	1	9x 16.5 cm	594HAIR		
NAMIBIA	8155	25	1	9x 16.5 cm	595HAIR		
MEXICO	10090	25	1	9x 16.5 cm	627HAIR		225
MEXICO	10162	25	1	9x 16.5 cm	628HAIR		
MEXICO	10364	25	1	9x 16.5 cm	10364HAIR		
MEXICO	10513	25	1	9x 16.5 cm	10513HAIR		
MEXICO	11162	25	1	9x 16.5 cm	11162HAIR	9	
MEXICO	11295	25	1	9x 16.5 cm	11295HAIR		
MEXICO	11296	25	1	9x 16.5 cm	11296HAIR		
MEXICO	11355	25	1	9x 16.5 cm	11355HAIR		
MEXICO	11472	25	1	9x 16.5 cm	11472HAIR		

AFRICAN ELEPHANTS (Loxodonta africana)

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United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

National Center for Import and Export

Animal Products

4700 River Road Unit 40 Riverdale, MD 20737

Telephone: (301) 851-3300

FAX: (301) 734-8226 Virginia R. Pearson / Fox Chase Cancer Center Rall Laboratory 333 Cottman Avenue Philadelphia, PA 19111

Tuesday, February 28, 2017

Dear Virginia R. Pearson:

Our office has reviewed your request to renew your Veterinary Services permit 111798 to import Pathological and diagnostic samples from wild and captive African and Asian elephants, including liquid or dried DNA samples extracted from blood, serum, saliva, trunk washings, dung, biopsies, and necropsy tissue. Your request has been assigned application reference number 17027083.

Our office has recently reviewed our requirements for the importation of material derived from elephants, and determined that there is minimal risk of introduction of foot-and-mouth disease (FMD) virus from these materials. As such, an import permit is not required. This change will bring us in line with our requirements for live animals, which currently allow for elephants to be imported with no restrictions related to FMD. Any materials collected from animals infected with or exposed to livestock or poultry pathogens are still subject to permitting by the Organisms and Vectors staff.

Please note that you will be required to show documentation to U.S. Customs and Border Protection CBP) Agricultural Specialists indicating the species from which the material is derived. Additionally, this change does not eliminate the need for permits from other agencies which may have jurisdiction over the material you wish to import, such as the U.S. Department of Interior, Fish and Wildlife Service (FWS).

Please visit the FWS website at www.FWS.gov/permits and/or www.FWS.gov/le/travelers.html to determine if they have additional requirements for this material.

If you have further questions, please contact us at AskNIES.products@aphis.usda.gov or (301) 851-3300, Option 1, or, for questions about infected materials, please contact Organisms and Vectors staff at OV@aphis.usda.gov or (301) 851-3300, Option 3.

Sincerely,



Safeguarding Animal Health

APHIS is an agency of USDA's Marketing and Regulatory Programs An Equal Opportunity Provider and Employer Federal Relay Service (Voice/TTY/ASCII/Spanish) 1-800-877-8339



International in the phant Foundation P.O. Box 366. Azle. Texus 76098 USA IEF Delephantconservation.org: 817-597-0956

Federal Tax Exempt 1D 75-2815706

Dedicated to saving elephants by providing funds and scientific expertise to support elephant conservation programs worldwide

8 June 2022

USFWS/Division of Management Authority Branch of Permits, MS: IA 5275 Leesburg Pike Falls Church, VA 22041-3803

JUN 14 PH12:19

Dear Management Authority:

It is with the most sincere appreciation that I attach the annual report for permit number 21US09806C/9 for the importation of biological samples from African elephant (*Loxodonta africana*), African Forest Elephant (*Loxodonta cyclotis*) and Asian elephant (*Elephas maximus*) provided to the International Elephant Foundation.

Enclosed are the:

- Permit application form 3-200-52 renewal of the permit for 2022-2023
- Original import permit 21US09806C/9
- Annual report for Permit 21US09806C/9
- documentation of permit use
- \$100 application fee

Thank you for issuing the permit and thereby assisting our research and conservation efforts. Our ability to contribute to valuable scientific investigations that enhance the long-term survival of elephants is dependent upon our ability to receive biological samples into United States laboratories from around the world. We are extremely grateful to USFWS and the exceptional agents that assist us in reviewing documents prior to and through the shipping and clearance process for making this possible.

Please do not hesitate to contact me should you require any additional information.

Sincerely,

Deborah Olson Executive Director



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



□New ■Reissue/Renew □Amendment

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

A. Complete if applying as an individual 1.a. Last name		1.b. First name	1.b. First name		1.d. Suffix
2 Date of birth (mm/dd/yyyy)	5.a. Telephone number	5.b. Alternate telephone number	6. E-mail address		- <u></u>

B. Complete if applying on behalf	f of a busine	ss, corporation, public a	gency, Tribe, or institution	on	
1.a. Name of business, agency, Tribe, International Elephan		1.b. Doing business as (dba)			
2. Tax identification no.3.a. Description of business, agency, T75-2815706conservation of all ele					3.b. Website URL (if applicable) www.elephantconservation.org
4.a. Principal officer (P.O.) last name 4.b. P.O. first Debora			4.c. P.O. middle initial J		4.b. P.O. Title Executive Director
5. Primary contact name Deborah Olson			6. Primary e-mail address dolson@elephantconservation.org		
7.a. Business telephone number 817-597-0956				8.a. Primary contact telephone no. 817-597-0956	

C. All applicants complete address information								
1.a. Physical address (Street address; Apartment #, Suite #, or Room #; no P.O. Boxes)								
c/o Fort Worth Zoo, 1989 Colonial Parkway								
1.b. City	1.b. City 1.c. State 1.d. Zip code/Postal code 1.e. County/Province 1.f. Country							
Fort Worth	Fort Worth TX 76110 Tarrant USA							
2.a. Mailing Address (include if different than physical address; include name of contact person if applicable) P.O. Box 366								
2.b. City 2.c. State 2.d. Zip code/Postal code 2.e. County/Province 2.f. Country								
Azle	ТХ	76098	Tarrant	USA				

D.	All	applicants MUST complete
	1.	Include a check or money order, payable to the U.S. FISH AND WILDLIFE SERVICE, a nonrefundable processing fee [50 CFR 13.11(d)(4)]. Federal, Tribal, State, and local government agencies, and those acting on behalf of such agencies, are exempt from the processing fee – attach documentation of fee exempt status as outlined in instructions. (50 CFR 13.11(d))
	2.	If you are requesting a reissue/renew/amendment, what is your permit/file number? 21US09806C/9
	3.	Certification: I hereby certify that I have read and am familiar with the regulations contained in Title 50, Part 13 of the Code of Federal Regulations and the other applicable parts in subchapter B of Chapter I of Title 50, and I certify that the information submitted in this application for a permit is complete and accurate to the best of my knowledge and belief. I understand that any false statement herein may subject me to the criminal penalties of 18 U.S.C. 1001.
	(1000

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

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

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

010005

Date (mm/dd/yyyy)

11

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

General Information

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

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

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

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

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

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

Questions

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

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

This form should NOT be used for:

- Captive Bred Wildlife Registration (use application <u>3-200-41</u>)
- ESA Plants (use application <u>3-200-36</u>)

Electronic Information Submission

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

All Applicants Must Complete

- 1. Name and address where you wish the permit to be mailed, **if different from physical address**. If you would like expedited shipping, please enclose a self-addressed, pre-paid, computer-generated, courier service airway bill. If unspecified, all documents will be mailed via regular mail through the U.S. Postal Service.
- 2. Point of contact if we have questions about the application (name, phone number, and email).
- 3. Have you or any of the owners of the business (if applying as a business, corporation, or institution), been assessed a civil penalty or convicted of any criminal provision of any statute or regulation relating to the activity for which the application is filed; been convicted, or entered a plea of guilty or nolo contendere, for a felony violation of the Lacey Act, the Migratory Bird Treaty Act, or the Bald and Golden Eagle Protection Act; forfeited collateral; OR are currently under charges for any violation of the laws mentioned above?

```
__No __Yes
```

If you answered "Yes" to Question 3, provide: a) the individual's name; b) date of charge; c) charge(s); d) location of incident; e) court, and f) action taken for each violation. Please be aware that a "Yes" response does not automatically disqualify you from getting a permit.

Proposed Activity

□ Import

□ Export

- □ Re-export (e.g. export of a specimen that was previously imported into the United States)
- 4. The **current** location of the samples (if different from the physical address provided):

Name: Address: City:

State/Province:

Postal Code:

Country:

5. Recipient/Sender:

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

Name:

Address:

City:

State/Province:

Postal Code:

Country:

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

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

Source of Specimen

- 7. For each biological sample taken from a captive-born/captive hatched animal(s), provide a signed and dated statement from the breeder or appropriate documentation (e.g. Species 360 report) that includes the following:
 - a. Scientific name (genus, species, and *if applicable*, subspecies),
 - b. Common name,
 - c. Name and address of the facility where the animal was bred and born;
 - d. Birth/hatch date (mm/dd/yyyy),
 - e. Identification information (studbook #, microchip, leg band, etc.),
 - f. Name and address of facility where the parental stock is located; and
 - g. A statement from the breeder that the animal was bred and born at the breeder's facility (including the facility's name and address), and
 - h. If not the breeder, documentation demonstrating the history of transactions (e.g., chain of custody or ownership of the sample(s), *if applicable*).

See next page

To Whom it may concern:

Asian elephant Luna studbook #780 was bred & born at African Lion Safari, 1386 Cooper Road, Cambridge, Ontario, N1R 5S2 Canada on August 17, 2018.

Asian elephant Onyx studbook #781 was bred & born at African Lion Safari, 1386 Cooper Road, Cambridge, Ontario, N1R 5S2 Canada on August 18, 2018.

Asian elephant Sunita studbook #782 was bred & born at African Lion Safari, 1386 Cooper Road, Cambridge, Ontario, N1R 5S2 Canada on November 14, 2018.

All parental stock located at:

African Lion Safari

1386 Cooper Road

Cambridge, Ontario

N1R 5S2

Canada

vortie Dray

Charlie Gray Superintendent of elephants African Lion Safari

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

- a. Scientific name (genus, species, and *if applicable*, subspecies),
- b. Common name,
- c. Specific location (e.g., county, state, province, country) where the samples were taken from the wild,
- d. The name of the individual(s) who collected the animal/samples and their authorization to do so including (but not limited to) copies of foreign and domestic (Federal, State, and/or Tribal) government collecting permits, licenses, contracts, and/or agreements.
- e. Method of collection: sampling protocol, approximate length of time held in captivity, any injury and/or mortality experienced during collection, transport, or holding;
- f. Information related to any remuneration, either financial or in-kind, provided for acquiring the sample(s);
- g. Efforts to use captive specimens (e.g., captive-born, captive-held) in lieu of taking samples from wild animals.

- 9. For **each biological sample being re-exported** (e.g., exporting a specimen that was previously imported into the United States), provide:
 - a. A copy of the **canceled** CITES export or re-export document issued by the appropriate CITES office in the country from which the wildlife was imported;
 - b. A copy of your Declaration for Importation or Exportation of Fish or Wildlife (Form 3-177), **cleared** by USFWS Office of Law Enforcement.
 - c. A copy of the ESA permit that authorized the original import.
 - d. If you did not make the original import, please provide documentation outlining chainof-ownership since import, including:
 - i. A copy of the importer's CITES, ESA, and declaration documents (a, b, & c above) and,
 - ii. Subsequent invoices (or other documentation) showing the history of transactions leading to your ownership of the sample(s) after import (provenance).

Not Applicable

Description and Justification For Requested Activity

10. Describe the purpose of the scientific research and include:

- a. A copy of the research proposal (outlining the purpose, objectives, methods).
- b. How long the research has been (or will be) conducted,
- c. Detailed information on sampling methods including:
 - i. who will be taking the samples
 - ii. equipment and methods used
 - iii. measures taken to prevent injuries and mortalities during collection

All above attached next pages

d. A copy of the study's Institutional Animal Care and Use Committee (IACUC) form (if applicable),

Not applicable

e. Peer-reviewed scientific papers published from this research

Not applicable

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

This is new research

PROJECT ABSTRACT

Evaluation of plasma D-dimer concentration in juvenile Asian (*Elephas maximus*) and African elephants (*Loxodonta africana*) with and without elephant endotheliotropic herpesvirus hemorrhagic disease.

Background: Elephant endotheliotropic herpesvirus hemorrhagic disease (EEHV-HD) is the leading cause of mortality in juvenile Asian elephants (*Elephas maximus*) and is increasingly reported as a cause of morbidity and mortality in juvenile African elephants (*Loxodonta africana*). Disseminated intravascular coagulation (DIC) has recently been evidenced in cases of EEHV-HD. DIC is the abnormal and excessive activation of hemostasis. It can be divided in two distinct phases: non-overt

(compensated) DIC and overt (decompensated) DIC. Since overt DIC is, by definition, the phase where clinical signs are visible, EEHV-HD likely corresponds to overt DIC. Identification of parameters allowing for diagnosis of non-overt EEHV-HD prior to the onset of clinical signs is crucial. D-dimer concentration has been shown to increase in cases of DIC in various species. While we recognize that no single laboratory test is specific and sensitive enough to make a definitive diagnosis of non-overt and overt DIC, D-dimer concentration is shown to be higher in overt DIC compared to non-overt DIC, and higher in non-overt DIC compared to non-OVERT DIC patients. Determination of D-dimer concentration may be helpful for the diagnosis of non-overt EEHV-HD.

Objectives and Hypotheses: The goal of this project is to evaluate plasma D-dimer concentration in healthy juvenile Asian and African elephants, elephants with EEHV viremia, and elephants with EEHV-HD.

- <u>Null hypothesis 1:</u> D-dimer concentration will not be statistically different between healthy juvenile Asian elephants, viremic juvenile Asian elephants, and juvenile Asian elephants with EEHV-HD.
- <u>Null hypothesis 2</u>: D-dimer concentration will not be statistically different between healthy juvenile African elephants, viremic juvenile African elephants, and juvenile African elephants with EEHV-HD.

Animals and Procedures: Our study population consists of all Asian elephants and all African elephants under the age of 15, housed in USA, Canada, Europe, Asia and Africa. For each species, elephants will be enrolled in one of three groups: control group (EEHV negative, healthy elephants), group 1 (EEHV positive, healthy elephants) and group 2 (EEHV-HD elephants). Routine venipuncture will be performed under behavioral restraint. Animals in the control group will be sampled once, while animals in group 1 and 2 will be sampled daily or at the clinician's discretion until resolution of EEHV viremia or EEHV-HD. Each blood sample will be submitted for plasma D-dimer concentration assay, a partial blood count including a platelet count, and EEHV qPCR. The distribution of D-dimer values will be compared between groups. Prevalence of EEHV viremia and EEHV-HD will be calculated for the year 2022.

Expected Relevance: Identification of parameters allowing for diagnosis of EEHV-HD prior to the onset of clinical signs is crucial. The data generated from this research has the potential to help zoo and wildlife veterinarians with the early diagnosis of non-overt EEHV-HD.

PROJECT NARRATIVE

Rationale

EEHV-HD pathophysiology

A recent investigation on the pathogenesis of EEHV-HD has shown that elephants with the disease have disrupted endothelia, resulting in increased platelet endothelial cell adhesion molecule 1 (PECAM-1) and von Willebrand factor (vWF) expression in certain visceral organs compared to EEHV-negative controls.⁸ Expression of these glycoproteins varied with EEHV genotype and organ evaluated.⁸ Activation of vWF results in platelet aggregation at the site of endothelial injury, and ultimately formation of a platelet plug.⁸ Excessive endothelial damage and platelet plug formation, evidenced by increased PECAM-1 and vWF expression, can lead to thrombocytopenia and thrombosis.⁸ Interestingly, thromboemboli in blood vessels and extravascular space of the lungs, heart, kidneys and intestine is also seen in EEHV-HD.⁸ Similarly, a retrospective study including 31 Asian elephant with EEHV-HD-related fatalities (based on positive EEHV-PCR and compatible macroscopic lesions) revealed the presence of microthrombi in 63% of cases.⁹ This, along with thrombocytopenia and bleeding, is in alignment with the definition of disseminated intravascular coagulation (DIC).^{8,9}

Disseminated Intravascular coagulation (DIC)

Hemostasis can be divided in three distinct processes, which are all taking place at the same time: primary hemostasis (platelet clot), secondary hemostasis (crosslinked fibrin or fibrin clot), and fibrinolysis (dissolution of the fibrin clot). DIC is the excessive and abnormal activation of hemostasis, with subsequent generation of excess thrombin and formation of microvascular thrombi.¹⁰ In non-overt DIC, thrombin generation is contained by inhibitors, and the patient is considered at risk of thrombosis, or may be already experiencing subclinical thrombosis. After some time, inhibitors become overwhelmed and thrombin generation resumes uncontrollably, leading to overt DIC and widespread thrombosis. Eventually, platelets and clotting factors are depleted, fibrinolysis dominates, the patient enters a hypocoagulable state and bleeding occurs.

Diagnosis of DIC can be challenging and is based on clinical signs and laboratory tests. Like the name suggests, clinical signs seen in non-overt DIC are usually absent or depend on the underlying condition causing DIC, and are therefore non-specific. Laboratory tests such as aPTT and PT are insensitive at this stage, and the only tests readily available to veterinarians are D-dimer assays and viscoelastographic assays. The latter, however, is affected by a myriad of variables such as sample collection, method of assay activation, and anemia.¹¹ Thus, diagnostic utility of viscoelastic assays is limited. In addition to signs created by the underlying cause of DIC and microvascular thrombosis, the presenting clinical sign of overt DIC is typically hemorrhage (epistaxis, petechiae, bruising, etc.). Abnormal laboratory findings at this stage include thrombocytopenia, prolonged aPTT, PT, TCT, hypofibrinogenemia, and increased fibrinogen degradation products (FDPs) and D-dimers. Thromboelastographic (TEG) abnormalities in cases of overt DIC are complex, since hypercoagulable tracings could be due to anemia and hyperfibrinogenemia, and hypocoagulable tracings may reflect any combination of thrombocytopenia, coagulation factor deficiencies, and hypofibrinogenemia.¹²

Coagulation testing in elephants

There exists one report commenting on the use of TEG during the clinical management of EEHV-HD in a juvenile Asian elephant, which revealed that the calf was initially in a hypocoagulable state, with some

improvement after aggressive medical management.¹³ Since then, efforts have been made to perform TEG in healthy Asian elephants and establish reference intervals, which are now available for veterinarians when managing EEHV-HD cases.^{14,15} However, hypocoagulable tracings are expected in thrombocytopenic animals, a hallmark of EEHV-HD.^{6,8,11,12,16,17} TEG may not give additional information about overall hemostatic state in such animals, making it difficult to justify its use for the clinical management of EEHV-HD when thrombocytopenia or anemia are present. In addition to TEG, other coagulation tests have been investigated in healthy Asian elephants, with reference intervals available for PT, aPTT and fibrinogen.¹⁸ Fibrinolysis, on the other hand, remains fairly unexplored in elephants. The potential role of hyperfibrinolysis in EEHV-HD has not been evaluated.

D-dimers

D-dimer is the plasmin-mediated degradation product of crosslinked fibrin, and an increase in circulating levels of D-dimer can develop in association with thrombosis and subsequent fibrinolysis. D-dimer concentration is a sensitive diagnostic test for DIC in dogs (85-100%), and is one of the few widely available laboratory test for non-overt DIC in veterinary medicine.^{19,20} In the face of DIC, D-dimer may increase before changes develop in other coagulation assays such as PT and aPTT.²⁰ In a study investigating D-dimer concentrations in domestic dogs, median D-dimer concentrations were significantly higher in dogs with DIC compared with healthy dogs.¹⁹ Another study found that various Ddimer assays could be used to differentiate between healthy dogs and dogs with DIC, acute thromboembolic disease or hemorrhage.²¹ This study highlights the fact that D-dimer, albeit sensitive, is a non-specific test for DIC. Increases in D-dimer can be seen in other disease processes such as neoplasia, inflammatory disease, and thrombotic disorders. However, considering that EEHV-HD is the leading cause of death in juvenile Asian elephants in Europe and North America, that thromboembolic disorders other than EEHV-HD are uncommonly reported in juvenile elephants, and that DIC is now known to occur with EEHV-HD, an elevation of D-dimer concentration in a juvenile elephant could be an indicator for non-overt EEHV-HD. Since early detection and intervention is the mainstay of successful clinical management of EEHV-HD, evaluation of plasma D-dimer concentration in elephants at risk of EEHV-HD is clinically warranted and urgent.

The authors have conducted a pilot study evaluating the plasma D-dimer concentration in healthy adult Asian and African elephants. While the data is still under evaluation and a larger sample size would be required to establish reference ranges, our study shows that D-dimer analysis is an accessible and affordable way to better characterize hemostasis in elephants.

Study population

Our study population is defined as all Asian and African elephants housed in USA, Canada, Europe, Asia and Africa.

- Inclusion criteria: Elephants must be between 1 and 15 years of age. There are currently 34 Asian elephants and 35 African elephants in the American and Canadian SSP population alone corresponding to this criterion.
- *Exclusion criteria*: Individuals outside of the provided age brackets. Any individual with an unknown EEHV status, unknown platelet count, and unknown health status will be excluded from the study. Animals with diseases known to potentially increase D-dimer concentration, such as thrombotic, neoplastic, and infectious diseases, will be excluded from the study.

EEHV-HD has been seen in both captive and free-ranging juvenile elephants. Although the study population of the project is strictly captive elephants, results from our study could be used to manage free-ranging or semi-free-ranging elephant calves with EEHV viremia or EEHV-HD.

Project goals

<u>Null hypothesis 1:</u> D-dimer concentration will not be statistically different between healthy juvenile Asian elephants, viremic juvenile Asian elephants, and juvenile Asian elephants with EEHV-HD.

<u>Null hypothesis 2:</u> D-dimer concentration will not be statistically different between healthy juvenile African elephants, viremic juvenile African elephants, and juvenile African elephants with EEHV-HD.

Objectives

Early diagnosis and treatment are key to the successful management of EEHV-HD. Plasmatic D-dimer concentration may help in the diagnosis of EEHV-HD before the onset of clinical signs, enabling the zoo and wildlife clinician to start therapy before irreversible damage has been done to internal organs. Early and aggressive treatment for EEHV-HD have been associated with successful outcomes. Therefore, evaluating plasmatic D-dimer concentration in juvenile elephants could lead to early diagnosis of this devastating disease, early onset of aggressive therapy and potentially better outcomes/survival of captive and free-ranging elephant calves.

By simultaneously measuring plasmatic D-dimer concentration, platelet count and EEHV viremia in individual elephant calves, we would be able to determine association between these variables and disease progression.

Project design, methodology & work plan

Study design

Prospective experimental study. For each species, elephants will be enrolled in one of the following groups:

- Control group: EEHV negative, normal platelet count, clinically healthy juvenile elephant
- *Group 1 (non-overt DIC or EEHV viremic):* EEHV positive, normal platelet count, clinically healthy juvenile elephant
- *Group 2 (overt DIC or EEHV-HD):* EEHV positive, thrombocytopenic, juvenile elephant with clinical signs of hemorrhagic disease.
 - Clinical signs of EEHV-HD: lethargy, lameness, changes in mentation, hyperemic oral mucosa, tongue cyanosis, edema of the head, neck and trunk, and decreased appetite.

Although easier to implement, a retrospective study design or case-control study was not considered for the purpose of our investigation because citrated plasma, required for D-dimer analysis, is not routinely banked and samples would not be available for analysis. With a prospective experimental study design taking place over several years, we will be able to enroll enough subjects in groups 1 and 2 for statistical analysis. We will also be able to establish reference ranges for plasmatic D-dimer concentration if enough elephants are enrolled in the control group.

Study enrollment

Elephant managers and veterinarians holding juvenile Asian and African elephants will be asked to enroll their elephant calves in our study. They will be provided a summary of our research proposal.

Sample collection and analysis

As per their respective institution's preventive medicine programs, routine venipuncture will be performed on elephants under behavioral restraint (biomedical training). Trained zoo veterinary or husbandry staff will be performing the blood draws. Animals in the control group will be sampled once. Animals in group 1 and 2 will be sampled daily or at the clinician's discretion until resolution of EEHV viremia or EEHV-HD. Each blood sample will be submitted for the following analysis:

- D-dimer concentration assay (immunoturbidimetric method): 1ml of citrated plasma will be sent to the Comparative Coagulation Lab at Cornell University. Samples can be frozen at -80°C and sent for batch analysis at the clinician's discretion.
- Partial blood count: 1ml of whole blood (EDTA) and 2 dry unfixed unstained blood smears will be shipped to the Comparative Coagulation Lab at Cornell University. Analyte will include white blood cell count, red blood cell count, hemoglobin, platelet count, PCV, MPV, RDW, MCH, MCHC and MCV. Alternatively, in-house CBC can be performed at the clinician's discretion (i.e. if Ddimer samples are to be stored and batched).
- EEHV qPCR: 1-2ml of whole blood (EDTA) and 0.5-1ml of serum will be shipped to the National Elephant Herpesvirus Lab at the Smithsonian's National Zoo. Quantitative viral DNA for all appropriate genotypes (EEHV 1, 4 and 5 for Asian elephants and EEHV 2, 3-4 and 6 for African elephants) will be assessed.

Data recording and analysis will be performed by Dr. Bercier.

Statistical analysis

The prevalence of EEHV viremia and EEHV-HD in North America is unknown. We use an estimate of 20% for the prevalence of EEHV viremia (a similar value is used for the two species of elephants). With an estimated number of subjects of 50, we will be able to estimate the prevalence of EEHV viremia with a margin of error of 11% and a level of certainty of 95%. We expect to enroll at least 10 subjects in groups 1 and 2 after combining the two species. If we get enough subjects in group 2, we can use the Kruskal-Wallis test to compare the distribution of D-dimer values among the three groups. Pearson's correlation could be used to examine the association between platelet count and D-dimer, and between EEHV viral load and D-dimer. A paired t-test could be used to compare changes through time in calves initially healthy that later become viremic. If the number of viremic and/or EEHV-HD cases turns out to be too small to allow for a statistical comparison, descriptive statistics will be provided for healthy juvenile elephants and comparisons/comments will be made for viremic and/or EEHV-HD elephants. Pooling of all EEHV positive subjects (group 1 and 2) and across species might also be an option.

Monitoring & evaluation procedures

Most clinicians routinely monitor at-risk juvenile elephants by performing weekly CBC and EEHV qPCR. If a clinician is faced with a case of EEHV viremia or EEHV-HD, citrated plasma will be banked for batched D-dimer analysis at a later time. Dr Bercier will gather all results and perform statistical analysis after the study has been conducted. No further monitoring will be required at the end of the program. The results from this project will be made available to other zoo veterinarians, particularly those who care for elephants. Wildlife veterinarians could also benefit from our findings, since EEHV-HD is also reported in free-ranging populations of elephants. We have the intention to present our preliminary results at the 55th AAZV Annual Conference (deadline for abstract submission around April 2023). We would like to publish our final results as an original research article in the Journal of Zoo and Wildlife Medicine in the year following study completion.

This study design could be used by other veterinarian and scientists who which to conduct cohort studies or multi-institutional prospective experimental studies.

Sustainability

If judged significant, plasmatic D-dimer concentration could be used as a tool for monitoring the health of at-risk juvenile elephants. Since EEHV-HD cases are sporadic, zoo and wildlife clinicians could continue to collect data routinely and contribute to this research well beyond the grant period. Other blood parameters, such as fibrinogen degradation products, thrombin antithrombin complex, or fibrin monomer complex, could be evaluated using the same study design.

Safeguarding

Once a zoological institution agrees to enroll in the study, the process for institutional animal care and use committee (IACUC) or equivalent animal welfare committee will be engaged. A letter of support from the institution's director will be obtained if the institution does not have an IACUC or welfare committee in place. Sample collection will not be allowed on any elephant before institutional IACUC approval. However, if venipuncture and EEHV viremia monitoring is already part of the institution's preventive health program, samples for this project could be obtained during routine blood draws and IACUC approval, for this specific situation, would not be required. The number of participating institutions has yet to be determined and will depend on research funding and the institution's interest in participating in this research.

TIMELINE

This project was funded in 2022 by the Wild Animal Health Fund and is currently underway. Because EEHV cases are sporadic, data collection and analysis need to be obtained over the course of several years in order to produce reliable and significant results. The IEF grant portion would allow us to continue our research during the years 2023-2024.

- May 1st 2023 July 1st 2023: institution enrollment and IACUC approval.
- <u>July 1st 2023 to October 1st 2024</u>: data collection, abstract submission for AAZV 56th Annual Conference.
- October 1st 2024 to November 1st 2024: statistical analysis, final report to WAHF.
- <u>November 1st 2024 to November 1st 2025</u>: manuscript writing and publication in a peerreviewed journal (JZWM).

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LETTERS OF SUPPORT

See attachments.

QUALIFICATIONS OF PRINCIPAL AND CO-INVESTIGATORS

Dr Bercier has approximately six years of experience working in a zoo/aquarium setting as a veterinarian. She has extensive experience working with African elephants in a clinical setting. Dr Bercier has also spent two years in an academic setting and conducted a pilot study evaluating D-dimer concentration in healthy adult Asian and African elephants during that time period.

Dr Brooks participated in this pilot study as well. Dr Brooks' career has focused on the development and characterization of animal models for hemostatic disorders. She is a recognized expert in the field of comparative hemostasis and her laboratory is a unique referral center for assay development and diagnosis of hemostatic defects and pre-clinical hemostasis testing. She has worked productively in numerous collaborations and in consultation with biomedical and veterinary researchers.

Erin Latimer has been a research specialist for the Department of Wildlife Health Sciences at the Smithsonian's National zoo since 2004. She is the head of the National Elephant Herpesvirus Laboratory and has authored more than 20 peer-reviewed publications relevant to EEHV.

PERMITS/IACUC

No permits are required for institutions in the USA. IACUCs will be acquired prior to sample collection.

11. Please provide a detailed description on how the proposed activities will **enhance or benefit the wild population within its native range** (e.g., direct or indirect **conservation efforts**) and provide documentation (e.g., signed memorandums of understanding) demonstrating your commitment to supporting the program and how the program contributes directly to the species identified in your application.

The International Elephant Foundation (IEF) is a non-profit 501(c)(3) corporation of individuals and institutions dedicated to the conservation of African and Asian Elephants worldwide. Our mission is to create a sustainable future for elephants by supporting elephant conservation, education, research, and management programs internationally. Through our passion, expertise, knowledge, and partnerships we inspire and engage people to ensure a vibrant future with elephants everywhere. Since our inception, IEF has provided over 8 million dollars to our mission. The many past and current projects we have supported can be found at https://elephantconservation.org/projects/

Although EEHV is not the only elephant health concern for the International Elephant Foundation (IEF), it is one of our highest funding priorities with IEF providing Evaluation of plasma D-dimer concentration in juvenile Asian (*Elephas maximus*) and African elephants (*Loxodonta africana*) with and without elephant endotheliotropic herpesvirus hemorrhagic disease, a grant of \$16,000 in 2023. IEF is also funding:

- EEHV Viral Genomics and Pathogenesis (2013-present): The three most common and useful techniques for studying viruses are not applicable for EEHV. However studying the genomes of EEHVs by PCR amplification and DNA sequencing directly from necropsy tissue and other clinical samples provides information about the genetic make-up of each virus and about the genes and pathways that they utilize to take control of particular types of host cell. That information allows us to generate additional clinical reagents such as specific antibodies and target antigens, as well as cloned expression vectors for viral enzymes that can be used in laboratory research and pathological diagnosis. The ultimate goal is to identify viral immediate-early genes, latency genes and immune evasion genes and other potential novel viral genes or pathways that will provide insights into the mechanisms of viral pathogenesis and will also help for generating engineered attenuated vaccine strains or new targets and approaches for better antiviral drugs.
- Realization of an Effective Vaccine Against Elephant Endotheliotropic Herpesvirus (2019-present): With IEF support among others, the Ling laboratory in Houston, Texas has spent the last several years developing the tools needed to discover what parts of the EEHV virus might be useful for developing a vaccine that can induce protective immunity for Asian elephants. This project uses those tools to develop and evaluate an anti-EEHV vaccine and the possibility of adapting it for use in African elephants as well.
- Development of EEHV-specific nanobodies as treatment for EEHV-hemorrhagic disease (2023): Two EEHV-dedicated research groups were able to develop sensitive diagnostic assays for detection of EEHV-specific antibodies. Of the assays, the gB ELISA detects antibodies elicited against multiple EEHV species, while a recently developed set of gH/gL ELISAs can differentiate between infections of the Asian elephant EEHV (sub)species. Observations strongly suggest that antibodies have an important role in protection against EEHV-HD. This would open up the opportunity to prevent and possibly treat the disease by direct administration of neutralizing antibodies, i.e. antibodies that can

inhibit (EEHV) infection of cells and thereby control dissemination of the virus in the animal, to animals at risk. Although less common than vaccination, direct use of antibodies to prevent disease, also known as passive immunization, is still commonly used in human medicine as (postexposure) prophylaxis against various important pathogens, including herpesviruses.

Emerging and re-emerging infectious diseases (encephalomyocarditis virus, tuberculosis, and toxoplasmosis) have been reported over the last decades. Elephant Endotheliotropic Herpesvirus (EEHV), an emerging disease, was first described in captive elephants in North America and Europe, with a mortality rate of ~ 65% in calves in human care. EEHV is a leading cause of acute fatal haemorrhagic disease (EEHV HD) in elephants in North America, Europe, and Asia. More recently, EEHV has been identified in wild elephant populations but the extent of morbidity and mortality in the wild in Asia and Africa has not been elucidated.

EEHV, a global threat to elephants, is not unique to location or management. There have been confirmed lethal cases in multiple Asian range countries including Myanmar, Laos, Malaysia, India, Thailand, Indonesia, Borneo, Nepal, and Cambodia [Elephant Endotheliotropic Herpesvirus (EEHV) in Asia Guidelines for Management 2nd Edition compiled by Sonja Luz and Lauren Howard, 2017. Page 5]. From 2003-2019 there were 142 known, reported cases of EEHV in Asia, with only 40 animals surviving. 18 of these cases were from wild populations in India. [Lauren Howard, EEHV Primer Jan 2022 IEF Symposium]. Given the lack of resources and testing it is likely that the number of elephants fallen ill or lost to EEHV in African elephants, but this has changed. From 2019-2021 there were 13 known cases of EEHV illness in African elephants ranging from 4 to 42 years old. [Lauren Howard, EEHV Primer Jan 2022 IEF Symposium].

The realization that EEHV threatens all elephant populations has produced an increased need for global testing for early detection, testing of samples from deceased animals to determine if EEHV was a factor and to enhance our knowledge base. For example, a study from October 2022 tested a serological assay specific to one African elephant strain of EEHV, a tool that can be used to screen for EEHV immune status in African elephant calves in range countries who have been orphaned by poachers, have been separated by their herds, required emergency veterinary care from snares and traps, and are being rehabilitated for release. [Pursell T, Spencer Clinton JL, Tan J, Peng R, Qin X, Doddapaneni H, Menon V, Momin Z, Kottapalli K, Howard L, Latimer E, Heaggans S, Hayward GS, Ling PD. Primary Infection May Be an Underlying Factor Contributing to Lethal Hemorrhagic Disease Caused by Elephant Endotheliotropic Herpesvirus 3 in African Elephants (*Loxodonta africana*). Microbiol Spectr. 2021 Oct 31;9(2):e0098321. doi: 10.1128/Spectrum.00983-21. Epub 2021 Oct 20. PMID: 34668724; PMCID: PMC8528115.]

IEF has been working in Uganda since 2012 supporting the construction of 14 Ranger stations in both Murchison Falls Conservation Area and Queen Elizabeth Protected Area (QEPA), funding community programs and a soon to be released manuscript on a high-resolution aerial survey of wildlife in QEPA. It has been reported to IEF that elephant calves and some adults have been dying of a rare unknown infection which some scientists assume to be of viral origin but to date no attempts have yet been made to examine the current EEHV status in free-ranging African savannah and forest elephants. However, The Uganda Wildlife Authority (UWA), with assistance from the

U.S. Defense Threat Reduction Agency (DTRA), recently established a state-of-the-art diagnostic and research laboratory in the middle of the Queen Elizabeth Protected Area (QEPA). This facility comes at a time when the country is wanting to improve on the surveillance and diagnosis of wildlife diseases with the intent of promoting wildlife health and conservation.

The UWA-DRL's biobank provides research and diagnostic potential for samples collected from wildlife, especially those whose protection and conservation are emphasized by the government. In Uganda elephant numbers have increased greatly from as low as 800 individuals in the 1980's to currently more than 5,000. Despite the increasing numbers, elephants in Uganda still face poaching pressure, and mortalities and morbidities due to unknown diseases. The potential for EEHV to threaten the recovery of Uganda's elephant population is great.

Because range countries often lack resources to test and monitor potential cases, there is limited data to inform responsible authorities and wildlife agencies to guide policy. EEHV advisory groups have been set up in Asia and Africa [established at the 2019 16th IEF International Elephant Conservation and Research Symposium] with a high level of international consultation and cooperation. The success of these groups is contingent on sharing knowledge, samples and research across international lines, including to those labs in the United States that are working on sequencing the genomes of the multiple strains of EEHV, developing a vaccine, and testing less invasive methods of early detection. Access to samples is vital to the research that will ultimately stop EEHV and remove it as a threat for wild populations. Without this work EEHV will be among poaching, human-elephant conflict, and habitat loss in the list of reasons why elephants go regionally and ultimately extinct.

Dear Marjorie,

The International Elephant Foundation is pleased to announce that your project "Plasma Ddimer Concentration in Juvenile Elephants with and without EEHV-HD" was chosen for funding in 2023 for the amount of US\$ 15,750. This year's Conservation Grant process was extremely competitive and we are excited that you have been selected. Since it has been a number of months since the drafting of your proposal, please take time right now to review your project as submitted and notify us of any changes/modifications that must be made.

Within the next 2 weeks I will be sending you a grant agreement to review. When it arrives, please review and sign, or propose any changes.

Again, Congratulations! We look forward to working with you!

Best wishes, Sarah Conley

> Sarah Conley Conservation Coordinator International Elephant Foundation www.elephantconservation.org

INTERNATIONAL ELEPHANT FOUNDATION

The International Elephant Foundation supports conservation, awareness and scientific programs that enhance the survival of elephants and protect their habitats worldwide.

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Technical Expertise & Authorizations

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

MARJORIE BERCIER, DMV, Dipl. ACZM

IER/HERS QUÉBEC, CANADA J2J 2H7 MBERCIER@ZOODEGRANBY.COM

EDUCATION

American College of Zoological M	Iedicine	Nov 2018
University of Montreal	July 2012 -	July 2013
University of Montréal	Sept 2007 -	- May 2012
University of Montréal	Sept 2006 -	- May 2007
CÉGEP André-Laurendeau	Sept 2003 -	Sept 2006

ACZM diplomate

Zoological Medicine internship, IPSAV Doctor of Veterinary Medicine, DMV Environmental Geography program, Minor Natural Science program, DEC

PROFESSIONAL ACTIVITIES

Start	End	Employment	Institution
July 2021	Present	Associate Veterinarian	Zoo de Granby 525, rue St-Hubert, Granby, Québec, Canada, J2G 5P3.
Sept 2019	July 2021	Clinical assistant professor, Zoological Medicine (ZCAM) service	Cummings School of Veterinary Medicine (CSVM), Tufts University 55 Willard Street, North Grafton, MA 01536.
Feb 2020	March 2020	Relief Veterinarian	Zoo New England 1 Franklin Park Rd, Boston, MA 02121
April 2019	August 2019	Locum Clinician, ZCAM service	CSVM, Tufts University
January 2018	March 2019	Associate Veterinarian	Cheyenne Mountain Zoo 4250 Cheyenne Mountain Zoo Road, Colorado Springs, CO 80906.
July 2017	December 2017	Clinical Instructor	Disney's Animal, Science and Environment, Animal Health Team Disney's Animal Kingdom, EPCOT's The Seas with Nemo and Friends, 1200 N. Savannah Cir. E., Bay Lake, Fl, 32830
July 2014	July 2017	Zoological Medicine Resident	University of Florida, Gainesville, USA. Small Animal Hospital, Zoological Medicine Service: 2089, SW 16 th avenue, Gainesville, Fl 32608, USA. White Oak Conservation Center: 581705 White Oak Road, Yulee, Fl 32097, USA.
July 2013	July 2014	Veterinarian	Le Gardeur Veterinary Hospital, Le Gardeur, Qc. 413, St- Paul, Le Gardeur, Québec, Canada, J5Z 2H9.
	Veterinarian	DMV veterinary referral center, Montréal, Qc. 2300, 54 ^e avenue, Lachine, Québec, Canada, H8T 3R2.	
July 2012	July 2013	Zoological Medicine Intern	University of Montréal, St-Hyacinthe, Qc 3200, Sicotte, St-Hyacinthe, Québec, Canada, J2S 2M2.
PUBLICA	TIONS – PE	EER REVIEWED	

• Mayer CC, Richard JN, Lin C-M, Conrado FO, Hahn S, Graham JE, **Bercier M**. Intracoelomic Teratoma in an Eclectus Parrot (*Eclectus roratus*). J Avian Med Surg, 2021, 35(2), 217-226.

- **Bercier M**, LaDouceur EB, Citino SB. Clinical findings, pathology, biosecurity and serosurveillance of coxiellosis in white rhinoceroses (*Ceratotherium simum*) at a conservation center: 2 cases. J Zoo Wildl Med, 2021, 52(1): 389-395. https://doi.org/10.1638/2020-0081
- **Bercier M**. Chapter 12 The rabbit emergency: triage, techniques and hospitalization. Under revision for the book: Rabbit Medicine and Surgery by Nico Shoemaker and Yvonne Zeeland.

- Bercier M. Gerontology of psittacines. Vet Clin North Am Exot Anim Pract. 2020; 23(3): e1-e14.
- Gillis J, **Bercier M**, Rattner F, Citino S. 63, XX, SRY negative, pseudohermaphroditism in a Somali wild ass (*Equus asinus somalicus*). Veterinary Record Case Reports 8: ee001164. Doi: 10.1136/vetreccr-2020-001164
- Black LJ, **Bercier M**. Chapter 133: Hematology of Serpentes. In: Weiss DJ, Wardrop KJ (eds). Schalm's Veterinary Hematology, 7th edition. Under review by editors.
- **Bercier M**, Heard DJ, Goe AM, Epperson E, Abbott JR, and Wellehan JFX. Granulomatous encephalomyelitis in a false gharial (*Tomistoma schlegelii*) associated with a novel *Chlamydia* species. J Zoo Wildl Med, 2017, 48(2): 563-567.
- **Bercier M**, Zoll W, Rosenberg JF, Giglio R, McCoy L, Castleman WL, Johnson MD, and Heard DJ. Gastric intussusceptions in a red ratsnake (*Pantherophis guttatus*) associated with cryptosporidiosis. Case Reports Vet Med, 2017, Article ID 4270904, 5 pages.
- Flanders AJ, Rosenberg JF, **Bercier M**, Leissinger MK, Black LJ, Giglio RF, Craft SL, Zoll WM, Childress AL, and Wellehan JFX. (2017). Antemortem diagnosis of coxiellosis in a blue and gold macaw (*Ara ararauna*). *J Avian Med Surg* 2017;31:364-372.
- **Bercier M**, Langlois I, Dunn M, Helie P, Burns P, and Gara-Boivin C. Cytological analysis of bronchoalveolar lavage fluid acquired by bronchoscopy in healthy ferrets: A pilot study. *Can J Vet Res* 2016;80:74-80.
- Bercier M, Alexander K, Gorow A, and Pye GW. Computed tomography and magnetic resonance for the advance imaging of the normal nasal cavity and paranasal sinuses of the koala (*Phascolarctos cinereus*). J Zoo Wildl Med, 2014, 45(4): 766-774.
- Bercier M, Guzman D S-M, Stockman J, Zwingenberger A, Vapniarsky N, Lowenstine L and Hawkins MG. Salivary gland adenocarcinoma in a domestic rabbit (*Oryctolagus cuniculus*). J Exotic Pet Med, 2013, 22(2): 218-224.
- Bercier M, Wynne J, Klause S, Stadler CK, Gorow A, and Pye GW. Nasal mass removal in the koala (*Phascolarctos cinereus*). J Zoo Wildl Med, 2012, 43(4): 898-908.

PUBLICATIONS – PROCEEDINGS AND POSTERS

- American Association of Zoo Veterinarian (AAZV) Infectious disease manual 2019.
- American Association of Zoo Veterinarian (AAZV) annual conference. October 2018. 15min presentation and published abstract. Abstract presented by Scott Citino, DVM, Dipl. ACZM. Investigation of a Q fever outbreak in a breeding herd of white rhinoceros (*Ceratotherium simum*).
- AAZV annual conference. September 2017. Poster. Limitations and impacts of the renal portal system on the determination of the glomerular filtration rate using contrast-enhanced computed tomography and plasma clearance of iohexol in bearded dragons (*Pogona vitticeps*). Proceedings Annual Conference AAZV, 2017. P.185.
- AAZV annual conference. October 2015. 15 min presentation and published abstract. A Novel *Chlamydophila* species in a False Gharial (*Tomistoma schlegelii*) with Cervicothoracic Scoliosis and Granulomatous Encephalomyelitis. Proceedings Annual Conference AAZV, 2015. P.112.
- AAZV annual conference. October 2015. 15 min presentation and published abstract. Retrospective Study of Morbidity and Mortality of Wild Gopher Tortoises (*Gopherus polyphemus*) admitted to the University of Florida (1998-2014). Proceedings Annual Conference AAZV, 2015. P.9.
- Association of Exotic Mammal Veterinarian annual conference. October 2014. 15 min presentation and published abstract. Cytologic Analysis of Bronchoalveolar Lavage Fluid Acquired by Bronchoscopy in Healthy Ferrets and Standardization of Cellular Counts Using Urea as a Marker of Dilution.
- AAZV annual conference. October 2013. Poster. Computed tomography, magnetic resonance imaging and gross cross-sectional views of the normal anatomy of the koala (*Phascolarctos cinereus*) nasal cavity and paranasal sinuses. Proceedings Annual Conference AAZV, 2013. P.155.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors. Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME		POSITION TITLE	
Brooks, Marjory B.		r of Practice	
eRA COMMONS USER NAME			
MBRooks			
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
Cornell University, Ithaca NY	BS	1977	Animal Science
NYS College of Veterinary Medicine, Ithaca, NY	DVM	1981	Veterinary Medicine
The Animal Medical Center, NY, NY	Internship	1984-1985	General medicine & surgery
The Animal Medical Center, NY, NY	Residency	1985-1987	Internal medicine-oncology
American College of Veterinary Internal Medicine	Diplomate	1988	Internal Medicine

A. Personal Statement

My career has focused on the development and characterization of animal models of hemostatic disorders. I am a recognized expert in the field of comparative hemostasis and my laboratory is a unique referral center for assay development and diagnosis of hemostatic defects and pre-clinical hemostasis testing. I have successfully adapted and/or developed techniques to study coagulation and platelet activation response and have worked productively in numerous collaborations and in consultation with biomedical and veterinary researchers.

B. Positions and Honors

Positions and Employment

1981-1983	Staff clinician, Henry Bergh Hospital of the ASPCA, NY, NY
1984-1985	Companion Animal Internship, The Animal Medical Center, NY, NY
1985-1986	Residency in Oncology and Internal Medicine, The Animal Medical Center, NY, NY
1986-1988	Staff Oncologist, The Animal Medical Center, NY, NY
1988-1994	Research Scientist, Wadsworth Center for Laboratories & Research, Albany, NY
1994-2009	Asst. Dir., Comparative Coagulation Lab, Animal Health Diagnostic Center, Ithaca, NY
2009-	Director, Comparative Coagulation Lab, Animal Health Diagnostic Center, Ithaca, NY

Other Experience and Professional Memberships

- 1981- Member, American Veterinary Medical Society
- 1988- Member, American College of Veterinary Internal Medicine
- 1994- Member, American Association of Blood Banking
- 2005-2009 Board member, Association of Veterinary Hematology and Transfusion Medicine
- 2010- Committee co-chair, HESI Cardiac Safety –Biomarkers Working Group

<u>Honors</u>

1986	Outstanding Resident Research Project Award, Animal Medical Center, NY, NY
1988	Diplomate, American College of Veterinary Medicine
2006	Dr. Jack Mara Am. College of Vet. Emergency and Critical Care Scientific Award
2020	DACC Award for Outstanding Contributions to Animal Clinical Chemistry

C. Selected peer-reviewed publications (From a total of 123 publications)

- Lynch AM, Ruterbories L, Griffith E, Hanel RM, Stablein AP, Brooks MB. The influence of feeding and gastroprotectant medications on the anticoagulant effect of orally administered rivaroxaban in normal dogs. J Vet Emerg Crit Care 2021;31:59-65.
- Cremer SE, Catalfamo JL, Goggs R, Seemann ES, Kristensen AT, Szklanna PB, Maguire PB. Brooks MB. The Activated Canine Platelet Secretome (CAPS): A Translational Model of Platelet Activation Response. Res Pract Thromb Haemost 2021;5:55-68.
- 3. Foote ML, **Brooks MB**, Archer TM, Wills RW, Mackin AJ, Thomason, JM. Effects of leukoreduction on clotting times and coagulation factor content in units of canine fresh frozen plasma. Am J Vet Res 2019;80:846-851.
- 4. Drinkhouse M, **Brooks MB**, Stefanovski D, Marryott K, Callan MB. Influence of Canine Donor Plasma Hemostatic Protein Concentration on Quality of Cryoprecipitate. J Vet Intern Med. 2019 Jan;33(1):124-131.
- Serpa PBS, Brooks MB, Divers T, Ness S, Birschmann I, Papich MG, Stokol T. Pharmacokinetics and pharmacodynamics of an oral formulation of apixaban in horses after oral and intravenous administration. Front Vet Sci 2018:5;304
- Stokol T, Serpa PBS, Brooks MB, Divers T, Ness S. Subcutaneous administration of low-molecularweight-heparin to horses inhibits Equine Herpesvirus Type 1-induced platelet activation. Front Vet Sci 2018:5;106
- Makielski KM, Brooks MB, Wang C, Cullen JN, O'Connor AM, LeVine DN. Development and implementation of a novel immune thrombocytopenic bleeding score in dogs. J Vet Intern Med 2018;32:1041-1050
- 8. Jeffery U, **Brooks MB**, LeVine DN. Development of a turbidimetric canine plasma fibrinolysis assay. The Veterinary Journal 2017:229;19-25.
- LeVine DN, Cianciolo RE, Linder KE, Bizikova P, Birkenheuer AJ, Brooks MB, Salous AK, Nordone SK, Bellinger DA, Marr H, Jones SL, Fischer TH, Deng Y, Mazepa, M, Key NS. Endothelial alterations in a canine model of immune thrombocytopenia. ePub Platelets. 2017 Nov 28:1-10
- Barratclough A, Conner BJ, Brooks MB, Stablein A, Gerlach TG, Reep RL, Ball RL, Floyd RF. Identifying coagulopathies in the pathophysiology of cold stress syndrome in the Florida manatee (Trichechus manatus latirostris). Diseases of Aquatic Organisms 2017;125:179-188.
- 11. **Brooks MB**, Stablein A, Johnson LM, Schultze AE. Pre-analytic Processing of Rat Plasma Influences Thrombin Generation and Fibrinolysis Assays. Vet Clin Pathol 2017;46:496-507.
- Ness SL, Frye AH, Divers TJ, Rishniw M, Erb HN, Brooks MB. A Randomized, Placebo-Controlled, Blinded Study of the Effects of Yunnan Baiyao on Parameters of Equine Hemostasis. Am J Vet Res 2017;78:969-976
- 13. Brooks MB, Turk JR, Guerrero A, Narayanan PK, et al. Non-Lethal Endotoxin Injection: A Rat Model of Hypercoagulability. PLoS One. 2017 Jan 12;12(1):e0169976
- 14. Hernandez D, Yeo WM, **Brooks MB**, Ness SL, Divers TJ, Stokol TS. Effect of various anti-platelet drugs on ex vivo Equine Herpesvirus type 1-induced platelet activation. Am J Vet Res 2016;77:1366-1373.
- 15. Dixon-Jimenez AC, Brainard BM, **Brooks MB**, Nie B, et al. Pharmacokinetic and pharmacodynamic evaluation of oral rivaroxaban in healthy adult cats. J Vet Emerg Crit Care 2016;26:619-629
- 16. Manion JS, Thomason JM, Langston VC, Claude AK, Brooks MB, Mackin AJ, Lunsford KV. Anticoagulant effects of inhaled unfractionated heparin in the dog as determined by partial thromboplastin time and factor Xa activity. J Vet Emerg Crit Care 2016;26:132-136.
- Fletcher D, Rozanski L, Delaforcade A, Brainard B, Brooks MB. Assessment of the relationships among coagulopathy, hyperfibrinolysis, plasma lactate, and protein C in dogs with spontaneous hemoperitioneum. J Vet Emerg Crit Care 2016;26:41-51.
- 18. Frye C, Enders A, **Brooks M**, Struble AM, Wakshlag J. Assessment of canine platelet-rich plasma produced with a commercial centrifugation and platelet recovery kit. Vet Comp Orthop Traumatol 2016;29:14-19.
- Brooks MB, Catalfamo JL, MacNguyen R, Tim D, Fancher S, McCardle JA. A TMEM16F Point Mutation Causes an Absence of Canine Platelet TMEM16F and Ineffective Activation and Death-Induced Phospholipid Scrambling. Journal of Thrombosis and Haemostasis 2015;13:2240-2252.

Curriculum Vitae

Name of Co Investigator: Erin Latimer Position Title: Research Specialist

Education and Training:

Degree	Major	Institution
B.S.	Bioch. and Nutrition	VA Tech
M.S.	Bacteriology	Univ. of Wisconsin-Madison

Appointments:

2004-present	Research Specialist, Department of Wildlife Health Sciences, Smithsonian's National Zoo
1993-1998	Research Associate, Biological Carcinogenesis and Development Program, NCI-Frederick
	Cancer Research and Development Center
1001 1000	

1991-1993 Research Scientist, TechLab, Inc.

List Selected Peer Reviewed Publications Most Relevant to this Task:

- Edwards KL, Latimer EM, Siegal-Willott J, Kiso W, Padilla L, Sanchez CR, Schmitt D, Brown JL. Patterns of serum immune biomarkers during elephant endotheliotropic herpesvirus viremia in Asian and African elephants. doi: <u>https://doi.org/10.1101/2021.05.12.443748his</u>
- Pursell T, Spencer Clinton JL, Tan J, Peng R, Qin X, Doddapaneni H, Menon V, Momin Z, Kottapalli K, Howard L, Latimer E, Heaggans S, Hayward GS, Ling PD. Primary Infection May Be an Underlying Factor Contributing to Lethal Hemorrhagic Disease Caused by Elephant Endotheliotropic Herpesvirus 3 in African Elephants (*Loxodonta africana*). Microbiol Spectr. 2021 Oct 20:e0098321. doi: 10.1128/Spectrum.00983-21
- **3.** Fayette MA, Brenner EE, Garner MG, Bowman MR, **Latimer E**, and Proudfoot JS. Acute Hemorrhagic Disease Due To Elephant Endotheliotropic Herpesvirus 3a Infection In Five African Elephants (Loxodonta Africana) At One North American Zoological Institution. 2021. J Zoo Wildl Med. 2021; 52(1) 357-365. <u>https://doi.org/10.1638/2020-0126</u>
- 4. Grenus B, Latimer E, Cullinane A, Lyons P, Creighton G, and Nutter FB. Evaluation of The Efficacy of Two Different Sampling Sites for the Detection of Elephant Endotheliotropic Herpesvirus (EEHV) in Asian Elephants (Elephas Maximus) in Ireland. J Zoo Wildl Med. 2020;51(2):303-307. doi:10.1638/2018-0193
- 5. Bauer KL, Latimer E, Finnegan M. (2018) Long-term, intermittent, low-level elephant endotheliotropic herpesvirus 1a viremia in a captive Asian elephant calf. J Vet Diag Inv. 2018, Vol. 30(6) 917–919. https://doi.org/10.1177/1040638718803138
- 6. Zachariah A, Sajesh PK, Bathrachalam C, Megha M, Pandiyan J, Jishnu M, Kobragade RS, Long SY, Zong J-C, Latimer EM, Heaggans SY and Hayward GS. Extended Genotypic Evaluation and Comparison of Twenty-Two Cases of Lethal EEHV1 Hemorrhagic Disease in Wild and Captive Asian Elephants in India. PLoS ONE 2018 Aug 22:13(8):e0202438 DOI: 10.1371/journal.pone.0202438 eCollection 2018.
- Bronson E, McClure M, Sohl J, Wiedner E, Cox S, Latimer EM, Pearson VR, Hayward GS, Fuery A, Ling PD. (2017) Epidemiologic Evaluation of Elephant Endotheliotropic Herpesvirus 3b Infection in an African Elephant (Loxodonta Africana). J Zoo Wild Med. 2017 Jun;48(2):335-343. doi: 10.1638/2016-0063R.1
- **8.** Long SY, **Latimer EM**, and Hayward GS. Review of Elephant Endotheliotropic Herpesviruses and Acute Hemorrhagic Disease. ILAR Journal 2016 56 (3): 283-296. doi: 10.1093/ilar/ilv041
- **9.** Zong_JC, Heaggans SY, Long SY, **Latimer EM**, Nofs SA, Bronson E, Casares M, Fouraker MD, Pearson VR, Richman LK, Hayward GS. Detection of Quiescent Infections with Multiple Elephant Endotheliotropic Herpesviruses (EEHVs), Including EEHV2, EEHV3, EEHV6, and EEHV7, within Lymphoid Lung Nodules or Lung and Spleen Tissue Samples

from Five Asymptomatic Adult African Elephants. <u>J Virol.</u> 2016 Feb 30;90(6):3028-43. doi: 10.1128/JVI.02936-15.

- van den Doel PB, Prieto VR, van Rossum-Fikkert SE, Schaftenaar W, Latimer E, Howard L, Chapman<u>http://www.biomedcentral.com/1746-6148/11/203/ - ins8</u> S, Masters N, Osterhaus ADME, Ling PD, Dastjerdi A and Martina B. A novel antigen capture ELISA for the specific detection of IgG antibodies to elephant endotheliotropic herpes virus. *BMC Veterinary Research* 2015, 11:203 doi:10.1186/s12917-015-0522-6
- Ortega J, Corpa JM, Orden JA, Blanco J, Carbonell MD, Gerique AC, Latimer E, Hayward GS, Roemmelt A, Kraemer T, Romey A, Kassimi LB, and Casares M. Acute death associated with *Citrobacter freundii* infection in an African elephant (*Loxodonta africana*). J Vet Diag Inv. July 25, 2015 doi: 10.1177/1040638715596034
- 12. Zong JC, Latimer EM, Long SY, Richman LK, Heaggans SY, and Hayward GS. 2014. Comparative Genome Analysis of Four Elephant Endotheliotropic Herpesviruses EEHV3, EEHV4, EEHV5 and EEHV6 from Cases of Hemorrhagic Disease or Viremia. J. Virol. 2014, 88(23):13547. DOI: 10.1128/JVI.01675-14.
- **13.** Richman LK, Zong JC, **Latimer EM**, Lock J, Fleischer R, Heaggans SY, and Hayward GS. 2014. Elephant Endotheliotropic Herpesviruses EEHV1A, EEHV1B and EEHV2 from Cases of Hemorrhagic Disease are Highly Diverged from Other Mammalian Herpesviruses and May Form a New Subfamily. J Virol. 2014, 88(23):13523. DOI: 10.1128/JVI.01673-14.
- Zachariah A, Zong J, Long SY, Latimer E, Heaggans SY, Richman L, and Hayward GS. 2013. Fatal Herpesvirus (EEHV) Hemorrhagic Disease in Wild and Orphan Asian Elephants in India. J Wild Dis. 49(2):381-93.
- **15.** Stanton JJ, Zong JC, Eng C, Howard L, Flanagan JP, Stevens M, Schmitt D, Wiedner E, Graham D, Junge JE, Weber MA, Fischer M, Mejia A, Tan J, **Latimer EM**, Herron A, Hayward GS, and Ling PD. 2013. Kinetics of Viral Loads and Genotype Analysis of Elephant Endotheliotropic herpesviruse-1 infection in captive Asian elephants (Elephas maximus). J Zoo Wildl Med 44(1): 42-54.
- 16. Atkins L, Tan J, Mejia A, Nofs S, Flanagan JP, Howard L, Latimer E, Hayward GS, Stevens MR Hoffman DS and Ling PD. 2013. EEHV-5, a newly recognized elephant herpesvirus associated with clinical and subclinical infections in captive Asian elephants (Elephas maximus). J Zoo Wildl Med 44(1): 136-143.
- 17. Denk D, Redrobe S, Latimer E, Hayward GS, Cracknel J, Classens A, Steinbach F, McGowan S & Dastjerdi. 2012. Fatal elephant endotheliotropic herpesvirus type 5 infection in a captive Asian elephant. Vet. Rec doi: 10.1136/vr.e6833
- **18.** Latimer E, Zong JC, Heaggans SY, Richman LK, Hayward GS. 2011. Detection and evaluation of novel herpesviruses in routine and pathological samples from Asian and African elephants: Identification of two new probosciviruses (EEHV5 and EEHV6) and two new gammaherpesviruses (EGHV3B and EGHV5). Vet Microbiol. 147(1-2): 28-41.
- **19.** Stanton JJ, Zong JC, **Latimer E**, Tan J, Herron A, Hayward GS, Ling P. 2010. Detection of pathogenic elephant endotheliotropic herpesvirus in routine trunk washes from healthy adult Asian elephants (Elephas maximus) by use of a real-time quantitative polymerase chain reaction assay. Am J Vet Res. **71**(8): 925-33.
- 20. Garner MM, Helmick K, Ochsenreiter J, Richman LK, Latimer E, Wise AG, Maes RK, Kiupel M, Nordhausen RW, Zong JC, Hayward, GS. 2009. *Clinico-Pathologic Features of Fatal Disease Attributed to New Variants of Endotheliotropic Herpesviruses in two Asian Elephants (Elephas maximus)*. Vet Path. 46:97-104.



Smithsonian Conservation Biology Institute *Wildlife Health Sciences National Elephant Herpes Laboratory*

August 16, 2022,

This letter is to confirm the support of the National Elephant Herpes Laboratory (NEHL) for the project entitled "Evaluation of plasma D-dimer concentration in juvenile Asian (Elephas maximus) and African elephants (Loxodonta africana) with and without elephant endotheliotropic herpesvirus hemorrhagic disease". We will help facilitate enrollment of the elephant-holding institutions in the project, provide qPCR results for the enrolled elephants, and help coordinate sample submission for d-dimer testing to the Comparative Coagulation Lab, Cornell University College of Veterinary Medicine. We already have a collaborative relationship with most of the facilities that will be asked to enroll in the study, as we provide routine and diagnostic EEHV testing for most of the Asian and African elephant herds in the US.

Rapid aggressive treatment has been shown to be vital for a good outcome in cases of EEHV Hemorrhagic Disease (EEHV HD). This project may contribute towards the identification of another biomarker that can be measured early in the disease course to identify elephants that need more acute care.

I look forward to helping with this important project.

Erin Letimer

Erin Latimer, Research Specialist National Elephant Herpesvirus Laboratory

SMITHSONIAN CONSERVATION BIOLOGY INSTITUTE National Zoological Park MRC 5501 Department of Pathology Washington, D.C. 20013-7012 Telephone: 202-633-4252 Fax: 202-633-8717



Cornell University

Comparative Coagulation Section Marjory Brooks, DVM Section Director,

Animal Health Diagnostic Center 240 Farrier Rd. Ithaca, New York 14853 t. 607.253.3469 f. 607.253.3471 e. mbb9@cornell.edu

November 29, 2021

Dr. Marjorie Bercier Associate Veterinarian Zoo de Granby 525, rue St-Hubert, Granby, Qc, Canada J2G 5P3

Dear Dr. Bercier

I am happy to collaborate in your project "Evaluation of plasma D dimer concentration in juvenile Asian (Elephas maximus) and African elephants (Loxodonta africana) with and without elephant endotheliotropic herpesvirus (EEHV) hemorrhagic disease." Infection with EEHV often culminates in a fatal hemorrhagic syndrome, however screening tests to detect hemostatic imbalance early in the disease process are lacking. Your project addresses this gap in EEHV management by evaluating the diagnostic utility of D dimer determinations as an early indicator of this devastating thrombohemorrhagic syndrome.

My primary role in support of this project will be oversight of assays performed at the Comparative Coagulation Laboratory. We have previously performed measurement of plasma D dimer and fibrinogen levels in a variety of species for research and clinical veterinary applications. In addition to assay oversight, I look forward to contributing to the success of this project by assisting in data interpretation, and ultimately publication of the study results.

Sincerely,

Broche Magony

Marjory Brooks, DVM, Dip. ACVIM



www.eehvinfo.org

EEHV Advisory Group Letter of Endorsement

9th December, 2021

Dear Dr. Bercier,

Thank you for the submission of your research proposal titled 'Evaluation of D dimer concentration in juvenile Asian (*Elephas maximus*) and African elephants (*Loxodonta africana*) with and without elephant endotheliotropic herpesvirus (EEHV) hemorrhagic disease' for review and potential endorsement by the EEHV Advisory Group.

Members of the Advisory Group have reviewed your proposal for the validity of the hypothesis, usefulness of the results, study feasibility, timeline and potential for publication. We are pleased to inform you that the EEHV Advisory Group has fully endorsed this proposal.

This project was considered very important from a clinical and physiological standpoint, and will hopefully add to the dearth of information regarding EEHV diagnosis in elephants, resulting in more rapid detection of the thrombohemorrhagic state. The proposed work is in alignment with EEHV Advisory Group research priorities, and we anticipate it providing extremely valuable data to help better understand this potentially fatal disease.

Thank you for your research efforts to benefit elephants, and your assistance in increasing the body of knowledge regarding EEHV. Good luck with securing funding for this project and please let me know how the EEHV Advisory group can be of further assistance. In addition, please keep us informed regarding project development and results.

Yours sincerely,

P. Rapoda

Priya Bapodra BVetMed MSc Dipl. ACZM Chair of Research Committee, EEHV Advisory Group priya.bapodra@columbuszoo.org 13. Please indicate if this is a one-time shipment or if you anticipate needing to import/export/re-export samples multiple times within one year or over multiple years.

We anticipate needing to import samples multiple times within one year and over multiple years for the project Evaluation of plasma D-dimer concentration in juvenile Asian (*Elephas maximus*) and African elephants (*Loxodonta africana*) with and without elephant endotheliotropic herpesvirus hemorrhagic disease, as well as for additional scientific research projects which need African elephant (*Loxodonta africana*), African Forest elephant (*Loxodonta cyclotis*) and Asian elephant (*Elephas maximus*) biological samples. Examples of other such scientific research projects requiring samples include:

- Virginia R. Pearson, Visiting Scientist, Fox Chase Cancer Center, Philadelphia PA USA and Gary S. Hayward, Professor, PhD, Johns Hopkins University Medical School, Baltimore, MD USA, and Paul Ling, PhD, Baylor Medical School, Houston, TX USA; 2023 - awaiting samples from University of Zurich, Switzerland; Vienna Zoo, Austria; University of Utrecht, Belgium; other European Zoos; Chester Zoo, England; and other English Zoos, and Borneo, Malaysia; and other Asian countries including but not limited to Myanmar, Thailand, Laos, Cambodia; and Kenya, Botswana, South Africa, Namibia and other African countries for elephant herpesviruses genomic and vaccine research.
- 2) Joshua Schiffman, MD, PhD and Lisa Abegglen, PhD, Schiffman Laboratory, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA, 2023 awaiting samples from Kenya, South Africa and Singapore for studies of Neutrophil Extracellular Traps (NETs) therapeutics for EEHVs infection.
- 3) Natalia Prado-Oviedo, PhD, Smithsonian Center for Species Survival, Washington, DC USA, awaiting samples from Mexico, Netherlands, University of Copenhagen, Denmark, and other European and English Zoo to generate high coverage (30X-60X) genome sequences of African and Asian elephants to assess genetic variation to address individual and population health and species survival.

The exact number and type of samples will depend on the specific research project to be performed and availability of those needed samples. Examples of sample types include blood, serum, saliva, tissue, skin, urine, dung. Samples will be collected from routine GPS collaring immobilizations for monitoring wild elephant movements in reserves and near villages, immobilizations by veterinarians for medical treatment of injured wild elephants, necropsies of dead elephants by natural causes or by poacher, and routine and emergency medical and husbandry treatment of captive-born elephants in human care. Some samples have already been collected and are currently stored in country of origin and some samples will be collected opportunistically throughout the year.

Exact packaging of samples will depend on specific research project to be performed. Examples of sample packaging include 2 ml GenTegraDN tubes or in Qiagen RNAprotect Cell and RNAlater Reagents, or dried on GenTegra Ahlstrom GenSaver Cards. Blood, tissue biopsies are typically packaged in vials envelopes, on slides, or in 2ml tubes or approximately 2-4" cubes in 50ml tubes in a freezer box frozen on dry ice in large styrofoam boxes or in cryogenic shipping containers.

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

Samples will be shipped

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

CITES Appendix I & Marine Mammal Species

For import of CITES Appendix-I listed species, provide information to show the import is not for primarily commercial purposes as outlined in <u>Resolution Conf. 5.10 (Rev CoP15)</u>.
 b) Scientific purposes: Article VII, paragraph 6, of the Convention uses the term "non-commercial loan, donation or exchange between scientists or scientific institutions". Thus, the Convention acknowledges that scientific purposes may justify a special departure from the Convention's general procedure. The import of specimens of an Appendix-I species may be permitted in those situations where the scientific purpose for such import is clearly predominant, the importer is a scientist or a scientific institution registered or otherwise acknowledged by the Management Authority of the country of import, and the resale or commercial exchange of the specimens, or their exhibit for economic benefit is not the primary intended use

All samples imported under this permit will be for scientific research purposes only. No economic benefit will be derived for the research.