IEF Interim report—Ling 2021

a. Project title: Realization of an Effective Vaccine Against Elephant Endotheliotropic Herpesvirus

b. Interim report

c. Investigators

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d. Project Start Date: January 1, 2020

e. Project completion date: December 31, 2022

2. Conservation needs this project addresses

EEHV is the single largest cause of death for captive juvenile Asian elephants in North America and Europe. Furthermore, EEHV-associated deaths have now been documented in wild Asian elephants in their natural range countries, adding yet another threat to this endangered species. Recent EEHV-associated deaths documented in captive African elephants are now raising concerns about EEHV in this species as well. In contrast to Asian elephants, much less is known about the EEHV viruses that are naturally found in African elephants.

Our lab has spent the last several years developing the tools needed to discover what parts of the EEHV virus might useful for developing a vaccine that can induce protective immunity. These tools will be used for evaluating anti-EEHV vaccines and will be adapted for use in African elephants.

The EEHV vaccine represents a truly innovative endeavor because it is considered an experimental vaccine developed specifically for use in an endangered species. If promising results are realized with vaccine trials in the Houston zoo elephants, we envision distribution of the vaccine to other institutions. Sequencing of the African elephant-specific EEHV types will provide the fundamental basis for multiple research activities, including adaptation of the tests and assays to the African elephant system that were developed for understanding Asian elephant anti-EEHV immune responses. Long-term, strategies developed for an anti-EEHV vaccine in Asian elephants will be leveraged for a similar vaccine in African elephants.

The first 12 months of this project have been significantly impacted by Covid 19. On March 20, 2020, Baylor College of Medicine shut down all nonessential research activities (known also as phase 0), which included our laboratory. On May 21, the school began phase 1 of a return to research plan, that restored activities/occupancy to 25% of normal operations. As of August 1 2020, the school moved to phase 2, restoring activities/occupancy to 50% of normal operations. Since January 2021, we have been operating close to normal, although staffing for some administrative positions and in our animal facility has been limited. The impact of this has been marginal, but in the case of our vaccine studies in mice, it may have led to a health issue with the mice (independent of the vaccine) that resulted in a compromised experiment and that we are in the process of repeating. While we are cautiously optimistic about the pandemic subsiding, the Delta variant or other emerging variants might stoke some headwinds for full recovery of our operations.

Nonetheless, we managed to complete sequencing of African elephant EEHV genomes 3A, 3B, and 2 and 6. In addition, we have sequenced samples representative for EEHV4B and 5B. Assembly and annotation of these genome sequences is in progress in collaboration with Dr. Gary Hayward (Johns Hopkins). This new sequencing information was used to develop a first generation serology assay for EEHV3, which was responsible for the deaths of 3 African elephants in 2019. We have now nearly completed our first vaccine study in mice with a prototype EEHV vaccine and hope to submit this for publication by the end of August. In addition, we have initiated a second preclinical vaccine trial in mice with a different vaccine platform and 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.

3. Goals and objectives of the study

Goals:

1. Develop an anti-EEHV vaccine for Asian elephants
2. Sequence EEHV viruses endemic within African elephants and develop tests to understand African elephant immunity to EEHV (as done previously for EEHV in Asian elephants)

Objectives:

Asian elephant EEHV vaccine-related activities

1. Preclinical studies in mice: Test two vaccine types (a recombinant vector and a subunit vaccine) expressing an EEHV protein known as glycoprotein B (gB) for immunogenicity and toxicity
2. Produce clinical-grade vaccine(s) sufficient to pass regulatory requirements
3. Test the vaccine(s) in Asian elephants housed at the Houston Zoo and monitor their ability to induce immune responses

African elephant EEHV activities:

1. Sequence the genomes of EEHV2, 3 and 6, which are endemic within African elephant populations
2. Utilize the information from genome sequencing to develop tests for understanding anti-EEHV immunity in African elephants, similar to those done already in Asian elephants.

Specific actions taken to achieve objectives

Elephant necropsy samples were identified and characterized by a variety of measures to determine suitability for sequencing. From this analysis, we submitted samples to the genome center at Baylor College of Medicine to determine the first genome sequences of EEHV2 and EEHV3A and EEHV3B and EEHV6. Preclinical vaccine studies, which involve testing of a prototype EEHV vaccine in mice, have been initiated and expanded. As mentioned previously, these studies have been delayed due to the Covid 19 pandemic. However, partial results from this study have now been completed and our ability to fully evaluate these first mouse experiments has been completed. A second vaccine trial in mice has now been initiated to compare and contrast with a second EEHV vaccine type.

4. Activities that differ from original proposal

Some small modifications have been made from our original proposal. We initially wanted to sequence the genome of EEHV3 from one of the cases at the Indianapolis zoo. However, it became apparent that the EEHV3 genomes from cases at the Indianapolis zoo were quite different from those of an earlier case at the Maryland zoo (a survivor) and a case at the Fresno zoo, even though both fell under the category of “EEHV3”. We decided to sequence this EEHV3 variant due to its divergence from the strain found in the Indianapolis zoo and some difficulties that our colleague, Gary Hayward at Johns Hopkins, was having with sequencing this variant. Tentatively, these EEHV3 strains are being referred to as EEHV3A and EEHV3B. At the moment, we think it may be possible that infections from 3A might not completely protect against disease caused by 3B and vice vera. This is similar to the situation observed for 1A and 1B in Asian elephants. Knowledge of the genome sequences from these two African elephant EEHV strains will help us generate tools to discriminate which EEHV strain, 3A or 3B, an elephant has been infected with and possibly identify whether an elephant is vulnerable to infection with one or the other or both. In addition, variant strains of EEHV4 and 5 have also been identified by Dr. Gary Hayward and we had in our possession samples representative samples for these strains, now called EEHV4B (EEHV4A prototype is from Baylor at the Houston zoo) and EEHV5B (EEHV5A prototype is from Vijay, an elephant from the UK). To finalize efforts to sequence major EEHV strains circulating in Asian elephants, we decided that these variant genomes should also be sequenced because they will provide foundational information about how EEHV has evolved and for the further design of tools required for evaluating immunity to EEHV.

5. Conservation outcomes for elephants, other wildlife, habitat and human communities, and other major findings and accomplishments to date

Below are summarized findings and accomplishments to date:

African elephant genome sequencing and serology tests

1. The full genome sequences of EEHV3A (Nyah- from the Indianapolis zoo), and EEHV3B (Ms Bets—Fresno zoo) have now been completed. Sequencing was accomplished by the Human genome sequencing center here at Baylor College of Medicine and Gary Hayward has done a considerable detailed analysis of the genomes. Briefly, the divergence of these genomes is somewhat greater than we see for EEHV1A versus 1B seen in Asians. One speculation is whether this represents two distinct EEHV3 species; one that coevolved with Loxodonta Africana and the other with Loxodonta Cyclotis. The level of divergence of these viruses hints at that, but more evidence is required to make a stronger case.

A draft of the complete annotated sequence of EEHV3A has already been submitted and is publicly available in GenBank, accession number MN373268.1.

Drafts of the EEHV2 and 6 genomes are attached to this report. We anticipate EEHV3B to be completed soon and that all three will be available in GenBank by the end of the year.

In summary, and in close collaboration with Gary Hayward at Johns Hopkins, we have completed the sequencing and annotation of the genomes for EEHV3A, EEHV2 and EEHV6. We are hopeful that the sequences for EEHV4B and EEHV5B will be completed in the coming weeks. In short, complete annotated genomes of all major EEHVs endemic within African and Asian elephants should be finished in the coming months. This effort will provide an essential source of information for investigation of EEHV biology in both species of elephants for years to come.

2. We have generated an EEHV3 serology test based on the immunoreactivity to the EEHV3 E34 protein, which we derived from sequencing the genomes of EEHVs endemic within African elephant populations. Initial results, which we have presented at the EAZWV conference in July 2020 and in the Journal of the Elephant Managers Association (JEMA) indicate that we can detect antibodies specific for EEHV3 from elephants who have been previously infected with EEHV3 and possibly other EEHVs. This test was used to determine that all three elephants that died from EEHV HD caused by EEHV3 were sero-negative for prior EEHV3 infection. These results suggest that lethal infection in these elephants resulted from primary infection. Notably, we have obtained similar findings in Asian elephants previously (Fuery et al , J. Virol 2020), indicating that both species may share similar dynamics for underlying factors that lead to EEHV-HD..

We have submitted a manuscript describing these results to the Journal of Virology and while it received a positive review, the journal editors felt that it might be a better fit for a sister journal, also published by the American Society for Microbiology (ASM) called Microbiology Spectrum. We are in the process of addressing a couple of minor comments raised by the reviewers and are hopeful for full acceptance shortly.

Asian elephant EEHV vaccine:

1. As outlined in our 2020 annual report, we initiated preclinical studies in mice to test a prototype EEHV vaccine. While analyses of these experiments was delayed due to the pandemic, we have now completed one preclinical trial in mice with our MVA-gB vaccine (i.e., recombinant virus vector vaccine) and just completed a second trial with a vaccine comprised of a purified gB protein (similar in concept to the Shringix vaccine for Shingles). Data for this vaccine's effectiveness at inducing an immune repose towards EEHV gB indicates that it is almost identical to the responses seen for the MVA gB vaccine. We are currently preparing a draft of these results for publication. We believe publication of these results will be critical for approval by regulatory agencies to test these vaccines in elephants.

6. Approximate number of humans/communities impacted by the project and approximate numbers of elephants impacted.

Knowledge of the complete genome sequences of the EEHVs endemic within African and Asian elephants will be a significant knowledge base for anyone doing research on EEHV. Our first generation serology assays will also be useful for screening elephants in captivity and those in the wild. When the Covid 19 pandemic ends and travel restrictions ease, we anticipate that we will collaborate with our colleagues in South Africa (Dr. Michelle Miller) for example, to conduct surveillance for anti-EEHV antibodies on collections of serum samples from wild elephants. Our vaccine development for EEHV in Asian elephants is in early stages. Success with this effort will impact all institutions that care for Asian elephants, especially ones that have breeding programs.

7. Problems discovered during grant period
Except for delays caused by the Covid 19 pandemic, we are on target to accomplish our original goals and objectives.

8. Project success evaluation.
We identified samples appropriate for determining the genome sequences of EEHVs 2, 3A, 3B, 6, 4B and 5B. Thus, we have nearly completed the goal of determining the genome sequences of most, if not all, of the major EEHVs endemic within African and Asian elephants. In addition, we developed a first generation serology assay for detecting anti-EEHV3 antibodies in African elephants. Thus, within the first year we have nearly completed goals outlined for research on African elephant EEHVs. A significant first step in the development of a vaccine is to test its ability to stimulate an immune response in animals and to assess it for potential toxic effects. In this regard, our MVA-gB recombinant vaccine showed no ill effects in mice and induced the mice to make antibodies and T cell responses towards the EEHV gB protein, which we believe will confer at least protection from lethal infection with EEHV (i.e., in an elephant). Initial results with a protein-based gB vaccine have yielded similar results and we look forward to testing whether or not using the two vaccine in combination might work even better.

9. What is the next step for this project and what are the implications for future conservation actions?
We intend to publish the results of our first two vaccine trials in mice, which both tested immune responses generated to the EEHV gB protein. Additionally, we would like to evaluate 2-3 other EEHV proteins identified from our earlier study as having the potential to elicit protective immunity against EEHV (Fuery et al 2018, J. Virol.) using similar strategies both separately and in combination with the gB vaccines already tested. Providing that we continue to obtain positive results, we would like to begin the process of identifying partners (in biotech) to manufacture these vaccines for testing in elephants.

10. Human interest story.

In our last report from December 2020, we outlined the story of how a single elephant from the Houston zoo, Tupelo, has taught us an extraordinary amount about EEHV. At the time, Tupelo and her mother Tess were both pregnant. Fast forward to today and Tupelo gave birth to a female, Winnie (3/10/2021), and Tess to a male (Teddy (5/16/2021), bringing the total herd size to 13 elephants. Tupelo's story is even more remarkable since Winnie was the result of AI, which more often than not results in males.

11. Organizations associated with this project and their roles.
Baylor College of Medicine: All vaccine related experiments were conducted, analyzed, and evaluated in Dr. Paul Ling's laboratory. All sequencing was done through the Human Genome Sequencing Center (HGSC) at Baylor College of Medicine with assistance from Dr. Vipin Kumar and Xiang Qin (also at BCM). Our collaborator Gary Hayward at Johns Hopkins has worked extensively in interpreting the sequencing results from Baylor.

Houston zoo, Indianapolis zoo, Fresno Chaffee zoo, Maryland zoo, Oakland zoo, San Diego Safari Park: These zoos provided serum samples or necropsy samples used for determination of genome sequences for African elephant EEHVs and for development of an EEHV3 serology assay.

12. Itemized financial report.
See separate page, as requested.

13. Five high resolution photos:
(attached in email).

14. 2 minute video clip
(attached in email)

15. Publications and/or conference presentations:

Manuscripts in preparation or under review:

1. Pursell, TP., Clinton JLS , Tan, J., Peng, RS, Qin, X., Doddapaneni, H., Menn, V., Momin, Z., Kottapalli, K., Howard, L., Latimer, E., Heaggans, S., Hayward, GS, and PD Ling. 2021. Primary infection may be an underlying factor contributing to lethal hemorrhagic disease caused by EEHV3 in African elephants (*Loxodonta africana*) (In revision, Microbiology Spectrum)

2. Pursell, TP, Clinton J., and **Paul D. Ling**. 2021. Modified Vaccinia Ankara vaccine expressing EEHV gB induces humoral and cellular immune responses in mice. (In preparation)

3. Clinton J., and **Paul D. Ling**. 2021. An EEHV gB subunit vaccine induces humoral and cellular immune responses in mice. (In preparation)

Conference presentations:

1. Ling, P.D. ,EEHV Advisory group meeting (5/14/2021) *EEHV serology*

2. Ling, P.D., AZVT Webinar (7/29/2021) *EEHV serology*

16. Media coverage.

None so far this year

17. Social media associated with work supported by IEF

None so far this year

Itemized Financial report

Personnel costs January 1, 2021-June 30, 2021: $20,731.38
Supplies/services January 1, 2021-June 30, 2021: $17,717.13
Total: $30,458.51
A general summary is below:

Layout	CJI3	/JDIGGS		TOTAL BY G/L	
Object		WBS 1383012508		INTERNATIONAL ELEPHANT FDN - Y	
Cost Element		40031600	To 73642000	PRIVATE AWARDS...	
Posting Date		01/01/2021	To 06/30/2021		

RefDocNo	Posting Date	Cost Elem.	WBS Element	DocTyp	BusA	Σ	ValCOArCur	Cost element name
		40031600					38,458.51-	PRIVATE AWARDS
		63270000					4,765.98	BENEFITS - STAFF
		67000000					15,975.40	SALARY - STAFF
		73524000					787.30	CCM CHARGES
		73530000					19.06	SERVICES - COURIER
		73548000					10,458.07	SERV.-LAB ANALYSIS
		73610000					400.19	ANIMAL PURCHSS
		73642000					6,052.51	CHEMICALS
							0.00	

Itemized costs, not including personnel are below (sequencing costs to the human genome center and animal and reagent costs for vaccine studies in mice:

Run Date: 07/30/2021 — Baylor College of Medicine
Run Time: 13:11:25 — Detailed Project Expense (Direct Cost Only)
For the Period 01/01/2021 - 06/30/2021

WBS Element: : 1383012508 INTERNATIONAL ELEPHA
Principal Investigator: 00037755 Paul Dalling Ling
Termination Date : 12/31/2021

Doc Type	Document Number	PO Number/ Req Number	Posting Date	Item Description	Vendor	GL Acct	Order	Actual
ZI	2500039007		03/31/2021	03202103775531-Paul D Ling		73524000	100000	256.20
ZI	2500039176		04/30/2021	04202103775530-Paul D Ling		73524000	100000	170.80
ZI	2500039176		04/30/2021	04202103775530-Paul D Ling		73524000	100000	25.00
ZI	2500039318		05/31/2021	05202103775531-Paul D Ling		73524000	100000	104.30
ZI	2500039538		06/30/2021	06202103775530-Paul D Ling		73524000	100000	231.00
				Sub Total for GL Acct 0073524000 SERVICES-COMPARATIVE MEDICINE CHARGES				787.30
KR	190546377		03/18/2021	FedEx 784536736029 03/09/2021	FEDEX ERS	73530000	100000	9.46
ZX	14267802		06/02/2021	00029546/124328/Courier/Shippi		73530000	100000	9.60
				Sub Total for GL Acct 0073530000 SERVICES-COURIER				19.06
ZI	2500038941		03/23/2021	PMAPC-PL-1953;Antibody Purific		73548000	100000	1,010.00
ZI	2500038941		03/23/2021	PMAPC-PL-1953;Extra Run (Antib		73548000	100000	120.00
ZJ	14245174		04/30/2021	TSF PYMT FOR HGSC120SSF INVOIC		73548000	100000	6,525.58
ZI	2500039141		04/30/2021	PMAPC-PL-1957;Extra Run (Antib		73548000	100000	120.00
ZI	2500039141		04/30/2021	PMAPC-PL-1957;Grow and Freeze		73548000	100000	103.00
ZI	2500039141		04/30/2021	PMAPC-PL-1957;Mycoplasma Testi		73548000	100000	60.50
ZI	2500039141		04/30/2021	PMAPC-PL-1953;IgG Quanificatio		73548000	100000	70.00
ZI	2500039141		04/30/2021	PMAPC-PL-1953;Produce 1-5 lite		73548000	100000	688.40
ZI	2500039141		04/30/2021	PMAPC-PL-1957;IgG Quanificatio		73548000	100000	35.00
ZI	2500039141		04/30/2021	PMAPC-PL-1957;Produce 1-5 lite		73548000	100000	688.40
ZI	2500039141		04/30/2021	PMAPC-PL-1957;Antibody Purific		73548000	100000	1,010.00
ZJ	14281263		06/23/2021	TSF PYMT FOR HGSC INVOICE #HGS		73548000	100000	27.19
				Sub Total for GL Acct 0073548000 SERVICES-LABORATORY ANALYSIS				10,458.07
ZI	2500039007		03/31/2021	03202103775531-Paul D Ling		73610000	100000	212.25
ZI	2500039318		05/31/2021	05202103775531-Paul D Ling		73610000	100000	187.94
				Sub Total for GL Acct 0073610000 SUPPLIES-ANIMAL PURCHASES				400.19

Legends for photos

1. Dr. Clinton analyzing mouse T cell responses to the gB subunit vaccine on a Fluorescent Activated Cell Sorter (FACS) instrument.

2. Jessica Watts, a new graduate student in the lab assisting Dr. Clinton with FACS analysis.

3. Paul Ling with partial elephant herd at the Houston zoo.

4. The two youngest elephants Teddy and Winnie with Tess.

5. Tess and her new calf Teddy.