IEF interim report—Ling 2019

a. Project title: Development of EEHV-specific serological assays

b. Final report

c. Investigators

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d. Project Start Date: January 2, 2019

e. Project completion date: December 31, 2019

2. Conservation needs this project addresses

EEHV is the leading cause of death for captive juvenile elephants in North America and Europe. Development of a robust EEHV-specific antibody test is necessary for generation and evaluation of an EEHV vaccine and will have significant impact on Asian elephant breeding populations in those areas of the world. EEHV hemorrhagic disease (HD) is also recognized in captive and wild range country elephants. In some cases, elephants in human care represent a significant proportion of the population in their natural range country (e.g., Thailand) and so an effective EEHV vaccine could aid in the care and management of large populations throughout the world. Furthermore, we envision that the EEHV-specific antibody test for Asian elephants could facilitate the development an antibody assay specific for EEHVs endemic within African elephant populations.

3. Goals and objectives of the study

Goal 1: To establish an improved EEHV antibody test for evaluating responses to EEHV gB-based vaccines.
To achieve this goal, we will generate a serological test using the Luciferase Immunoprecipitation system (LIPS) to measure antibody levels to the outer membrane glycoprotein gB. This assay will be critical for evaluating vaccine effectiveness in small animal studies and ultimately in elephants.

Goal 2: Develop antibody tests to distinguish infection histories between EEHV1 and EEHVs 4 and 5 within Asian elephant populations.
To achieve this goal, we will test whether antibodies to a unique protein encoded by EEHV1, known as open reading frame Q (ORF Q), can serve as a biomarker to differentiate prior infection with EEHV1 from infection with EEHVs 4 and 5, which are known to be endemic within Asian elephant populations. Serum samples from normal healthy adult elephants, from cases of clinical nonlethal infection, and from lethal cases caused by EEHV1 will be tested for antibodies towards ORF Q. Because ORF Q from EEHV1B strains appears to be relatively diverged from EEHV1A strains, we may be able to differentiate between infections between these virus types by measuring antibodies to representative ORF Q proteins from these viruses.

4. Specific actions taken to achieve objectives
All elephants described in the proposal have now been screened for antibody responses to glycoprotein B and ORF-Q using LIPS as outlined in the proposal.

5. Activities that differ from original proposal
None.

6. Conservation outcomes for elephants, other wildlife, habitat and human communities, and other major findings and accomplishments to date
The results from our actions (#4 above) indicate that we can detect general anti-EEHV responses and differentiate between these responses and those specific for EEHV1A and/or EEHV1B. All of the EEHV HD cases examined so far indicate that these elephants had no prior history of being infected with EEHV1A prior to illness and death caused by infection with EEHV1A. In addition, three elephants who endured clinical illness caused by either EEHV1A or EEHV1B were also seronegative for the virus type that caused illness. The accumulated evidence suggests that lethal hemorrhagic disease or clinical illness caused by infection with EEHV1A or 1B is due to primary infection with these viruses and not reactivation.

7. Approximate number of humans/communities impacted by the project and approximate numbers of elephants impacted.
At the present time, we believe that our current immunologic profiling assays can determine whether or not an Asian elephant has been previously infected with EEHV1A or 1B, which are the two strains that cause most of the EEHV related deaths. In doing so, we will be able to predict potential

vulnerability of elephants for getting primary infection with these viruses and possible illness. Such knowledge could impact all institutions that care for Asian elephants, especially ones that have breeding programs. We anticipate that our assays could be adapted for use in range countries and impact elephants there too. Thus, our work could impact humans who care for elephants in addition to the elephants themselves.

8. Problems discovered during grant period
None.

9. Project success evaluation.
Our initial short-term goal was to evaluate our EEHV-specific serological assay in a small cohort of elephants with known or partially known EEHV histories. The results indicate that the assays we developed have superior sensitivity and dynamic range compared to previously reported EEHV serology assays. Importantly, we can distinguish whether or not elephants have had prior infection with EEHV1A or 1B. We would like to expand the number of elephants screened using our assays to battle test their reliability and to provide valuable information to institutions caring for Asian elephants, particularly breeding herds.

10. What is the next step for this project and what are the implications for future conservation actions?
Future goals include screening more elephants in North American institutions, and expanding our assays to definitively detect infections from EEHV4 and 5. We would like to either implement our assays in range countries or facilitate import of samples from range countries to cast a wider net for understanding sero prevalence of EEHVs world-wide and as a tool for management of (captive) herds in range countries.

11. Human interest story.
Unfortunately, lab work does not always lend itself to funny stories or strange occurrences. The best I can come up with is that a long-standing issue in the field was whether or not the clinical illness or lethal disease caused by EEHV was due to primary infection or a reactivation. As our data emerged, it became clear, at least from this cohort of elephants, that disease was 100% associated with primary infection. Moreover, we have now been able to look at anti-EEHV maternal antibodies in a few calves and it's clear that they can get lots of anti-EEHV antibodies from their mothers and that these antibodies can decline by age 2 or so. While this was a predictable scenario (albeit among many), the EEHV-specific LIPS assay provided a quick and convenient way to address this issue clearly for the first time. When we presented this data to our partners at the Houston zoo, they were suitably impressed and agreed that the ramifications for elephant management were significant. For me personally, this study represents one of the more significant achievements for the lab and I hope will be an important tool for the elephant community writ large.

12. Progress and results summary.
Elephant Endotheliotropic Herpesvirus (EEHV) can cause lethal hemorrhagic disease in juvenile Asian elephants, both in captivity and in the wild. Most EEHV deaths are caused by two chimeric variants of EEHV1- EEHV1A and EEHV1B, while two other EEHVs endemic within Asian elephants (EEHV4 and EEHV5) have been recognized, but cause death less frequently. It remains unknown whether lethal infections caused by EEHV are due to primary infection or reactivation of latent virus. Furthermore, knowledge of the anti-EEHV antibody levels in young calves is limited. A serology assay capable of distinguishing between infections from the various EEHV types is needed to resolve these important issues. To address this, a Luciferase Immunoprecipitation Systems (LIPS) assay for antibody profiling was investigated using a panel of conserved EEHV recombinant proteins and proteins unique to EEHV1. The results show that elephants who died from EEHV1 hemorrhagic disease (HD) or developed EEHV-associated clinical illness were seronegative for the EEHV species that caused disease or illness suggesting that these events were associated with primary infection

rather than reactivation. We were also able to demonstrate that waning of EEHV1 specific antibodies can occur in the first two years of life, where there may be a potential threshold protective level of antibody required to prevent HD. Identification of these "diagnostic" proteins together with the LIPS assay itself, are likely to be extremely useful tools for the study of EEHV immunity and responses to candidate EEHV vaccines in the future.

13. Organizations associated with this project and their roles.
Baylor College of Medicine: All experiments were conducted, analyzed, and evaluated in Dr. Paul Ling's laboratory.
Houston zoo, Albuquerque zoo, Oklahoma City zoo and Feld Entertainment: These zoos and organizations provided serum samples from elephants housed at their institutions that were used to establish the EEHV-specific LIPS assays.

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

15. Five high resolution photos:
(attached in email). Please note that Baylor and Baylor/Tupelo photos were taken by Stacy Adams/Houston zoo. Baylor (age 9), Tupelo (age 8), and Joy (age 2) have been exceptional as they are some of the few elephants in which we have abundant serum samples over time from birth. Their samples were critical for helping us validate the EEHV-specific LIPS assays. The other two photos are of the GloMax Luminometer (purchased with IEF funds), which was used for all LIPS experiments conducted so far and a photo of Baylor the elephant with Dr. Ling.

16. 2 minute video clip
(attached in email)

17. Publications and/or conference presentations:
We have presented this IEF supported work at the 2019 EEHV workshop in Houston, the 44th International Herpesvirus Workshop in Knoxville TN in July 2019, the AZA annual conference in New Orleans in September 2019, and the IEF conference in South Africa. We now have published a paper in the Journal of Virology describing our findings.

Abstracts:

1.Serological detection of EEHV infection
Fuery, A., Hayward, G.S., and PD Ling
EEHV workshop 2019, Houston TX

2. Serologic detection of Elephant Endotheliotropic Herpesvirus (EEHV) infection
Fuery, A., Hayward, G.S., and PD Ling
IHW 2019, Knoxville TN.

3. Updates on Asian elephant EEHV serology test and African elephant EEHV genome sequencing efforts.
Paul D. Ling
Elephant Managers Association Conference, Denver, October 2019.

4. Serological detection of EEHV infections by a Luciferase Immunoprecipitation System assay.
Paul D. Ling
International Elephant Foundation Conference, Bela Bela, South Africa, October 2019

Manuscripts

Fuery, A., Pursell, T., Tan, J., Peng, R.S., Burbelo, P., Hayward, G.S., and **Paul D. Ling**. 2020. Lethal hemorrhagic disease and clinical illness associated with the elephant EEHV1 virus are caused by primary infection: Implications for the detection of diagnostic proteins. J. Virol. 94:e01528-19.

18. Media coverage.
No media coverage yet.

19. No social media postings with this project at the current time.

Itemized budget

GloMax Luminometer system: $6,916.13
Luciferase Substrate: $1,482
Tissue culture reagents: $1265
Gene synthesis: $2794.97
Midi plasmid kit: $685
Transfection reagent: 826
Total: $13,969.1

We will provide documentation from Baylor College of Medicine confirming these expenses, if needed.