



Noninvasive EEHV detection  
in semi-captive and free-ranging Asian elephants  
Project period January 1, 2019 to April 1, 2020

Final Report  
to the International Elephant Foundation  
Submitted June 1, 2020

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We are grateful for the support of the International Elephant Foundation as we have pursued a non-invasive testing protocol that can effectively detect Elephant Endotheliotropic Herpesvirus (EEHV) shedding in Asian elephants (*Elephas maximus*) when blood draws and trunk washes are not an option. The Houston Zoo and its partners have worked for more than a decade to address the threat of EEHV, developing a treatment protocol for EEHV hemorrhagic disease that has saved the lives of juvenile elephants, supporting colleagues in global efforts to combat EEHV, and working toward a vaccine side by side with Baylor College of Medicine.

### **Conservation Needs Addressed**

EEHV is the leading cause of death in Asian elephant calves in North America and Europe and it is known to occur in range countries, though the extent of this virus' impact is unknown. A deeper understanding of the most important viral disease of this species may offer insights on effective management strategies and facilitate the ability to clinically manage the disease when encountered. One of the challenges in evaluating EEHV epidemiology in range countries is the inability to collect samples from wild or untrained elephants, such as blood samples and trunk washes. This project aims to evaluate non-invasive testing methodologies using feces and chewed plants that would allow for PCR detection of the virus without touching the elephant. This will help lay the foundation for evaluating the prevalence of EEHV in semi-captive and free-ranging elephant herds in Myanmar. Moreover, semi-captive management strategies may impact elephant glucocorticoid levels and potential EEHV shedding, a long suspected, but poorly understood theory. This study will set up a longitudinal epidemiology study on the impacts of various semi-captive management strategies in Myanmar.

### **Goals and Objectives**

There have been no changes to the goals and objectives of our project since our application. Below are listed the project's goals and objectives alongside status updates for each objective.

#### **Phase 1 – Houston Zoo Herd Testing for EEHV**

GOAL: Optimize noninvasive sampling protocol to test for EEHV using samples from Houston Zoo elephants.

##### ***Objectives***

**1.1:** Collect samples of blood, trunk wash, oral swab, feces, and chewed vegetation coated with saliva, mimicking field conditions (delayed freezing) with a subset of samples.

**1.2:** Use qPCR to test samples for EEHV shedding and viremia.

**1.3** Devise optimal noninvasive protocol for use with untrained elephants in Asia.

##### ***Final Report Updates***

Sample collection is complete. The entire herd had samples collected for 5 months and 3 viremic calves had samples collected daily while they were shedding viral DNA, as detected in trunk washes.

DNA has been extracted from samples from 1 viremic calf, while the other 2 viremic calves are being processed in summer 2020. Moreover, PCR has been used to test some blood and trunk wash samples, primarily from the 3 viremic calves.

The optimal protocol will be devised following further action on Objective 1.1 and 1.2.

#### **Phase 2 - Myanmar Herd Testing for EEHV**

GOAL: Identify EEHV shedding prevalence in semi-captive and free-ranging elephant herds in the Bago Yoma mountain range of Myanmar.

### ***Objectives***

**2.1:** Use optimized noninvasive protocol to collect and store samples from semi-captive and free-ranging Asian elephants in Myanmar.

**2.2:** Use qPCR diagnostics to test samples for EEHV shedding.

### ***Interim Report Updates***

Sample collection in Myanmar is complete and followed the same protocol utilized at the Houston Zoo.

A PCR workshop was held in Myanmar in February 2020 to provide PCR supplies and build capacity for EEHV PCR testing within the country. Some of the collected research samples were processed during this workshop and the remainder are currently underway.

### **Phase 3 – EEHV and Stress**

GOAL: Use noninvasive sampling protocol together with environmental, management and demographic data to lay groundwork for a longitudinal epidemiological study on the impacts of management strategies on EEHV shedding in Asian elephant range countries.

### ***Objectives***

**3.1:** Use enzyme immunoassay to test fecal samples for glucocorticoids.

**3.2:** Collect covariate data on environmental factors, management practices, and elephant demographics.

**3.3:** Compare covariate data with sampling data to identify any patterns that should be explored in a future longitudinal epidemiological study.

### ***Interim Report Updates***

Sample collection at the Houston Zoo and Myanmar is complete.

Data collection at the Houston Zoo and Myanmar is complete.

This is planned after sample and data analysis.

### **Phase 4 – Dissemination of Findings**

GOAL: Disseminate findings to aid elephant managers and researchers.

### ***Objectives***

**4.1.** Disseminate information to Asian elephant community via the EEHV Asia Working Group.

**4.2.** Publish findings in peer-reviewed journal to reach larger scientific community.

### ***Interim Report Updates***

This will occur after the other goals of the study are complete.

This will occur after the other goals of the study are complete. Data from the 2018 pilot study has been accepted by the Journal of Zoo and Wildlife Medicine for publication (Jeffrey A, Evans TS, Molter C, Howard LL, Ling P, Goldstein T, and Gilardi K. Noninvasive sampling for detection of elephant endotheliotropic herpesvirus and genomic DNA in Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. JZWM 2020;51(2)).

### **Changes to Activities**

- In addition to sampling the entire Houston Zoo herd, we sampled 3 viremic calves daily during the study period. One calf had clinical EEHV-HD, while the other 2 were low-level viremic. This increased our sample size significantly and more supplies were needed to accommodate this daily testing.
- In February 2020, in addition to the PCR workshop to facilitate sample processing in Myanmar, an Elephant Health Workshop was held. The Elephant Health Workshop aimed to provide veterinarians, veterinary technicians, and mahouts in Myanmar a 2-day intensive workshop focused on clinical aspects of elephant health including EEHV, tuberculosis, overall wellness, clinical techniques, and diagnostic sample processing. Staff from the Houston Zoo, University of California – Davis, Smithsonian Conservation Biology Institute, Oregon Zoo, and Myanmar Timber Enterprise facilitated the workshop.

### **Conservation Outcomes**

This project is still in the sample analysis phase at both the Houston Zoo and in Myanmar, but it is anticipated that this work will lay the foundation for a long-term epidemiology study of EEHV in semi-captive and free-ranging Asian elephant herds in Myanmar. This will help increase vigilance for disease monitoring and clinical case management, as well as evaluate management practices that may impact glucocorticoid levels and EEHV shedding.

### **Impact on Humans and Elephants**

We expect that three populations of elephants, those in logging camps, tourism camps, and free-ranging herds will be impacted, in addition to the communities that care for elephants there.

The Houston Zoo not only cares for elephants in our own herd, but also cares for elephants around the world by supporting our colleagues and partners in the global elephant community. Through what we have learned about elephants in zoos, we have been able to develop life-saving treatment protocols and diagnostic tests that are shared worldwide. We can apply these advanced diagnostics in novel ways, such as testing feces and chewed plants, to assess otherwise inaccessible elephant populations. Moreover, the role of stress hormones in EEHV remains largely unknown and gaining a deeper comprehension by evaluating fecal samples, paired with EEHV qPCR has wide reaching management implications. Therefore, understanding the epidemiology of EEHV in range countries, like Myanmar, will set the stage for improving management and targeting at risk herds for vaccination in the future to sustain healthy elephant populations.

### **Challenges**

Collecting samples on a total of 3 viremic calves was a challenge to our initially set timeline and budget; however, we feel that the information garnered from these 3 cases will be important. These samples are scheduled to be analyzed this summer.

We have met some logistical challenges related to the ongoing COVID-19 pandemic and do anticipate some delays in our processing of the remaining samples, given laboratory closures and travel restrictions.

### **Successes**

We feel that this project has been successful so far. Our short-term goals to complete sample collection were achieved in 2019 and analysis on samples collected at the Houston Zoo and in Myanmar has begun. We are on track to complete these activities within the next several months, pending permissions to work given the ongoing COVID-19 crisis. Pilot data from 2018 will be published as in the June edition of the Journal of Zoo and Wildlife Medicine. This pilot data showed promise as EEHV was detected in several noninvasive African elephant samples

(San Diego Zoo Safari Park) and Asian elephant DNA was detected on hay samples from the Houston Zoo, which guided our current collection efforts. Our long-term goals are to disseminate our findings from the current data set, which is much more extensive and longer-term, and use them as a basis for an epidemiological study of EEHV in semi-captive and free-ranging elephants with the ultimate goal of mitigating and treating EEHV and EEHV-HD in these populations, and we remain confident that our project will make this valuable contribution to the study of EEHV.

Another success in this phase of the study, which developed organically to address the needs of our partners in Myanmar, was the PCR and Elephant Health Workshop. Although these workshops were not directly supported by this grant specifically, the samples that were processed during the PCR workshop were collected with the support of the International Elephant Foundation.

### **Next Steps**

As described above in the goals section, our next steps include continuing to analyze samples from the Houston Zoo and Myanmar, compare the datasets and propose an optimal protocol for non-invasive testing, and disseminating our data.

### **Story**

The Houston Zoo is fortunate to have one of the strongest breeding herds of Asian elephants in the United States, and the whole Houston community has been overjoyed to welcome two elephant calves in the last three years. It is a delight to watch the two calves frolic together and to see all of the cows in the herd, but particularly older sister Tupelo, look after these rambunctious youngsters. The importance of our EEHV work was underlined when Joy, our two-year-old, became ill in April 2019. Juvenile elephants in our herd are tested weekly for EEHV, allowing staff to catch active infections before elephants become symptomatic – at which point, it is often too late. We are grateful to have strong partners in Baylor College of Medicine – just across the street in the Texas Medical Center – and with their help, Joy’s infection was detected in time to provide life-saving treatments. Intensive treatment began within hours and continued round-the-clock for over a week. Incredibly, the herd matriarch, Methai, donated over 17 units of blood and plasma. It is truly remarkable how Joy’s whole community – researchers, keepers, vets, and even other elephants – were rallied to save her life. But we hope that, through our partnership with Baylor College of Medicine, a vaccine is just a few years away and crises like Joy’s will soon be a memory for elephants and their keepers all over the world. Read more about Joy’s illness on the Houston Zoo blog: <https://www.houstonzoo.org/blog/houston-zoo-elephant-calf-completed-treatment-for-deadly-virus/>

### **Lay Summary**

EEHV is the leading cause of death of young Asian elephants in human care in both North America and Europe. Its impact on wild populations is believed to be significant as well, but because we do not yet have widespread means to test for it, we cannot know for sure. Currently, our only accurate tests for EEHV require blood or trunk wash samples, and wild elephants are not trained to provide them.

With this study, the Houston Zoo, International Elephant Foundation, and a coalition of partners are working to develop a test that does not require invasive samples, meaning that an elephant does not need special training or to be touched in order to be tested. Basing our work on testing techniques that have successfully detected herpesviruses in rodents and primates, we are working to discover whether EEHV can be detected accurately in elephants’ droppings and

saliva left on chewed plants. Both of these sampling methods could potentially elephants both in human care and the wild.

As we collect samples for EEHV testing, we are also looking at covariant data – stress hormones in elephants’ fecal samples as well as stress factors like social changes in the herd – to discover whether stress may play a role in deadly EEHV infections.

This research is in the early stages, but if we are successful in developing a protocol for non-invasive EEHV testing, we hope it lays the groundwork for studies that will map EEHV throughout wild elephant populations in Asia. And when someday a vaccine is developed, these studies will tell us where vaccination is most needed. Meanwhile, our stress study will tell us a little more about mitigating EEHV everywhere.

### **Organizations and Roles**

- Houston Zoo – sample collection, processing, protocol development
- University of California Davis Wildlife Health Center – sample collection (Myanmar), protocol development, qPCR
- Baylor College of Medicine – protocol development, qPCR
- San Diego Zoo Safari Park – protocol development
- Smithsonian Conservation Biology Institute – protocol development, glucocorticoid analysis
- Myanmar Timber Enterprise – sample collection, processing, protocol development

### **Expenses**

To date, the project has spent \$4,121.83 on collection supplies at the Houston Zoo. The remaining \$3,278.17 has been spent on PCR supplies both for domestic processing and in Myanmar.

Expense	
9 boxes of Thermo Scientific MicroTest Tubes Reme M6 B/72	\$2,573.47
2 bags (x1000 count) cryovials	\$1,221.90
5 bags (x100 tubes) 50 ml conical tubes	\$255.00
2 boxes (x100 each) Dacron swabs	\$19.90
1 box (x500 count) Whirl packs	\$51.56
Supplies Subtotal	\$4,121.83
PCR supplies both for domestic processing and in Myanmar.	\$3,278.17
Total Expenses	\$7,400.00

### **Publications**

The information generated is intended to be published in the future and presented at conferences, including EEHV Workshops with the intention to present at the EEHV Asia Working Group. Distributions of materials to those working with elephants in range countries will be done in cooperation with the EEHV Asia Working Group. We will be happy to provide copies when they become available.

### **Media Coverage**

Though the Zoo's EEHV work over the years has been featured in a number of print and broadcast media, this specific project has garnered no media attention to date. When we have results ready to publish, we will work with the Zoo's PR department to determine whether our media partners might be interested in the story.

### **Find us Online**

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UC Davis Drayer Wildlife Health Center

Web: [whc.vetmed.ucdavis.edu](http://whc.vetmed.ucdavis.edu)

News: [whc.vetmed.ucdavis.edu/news-0](http://whc.vetmed.ucdavis.edu/news-0)

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San Diego Zoo

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Smithsonian Conservation Biology Institute

Web: [nationalzoo.si.edu/conservation](http://nationalzoo.si.edu/conservation)

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