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THE EEHV CONSORTIUM
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NEHL at the National Zoo
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The EEHV Consortium

at National Elephant Herpesvirus Laboratory **Update**

Using Serum Biomarkers to Investigate the Immune Response to EEHV

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USEFUL LINKS FOR EEHV PREPARATION

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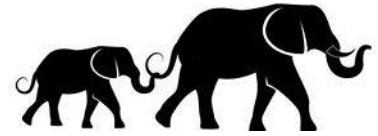
One of the greatest challenges with EEHV is the speed at which viremia and hemorrhagic disease can develop. Often, by the time the first clinical signs are seen, the virus has already taken hold and it can be an uphill battle to contain the vascular damage and associated effects that follow. One hypothesis for this rapid development is that the calf's immune system is too naïve to mount an adequate response, perhaps in combination with active suppression of the host immune system by the virus itself. However, surprisingly little is known about the interaction between the elephant immune system and EEHV, and it remains unclear why some calves succumb to hemorrhagic disease, while others are able to recover or experience only asymptomatic or latent infections. We question whether these varied responses are due to differences in immune system responses, or if there are some viral strains that are just more difficult to keep under control. Without a better understanding of basic immune processes in elephants, it will be difficult to predict if an individual will be able to successfully fight the infection.

At the Smithsonian Conservation Biology Institute's Endocrine Research Lab, we are using a number of assays to measure serum biomarkers, including cytokines and acute phase proteins, to help us better understand the elephant immune response. Cytokines are a group of protein mediators of the immune response, involved in activating and recruiting immune cells to the site of infection. Acute phase protein production is stimulated by cytokines as part of an inflammatory response to help the body deal with and repair tissue damage.

Measures of these immune biomarkers have shown promise in helping us understand how elephants fight other types of infection, and potentially could provide insight into the immune response to EEHV. Several cytokines and acute phase proteins have been shown to play an important role in other viral hemorrhagic diseases, such as Crimean-Congo hemorrhagic fever, Dengue hemorrhagic fever and Ebola. Together these proteins are key to a successful immune response, but overproduction, or an inappropriate balance of cytokines associated with cell-mediated and humoral branches of the immune response, could also be associated with pathology. By using a panel of multiple markers together, we hope to learn more about the elephant immune response, how it should be responding, and whether there are differences between cases of asymptomatic viremia, and hemorrhagic disease that result in survivors or fatalities.

In collaboration with the National Elephant Herpesvirus Lab, and supported by grants from the Smithsonian Institution Scholarly Studies Award Program and Smithsonian Women's Committee, we are using banked serum from past cases, along with samples collected surrounding any active cases of EEHV viremia or viral shedding, to determine whether changes in immune function can be detected. If we can determine where the immune response may be failing, this could provide important diagnostic and prognostic information, and help with designing and assessing the efficacy of targeted treatments to support the individuals' own immune system.

If you have any questions about this research, please contact edwardskl@si.edu or brownjan@si.edu.



EEHV Diagnostics Training in Southeast Asia

After a request for training in EEHV molecular diagnostics from the attendees of the 1st and 2nd EEHV Asia Working Group meetings in 2015 and 2016, Erin Latimer (Smithsonian's National Zoo), Supaphen Sripiboon (Kasetsart University), and Vijitha Perera (Elephant Transit Home) put together funding and planned a training workshop that was held at Kasetsart University in Thailand from Nov 13-15, 2017.

Trainees received hands-on instruction in DNA preparation and analysis, and polymerase chain reaction (PCR), as well as lectures on EEHV in Asia, PCR, sample handling, field necropsies, and calf monitoring. They received reagents to start the testing in their own laboratories. A Facebook page was started to facilitate further education and collaborations amongst the participants and lecturers. The workshop received an average rating of 4.7 (out of 5), for factors such as knowledge gained, value of the workshop, helpfulness of the organizers, etc. Requests for future training included information on DNA sequencing, developing monitoring and treatment protocols, sample collection/shipment, and collaboration between the EEHV diagnostic labs.

These new trainees will now be able to train additional colleagues in Asian range countries to further elephant conservation, EEHV monitoring, and EEHV research. Dr. Chia-Da Hsu of the Singapore Zoo went to Sumatra in March of 2018 to train colleagues from Syiah Kuala University, University of Gadjah Mada, and VESSWIC. Additional trips are being considered for Myanmar and Vietnam; funding for PCR equipment needs to be obtained for both of these countries before these trips come to fruition.

Planning for additional laboratories has been started for all of the range countries in Asia. A core group consisting of Supaphen Sripiboon, Erin Latimer, Chatchote Thitaram, Sonja Luz, Chia-Da Hsu, Amir Sadaula, and representatives from the Asian range countries will pursue funding, identify possible lab sites, and organize future training sessions.

The workshop and additional training trips were made possible by support from Asian Elephant Support, Kasetsart University, Zoological Parks of Thailand, Wildlife Reserves Singapore, and the Smithsonian Institution.



ASIAN ELEPHANT
SUPPORT



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Recent EEHV/Elephant Publications:

Fuery A, Leen AM, Peng R, Wong MC, Liu H, Ling PD. 2018. *Asian elephant T cell responses to elephant endotheliotropic herpesvirus*. J Virol 92:e01951-17. <https://doi.org/10.1128/JVI.01951-17>.

Palkopoulou E et al. *Genomic History of Extinct and Living Elephantids*. PNAS Mar 2018, 115 (11) E2566-E2574; DOI: 10.1073/pnas.1720554115

Kochagul V, Srivorakul S, Boonsri K, Somgird C, Sthitmatee N, Thitaram C & Pringproa K. *Production of antibody against elephant endotheliotropic herpesvirus (EEHV) unveils tissue tropisms and routes of viral transmission in EEHV-infected Asian elephants*. Sci Rep (2018) 8:4675 DOI:10.1038/s41598-018-22968-5.

Boonsri K, Somgird C, Noinafai P, Pringproa K, Javyamethakul T, Angkawanish T, Brown JL, Tankae P, Srivorakul S, and Thitaram C. *Elephant Endotheliotropic Herpesvirus Associated w/ Clostridium Perfringens Infection in Two Asian Elephant (Elephas Maximus) Calves*. JZWM. 2018 49 (1), 178-182. doi.org/10.1638/2017-0001R1.1





2017 NEHL Activities

Erin Latimer

Sample Testing

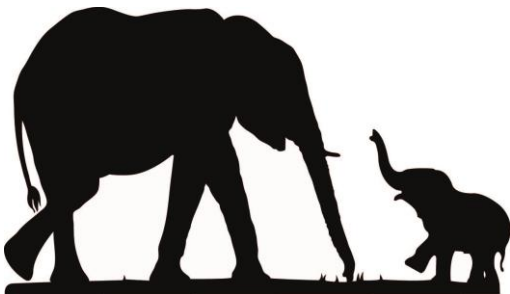
Over the last calendar year, the NEHL tested over 1000 samples, from 379 shipments from elephant-holding facilities. Most of these shipments were from EEHV Consortium members and most of the samples were for routine testing, either whole bloods from calves that we monitor weekly for early detection of viremia or trunk wash samples that we screen for EEHV shedding.

EEHV Cases in 2017

The US did not have any deaths due to EEHV in 2017. There were several calves with EEHV viremias due to different EEHVs with varying viral levels. One calf had an EEHV5 viremia (peak of 30,000 vge/ml) followed by an overlapping low level EEHV1 viremia. Another calf had an EEHV5 viremia with a peak level of 90,000 vge/ml. Both of these calves remained healthy and had no clinical signs.

One calf had EEHV1 Hemorrhagic Disease (peak viral levels of 500,000 vge/ml), followed about a month later by an EEHV5 viremia (peak viral levels of 23,000 vge/ml). Following an initial EEHV1 viremia report (8500 vge/ml) from routine weekly monitoring of EEHV, it was decided to initiate treatment and monitor EEHV1 levels once or twice daily. In addition to monitoring daily EEHV1 levels (NEHL), the five year-old patient was intensively monitored with CBC (including platelet counts), serum chemistries, and thromboelastography (TEG).

Treatment included nine liters of fresh plasma administered over the first ten days of treatment. The first day of treatment included both the recommended levels of Famvir and fresh plasma from another elephant (previously cross-matched), which had been administered Famvir at double the normal dose, with plasma collected ~1.5 hours post treatment (theoretically to provide the active form of the drug to the patient). Fresh plasma was administered again on day six of treatment. Famvir was administered rectally as well as rectal fluids including antibiotics, and electrolytes (at different time points). The intensive care provided by elephant staff, to administer the rectal fluids, electrolytes, and medications in addition to the collection of samples for testing and administration of plasma and fluids IV, was instrumental in the treatment and survival of the calf. *Details provided by the attending veterinarian.*



Meetings and Capacity-Building Here and Abroad

- In May 2017, I attended the 11th International EEHV Symposium in London. Details can be found [here](#).
- I went to Borneo on a State Department-funded capacity-building trip in July/Aug, to help the Sabah Wildlife Department (SWD) after the deaths of three Bornean calves in 2016 due to EEHV. I provided reagents, protocols, and training to three colleagues (two from SWD and one from EcoHealth Alliance) in the molecular diagnostics of EEHV.
- In November, I helped train ten Asian colleagues at Kasetsart University in Thailand on EEHV molecular diagnostics. The group of researchers, veterinarians, and pathologists were from Nepal, Thailand, Sri Lanka, Malaysia, and Singapore. Co-trainers were Dr. Vijitha Perera from Sri Lanka and Dr. Amm Sripiboon from Thailand. I put together funding from Asian Elephant Support, Wildlife Reserves Singapore, and Zoological Parks of Thailand to fund the travel/reagent costs of workshop participants. Following the training workshop, I attended the EEHV Asia Working Group meeting, also held in Thailand.
- I provided training and consultation to the Oklahoma City Zoo in setting up an onsite EEHV qPCR laboratory.
- A paper was published about the Maryland Zoo case of EEHV3b (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.
- I started collaborating with Janine Brown and Katie Edwards on the project *Using Serum Inflammatory Markers to Investigate the Immune Response to Elephant Endotheliotropic Herpesvirus* (see story on p1).