

EEHV Testing Capacity-Building in Asia, Facilitated by the EEHV Asia Working Group

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Elephant Endotheliotropic Herpesvirus (EEHV) is a major cause of death for young Asian elephants (*Elephas maximus*), with a fatality rate of up to 80% in elephants between the ages of 1–8 years in human care. More is known about the prevalence of EEHV in elephants in human care, but morbidity and mortality due to EEHV has also been seen in wild elephants in Asia. The hemorrhagic form of the disease (EEHV HD) occurs when the primary infection is not controlled by the immune system; this can occur in up to 20% of calves and it is not currently known why this occurs. Most of the deaths in Asian elephants due to EEHV HD are from EEHV1A, with deaths and serious disease also caused by EEHV1B, EEHV3A, EEHV4, and EEHV5. African elephants can carry the related EEHV2, EEHV3B, EEHV6, and EEHV7 viruses latently; there have been isolated deaths and disease due to EEHV2, EEHV3B, and EEHV6 in African elephants. Little is known about the prevalence and impact of EEHV on captive and wild elephant populations in Asian elephant range countries.

The EEHV Asia Working Group consists of veterinarians, researchers, animal husbandry experts, conservationists, and range country government experts, united to decrease elephant deaths from EEHV in Asia. At the group's inaugural meeting in Singapore in 2015 concerns were identified, as follows:

- The epidemiology of EEHV in elephants in Asia and its impact on populations is currently unknown. Within the last 10 years, 59 fatal cases of EEHV disease in Asian elephants have been identified within the eight range countries represented at our

meeting. Twelve of these deaths were wild elephants.

- The identification of EEHV-associated deaths in wild elephants in Asia is significant and it is the opinion of the Working Group that EEHV is a conservation concern requiring close monitoring and further study.
- Early diagnosis of EEHV-associated disease in young elephant calves allows early treatment and a better chance of a successful outcome. Therefore, an important consideration is that the examination, sample collection, and treatment of young calves depend on the ability to handle and manage the calf from a very young age (less than 1 year old).
- Laboratories are critical to the routine monitoring, detection, and post mortem evaluation of elephants affected by EEHV. Currently, of 13 Asian elephant range countries, only 3 (Thailand, Indonesia, and India), have laboratories capable of confirming EEHV.

At the second meeting in 2016, again held in Singapore, three focus areas were decided on: 1) Evaluation of EEHV Epidemiology and Risk Factors; 2) Establishment of cPCR and qPCR EEHV Laboratories; and 3) Surveillance of wild elephants for EEHV. At the time, still only three Asian range countries (Thailand, Indonesia, and India) had lab capacity capable of diagnosing EEHV. As local lab capacity is necessary for calf monitoring, diagnostics, and research support, a sub-group of the Working Group was tasked with increasing EEHV testing capacity in Asia. A regional workshop with a train-the-trainers format was decided on, to provide several

colleagues with the knowledge and skills to perform the EEHV molecular diagnostics in their own laboratories; these colleagues would also provide follow-up training to colleagues in other range countries in the same protocols that they were taught.

SurveyMonkey surveys were used to determine interest, level of training desired, and current lab capacity. In 2017, we were able to conduct the first International Workshop on Molecular Diagnosis for EEHV infection in Thailand. Funding was procured from Asian Elephant Support, Kasetsart University, Smithsonian's National Zoo, Wildlife Reserves Singapore, and Zoological Park Organization of Thailand. Kasetsart University hosted the workshop and provided laboratory space, housing for participants and local travel during the workshop. Supaphen Sripiboon (Kasetsart University), Erin Latimer (Smithsonian's National Zoo), and Vijitha Perera (Elephant Transit Home) provided lecture and/or laboratory training. Ten participants were chosen from the colleagues expressing interest; all had existing laboratory space and equipment and just needed EEHV-specific training and reagents. Nepal, Thailand, Sri Lanka, Malaysia, and Singapore were represented at the workshop. Follow-up work was planned to increase the number of colleagues trained and to procure funding to start, equip, and train new laboratories.

The workshop in November 2017 consisted of two days of lab and lecture. Trainees received hands-on instruction in DNA preparation and analysis, polymerase chain reaction (PCR) and real-time PCR, as well as lectures on EEHV in

Asia, molecular diagnostics, sample handling, field necropsies, and calf monitoring. They received reagents to bring back to their home 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.

One of our newly trained scientists, Dr. Chia-Da Hsu of the Singapore Zoo, provided training in March 2018 to four colleagues in Sumatra, Indonesia, using a similar format as our November workshop. In June 2018, Dr. Supaphen Sripiboon, our host for the November workshop, held another training session in Thailand for 24 Thai participants that are involved with the care of elephants in the wild and in human care.

By increasing the laboratory capacity for EEHV molecular diagnostics, range countries will be able to provide early diagnosis of EEHV in calves, in order to provide timely treatment and improve outcomes. Routine calf monitoring will be possible, as well as research studies to determine prevalence, improved treatment modalities, and the virus' pathology.

We have grant proposals in progress to secure funding for new testing laboratories and training in additional range countries.

