Elephant Endothelial Herpesvirus Publications (By date; most recent first)
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Sree Lakshmi, P., M. Karikalan, G. K. Sharma, K. Sharma, S. Chandra Mohan, K. Rajesh Kumar, K. Miachieo, A. Kumar, M. K. Gupta, R. K. Verma, N. Sahoo, G. Saikumar and A. M. Pawde (2023). "Pathological and molecular studies on elephant endotheliotropic herpesvirus haemorrhagic disease among captive and free-range Asian elephants in India." Microb Pathog **175**: 105972.

In the present research pathology and molecular diagnosis of elephant endotheliotropic herpes virus-haemorrhagic disease (EEHV-HD) among Asian elephants was studied. Out of 76 cases, 20 were positive for EEHV infection in PANPOL and POL1 based semi-nested PCR. Out of 20 samples, 10 samples were fatal cases of EEHV-HD while 10 were of either subclinical or latent infection. Acute onset haemorrhagic disease with EEHV-HD had anorexia, facial and neck swelling, cyanotic buccal mucosa and tongue, nasal and ocular discharge, and colic. The hallmark of gross finding in all cases were severe haemorrhagic lesions in the internal organs viz. cyanosis of tongue with multifocal petechial haemorrhages, diffuse epicardial and endocardial haemorrhages, swollen liver (rounded edges) with parenchymal haemorrhages, serosal and mucosal haemorrhages in gastrointestinal tract, congested kidneys with corticomedullary haemorrhages, highly congested meninges, and brain capillaries with haemorrhages. Microscopic findings in all the cases had severe vascular changes in the visceral organs. Microthrombi was present in the vasculature of tongue, heart, lung, liver, kidney, and brain. The endothelial lining of most of the blood vessels were swollen with apoptotic changes. Amphophilic to basophilic intranuclear inclusion bodies were observed in the endothelial cells. Immunostaining using anti-EEHV DNAPOL hyperimmune sera revealed intense positive signals in the endothelium of blood vessels and their walls. Quantification of viral load in necropsy tissue samples revealed highest in the heart (7.4 × 10(6)/µg of sample) and least in the brain (9 × 10(3)/µg of sample). The PCR amplicons from EEHV1 specific genes (POL1(U38) and TER were subjected to partial genome sequencing which had 99.9% similarity with the EEHV1A subtype. It was concluded that Asian elephants in India are latently infected for EEHV1 and in all the fatal EEHV-HD cases, EEHV1A subtype was the causative agent with characteristic pathomorphological changes in visceral organs.

Kosaruk, W., J. L. Brown, P. Towiboon, V. Punyapornwithaya, K. Pringproa and C. Thitaram (2023). "Measures of Oxidative Status Markers in Relation to Age, Sex, and Season in Sick and Healthy Captive Asian Elephants in Thailand." Animals (Basel) **13**(9).

Oxidative stress is a pathological condition that can have adverse effects on animal health, although little research has been conducted on wildlife species. In this study, blood was collected from captive Asian elephants for

the assessment of five serum oxidative status markers (reactive oxygen species (ROS) concentrations; malondialdehyde, MDA; albumin; glutathione peroxidase, GPx; and catalase) in healthy (n = 137) and sick (n = 20) animals. Health problems consisted of weakness, puncture wounds, gastrointestinal distress, eye and musculoskeletal problems, and elephant endotheliotropic herpesvirus hemorrhagic disease (EEHV-HD). Fecal samples were also collected to assess glucocorticoid metabolites (fGCMs) as a measure of stress. All data were analyzed in relation to age, sex, sampling season, and their interactions using generalized linear models, and a correlation matrix was constructed. ROS and serum albumin concentrations exhibited the highest concentrations in aged elephants (>45 years). No sex differences were found for any biomarker. Interactions were observed for age groups and seasons for ROS and catalase, while GPx displayed a significant interaction between sex and season. In pairwise comparisons, significant increases in ROS and catalase were observed in summer, with higher ROS concentrations observed only in the adult female group. Lower catalase activity was exhibited in juvenile males, subadult males, adult females, and aged females compared to subadult and adult elephants (males and females) in winter and the rainy season. There was a positive association between catalase activity and fGCMs ($r = 0.23$, $p < 0.05$), and a number of red blood cell parameters were positively associated with several of these biomarkers, suggesting high oxidative and antioxidative activity covary in red cells ($p < 0.05$). According to health status, elephants with EEHV-HD showed the most significant changes in oxidative stress markers, with MDA, GPx, and catalase being higher and albumin being lower than in healthy elephants. This study provides an analysis of understudied health biomarkers in Asian elephants, which can be used as additional tools for assessing the health condition of this species and suggests age and season may be important factors in data interpretation.

Kerr, T. J., J. van Heerden, W. J. Goosen, L. Kleynhans, P. E. Buss, E. Latimer and M. A. Miller (2023). "DETECTION OF ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS (EEHV) IN FREE-RANGING AFRICAN ELEPHANTS (LOXODONTA AFRICANA) IN THE KRUGER NATIONAL PARK, SOUTH AFRICA." J Wildl Dis **59**(1): 128-137.

Elephant endotheliotropic herpesvirus (EEHV) infection can cause acute, often fatal, EEHV hemorrhagic disease in free-ranging and human-managed Asian elephants (Elephas maximus) and human-managed African elephants (Loxodonta africana). However, significant knowledge gaps exist pertaining to the presence of EEHV in free-ranging African elephant populations. We retrospectively screened 142 opportunistically collected samples (blood, n=98; bronchoalveolar lavage (BAL) fluid, n=21; trunk wash (TW) fluid, n=23) obtained between 2010 and 2020 from 98 free-ranging African elephants in the Kruger National Park, South Africa, for the presence of different EEHVs, as well as determining the real-time quantitative PCR positivity rate in this population. With the use of validated, previously published DNA extraction and real-time quantitative PCR protocols provided by the National Elephant Herpesvirus Laboratory (Washington, DC, USA),

EEHV was detected in nine male African elephants from samples collected in 2011 (n=1), 2013 (n=1), 2018 (n=2), 2019 (n=4), and 2020 (n=1). Viral detection was more common in respiratory compared with blood samples. Six elephants tested positive for EEHV2 subtype (blood, n=2; BAL, n=3; TW, n=2), including one individual that tested positive on matched respiratory samples (BAL and TW). Four elephants tested positive for EEHV3-4-7 (blood, n=1; BAL, n=2; TW, n=1), whereas EEHV6 was not detected in any of the study animals. One elephant tested positive for both EEHV2 and EEHV3-4-7 in the same BAL sample. Even though the levels of viremia varied between 158 and 1,292 viral genome equivalents/mL blood and viral shedding of EEHV2 and EEHV3-4-7 was detected in respiratory samples, no clinical signs were observed in these apparently healthy elephants. These findings are consistent with reports of asymptomatic EEHV infection in human-managed African elephants.

Fomsgaard, A. S., S. A. Tahas, K. Spiess, C. Polacek, J. Fonager and G. J. Belsham (2023). "Unbiased Virus Detection in a Danish Zoo Using a Portable Metagenomic Sequencing System." Viruses **15**(6).

Metagenomic next-generation sequencing (mNGS) is receiving increased attention for the detection of new viruses and infections occurring at the human-animal interface. The ability to actively transport and relocate this technology enables in situ virus identification, which could reduce response time and enhance disease management. In a previous study, we developed a straightforward mNGS procedure that greatly enhances the detection of RNA and DNA viruses in human clinical samples. In this study, we improved the mNGS protocol with transportable battery-driven equipment for the portable, non-targeted detection of RNA and DNA viruses in animals from a large zoological facility, to simulate a field setting for point-of-incidence virus detection. From the resulting metagenomic data, we detected 13 vertebrate viruses from four major virus groups: (+)ssRNA, (+)ssRNA-RT, dsDNA and (+)ssDNA, including avian leukosis virus in domestic chickens (Gallus gallus), enzootic nasal tumour virus in goats (Capra hircus) and several small, circular, Rep-encoding, ssDNA (CRESS DNA) viruses in several mammal species. More significantly, we demonstrate that the mNGS method is able to detect potentially lethal animal viruses, such as elephant endotheliotropic herpesvirus in Asian elephants (Elephas maximus) and the newly described human-associated gemykibivirus 2, a human-to-animal cross-species virus, in a Linnaeus two-toed sloth (Choloepus didactylus) and its enclosure, for the first time.

Cook, K. A., P. D. Ling, K. A. Terio, W. A. Baumgartner, L. L. Howard and J. A. Landolfi (2023). "DETECTION OF ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS 1A IN ARCHIVAL TISSUE USING RNASCOPE(®) IN SITU HYBRIDIZATION." J Zoo Wildl Med **53**(4): 661-669.

Hemorrhagic disease due to elephant endotheliotropic herpesvirus infection (EEHV-HD) is an important cause of calf mortality in managed and free-ranging Asian (Elephas maximus) and African elephant (Loxodonta spp.)

populations. Consequently, infection has profound implications for elephant population growth and sustainability. The mechanisms of disease caused by EEHV (i.e., infection, dissemination, shedding, latency) are relatively undefined, in part because of a lack of robust validated assays for detecting viral gene products in relevant samples. To address this issue, we used RNAscope(®) in situ hybridization (ISH) based on EEHV1A DNA polymerase and terminase genes to detect EEHV1A RNA in archival formalin-fixed, paraffin-embedded Asian elephant heart and tongue from PCR-confirmed cases (n = 4) of EEHV-HD and Asian elephants (n = 2) that died from other causes. EEHV1A-positive cases had positive hybridization signal in endothelial cell nuclei of both tissues for both DNA polymerase and terminase. EEHV-negative cases lacked signal. In positive cases, the number of positive nuclei was manually assessed to provide an estimate of the viral load and compare sensitivity of the two probes. In all cases, heart had greater signal than tongue for both probes (Wilcoxon rank test; $P \leq 0.01$). Overall, terminase hybridization signal was greater than DNA polymerase signal (Wilcoxon rank test; $P \leq 0.01$). Results indicate RNAscope ISH is a valuable tool for detection of EEHV in archival samples and for confirming infection. Additionally, the terminase gene is the optimal target and heart is preferable to tongue for detection in cases of EEHV-HD. Results will inform future investigations of viral tropism in EEHV-HD cases due to EEHV1A.

Yang, N., M. Bao, B. Zhu, Q. Shen, X. Guo, W. Li, R. Tang, D. Zhu, Y. Tang, D. N. Phalen and L. Zhang (2022). "Elephant Endotheliotropic Herpesvirus 1, 4 and 5 in China: Occurrence in Multiple Sample Types and Implications for Wild and Captive Population Surveillance." Viruses **14**(2).

Elephant endotheliotropic herpesviruses (EEHVs) are important causes of death in both captive and wild Asian elephants (Elephas maximus). Nothing is known about the prevalence of EEHVs in wild or domestic elephants in China. To determine if EEHVs are present in elephants in China, 126 wild elephants from three populations and 202 captive individuals from zoos (n = 155) and the Wild Elephant Valley (n = 47) were screened using semi-nested polymerase chain reaction assays with EEHV-redundant and EEHV1/4/5-specific primers. EEHV1B and EEHV4 were detected in samples from both wild (EEHV1B:8/126; EEHV4:2/126) and captive (EEHV1B:5/155; EEHV4:9/155) elephants, while EEHV1A (six cases) and EEHV5 (one case) were only present in the captive elephants from the Wild Elephant Valley. EEHV1 was detected in blood and trunk and oral swabs; EEHV4 was detected in trunk and oral swabs as well as feces; EEHV5 was found in trunk and oral swabs. No significant age or sex association with EEHV1A, EEHV1B, or EEHV5 positivity was observed. An age association with EEHV4 positivity was found, with all unweaned elephants being EEHV4 positive, but an association with the sex of the elephant was not observed. These findings represent the first documentation of EEHV presence in captive and wild elephants in China. These findings also document EEHV1B and EEHV4 shedding in feces and demonstrate the utility of fecal screening as a tool for investigating EEHV4 infection in wild populations of elephants. It is recommended that EEHV

testing be included in surveillance programs for captive and wild elephants in China. © 2022 by the authors. Licensee MDPI, Basel, Switzerland.

Titus, S. E., S. Patterson, J. Prince-Wright, A. Dastjerdi and F. M. Molenaar (2022). "Effects of between and within Herd Moves on Elephant Endotheliotropic Herpesvirus (EEHV) Recrudescence and Shedding in Captive Asian Elephants (Elephas maximus)." Viruses **14**(2).

Haemorrhagic disease associated with elephant endotheliotropic herpesvirus (Elephantid herpesvirus, EEHV) infections is the leading cause of death for Asian elephant (Elephas maximus) calves. This study assessed the effect of captive herd management on EEHV shedding, as evidence of latent infection reactivation, focusing on: (1) the influence of social change on the odds of recrudescence; (2) the respective effects of between and within herd moves; and (3) characteristics of recrudescent viral shedding. Trunk and conjunctival swabs (n = 165) were obtained from six elephants at an EAZA-accredited zoo, collected during a period of social stability, and at times of social change. Longitudinal sampling took place at times of moving two bulls out of the collection and one new bull into an adjacent enclosure to the cow herd (between herd moves), and during a period of mixing this new bull with the cow herd to facilitate mating (within herd moves). Quantitative PCR was employed to detect EEHV 1a/b, 4a/b, and EF-1-α (housekeeping gene). Generalised estimating equations determined EEHV recrudescence odds ratios (OR) and relative viral DNA load. Sixteen EEHV 1a/b shedding events occurred, but no EEHV 4a/b was detected. All management-derived social changes promoted recrudescence (social change OR = 3.27, 95% CI = 0.412-26, p = 0.262; and between herd moves OR = 1.6, 95% CI = 0.178-14.4, p = 0.675), though within herd movements posed the most significant increase of EEHV reactivation odds (OR = 6.86, 95% CI = 0.823-57.1, p = 0.075) and demonstrated the strongest relative influence (post hoc Tukey test p = 0.0425). Shedding onset and magnitude ranged from six to 54 days and from 3.59 to 11.09 ΔCts. Differing challenges are associated with between and within herd movements, which can promote recrudescence and should be considered an exposure risk to naïve elephants.

Khammesri, S., C. Ampasavate, D. Hongwiset, R. Mektrirat, S. Sangsrijan, J. L. Brown and C. Thitaram (2022). "Pharmacokinetics and analytical determination of acyclovir in Asian elephant calves (Elephas maximus)." Vet Anim Sci **15**: 100227.

A therapeutic regimen that includes antiviral drugs is critical for the survival of Asian elephant (Elephas maximus) calves infected with elephant endotheliotropic herpesvirus hemorrhagic disease (EEHV-HD), with acyclovir showing considerable promise. The purpose of this study was to determine the pharmacokinetics and bioavailability of acyclovir following intravenous (IV) and oral (PO) administration in Asian elephants. A single dose of acyclovir (15 mg/kg, IV or 45 mg/kg, PO) was administered to four healthy elephant calves, with a minimum 2-week washout period between treatments. Serial plasma samples were collected after each injection for acyclovir analysis using a validated liquid chromatography-tandem mass

spectrometry (LC-MS/MS) technique. Maximum plasma acyclovir concentrations were 27.02 ± 6.79 µg/mL at 0.94 ± 0.31 h after IV administration, and 1.45 ± 0.20 µg/mL at 3.00 ± 0.70 h after PO administration. The half-life of the elimination phase (T(1/2)) was 5.84 ± 0.74 and 8.74 ± 2.47 h after IV and PO administration, respectively. After IV administration, acyclovir concentrations were higher than the half-maximal inhibitory concentration (IC(50)) of those found for herpes simplex virus (HSV) 1 and 2 in humans, and equid alpha herpesvirus-1 (EHV-1) for at least 12 h. By contrast, the bioavailability of oral administration was low, only 6.03 ± 0.87%, so higher doses by that route likely are needed to be effective. Due to the high concentration of plasma acyclovir after IV administration, the dose may need to be adjusted to prevent any negative side effects.

Jesus, S. A., A. Schmidt, J. Fickel, M. G. Doherr, K. Boonprasert, C. Thitaram, L. Sariya, P. Ratanakron and T. B. Hildebrandt (2022). "Assessing Coagulation Parameters in Healthy Asian Elephants (Elephas maximus) from European and Thai Populations." Animals (Basel) **12**(3).

The Asian elephant population is continuously declining due to several extrinsic reasons in their range countries, but also due to diseases in captive populations worldwide. One of these diseases, the elephant endotheliotropic herpesvirus (EEHV) hemorrhagic disease, is very impactful because it particularly affects Asian elephant calves. It is commonly fatal and presents as an acute and generalized hemorrhagic syndrome. Therefore, having reference values of coagulation parameters, and obtaining such values for diseased animals in a very short time, is of great importance. We analyzed prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen concentrations using a portable and fast point-of-care analyzer (VetScan Pro) in 127 Asian elephants from Thai camps and European captive herds. We found significantly different PT and aPTT coagulation times between elephants from the two regions, as well as clear differences in fibrinogen concentration. Nevertheless, these alterations were not expected to have biological or clinical implications. We have also sequenced the coagulation factor VII gene of 141 animals to assess the presence of a previously reported hereditary coagulation disorder in Asian elephants and to investigate the presence of other mutations. We did not find the previously reported mutation in our study population. Instead, we discovered the presence of several new single nucleotide polymorphisms, two of them being considered as deleterious by effect prediction software.

Iyer, M. L., C. M. Molter, J. P. Flanagan, K. L. Bauer, R. Bernardy, D. Hoffman, L. Parkinson, B. M. Brainard, T. S. Evans, T. Pursell and P. D. Ling (2022). "NOVEL DIAGNOSTIC AND THERAPEUTIC APPROACHES TO ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS 1A HEMORRHAGIC DISEASE IN A CAPTIVE JUVENILE ASIAN ELEPHANT (ELEPHAS MAXIMUS)." J Zoo Wildl Med **53**(1): 232-240.

Novel diagnostic and therapeutic methods were utilized in the successful management of severe elephant endotheliotropic herpesvirus hemorrhagic

disease (EEHV-HD) in a 1.9-yr-old captive Asian elephant (Elephas maximus). High levels of EEHV1A viremia were detected for 12 d. In addition to established EEHV treatments, therapies included famciclovir-fortified elephant whole blood and plasma, mesenchymal stem cells harvested from elephant umbilical tissue, and aminocaproic acid. Testing conducted to examine the effects of EEHV infection on hemostasis suggested marked intravascular coagulation with decreased plasminogen activity and increased D-dimer concentrations. Thromboelastography was used to assess the efficacy of aminocaproic acid and demonstrated hypofibrinolysis on samples taken after drug administration, as compared with samples from healthy adult Asian elephants. A serological assay for a novel EEHV1A-specific antibody marker (E52) was developed due to lack of seroconversion to a previously established EEHV1A-specific antibody marker (ORFQ) and showed a sustained increase after EEHV-HD illness.

Hoornweg, T. E., V. P. Perera, R. N. S. Karunarathne, W. Schaftenaar, T. A. N. Mahakapuge, A. W. Kalupahana, V. Rutten and C. A. M. de Haan (2022). "Young elephants in a large herd maintain high levels of elephant endotheliotropic herpesvirus-specific antibodies and do not succumb to fatal haemorrhagic disease." Transboundary and Emerging Diseases **69**(5): E3379-E3385.

Elephant endotheliotropic herpesviruses (EEHVs) have co-existed with elephants for millions of years, yet may cause fatal haemorrhagic disease (EEHV-HD), typically in elephants between 1 and 10 years of age. EEHV is omnipresent in (sub)adult elephants, and young elephants with low EEHV-specific antibody levels are at risk for EEHV-HD, suggesting that fatal disease may occur due to an insufficiently controlled primary infection. To further address this hypothesis, sera of three large elephant cohorts were subjected to a multiple EEHV species ELISA: (I) 96 Asian elephants between 0 and 57 years, including 13 EEHV-HD fatalities, from European zoo herds typically sized five to six elephants, (II) a herd of 64 orphaned elephants aged 0-15 years at the Elephant Transit Home in Sri Lanka and (III) 31 elephants aged 8-63 years, part of a large herd of 93 elephants at Pinnawala Elephant Orphanage, Sri Lanka. All Sri Lankan elephants showed high EEHV-specific antibody levels regardless of their age. While antibody levels of most European zoo elephants were comparable to those of Sri Lankan elephants, the average antibody level of the European juveniles (1-5 years of age) was significantly lower than those of age-matched Sri Lankan individuals. Moreover, the European juveniles showed a gradual decrease between 1 and 4 years of age, to be attributed to waning maternal antibodies. Maintenance of high levels of antibodies in spite of waning maternal antibodies in young Sri Lankan elephants is likely due to the larger herd size that increases the likelihood of contact with EEHV-shedding elephants. Together with the observation that low levels of EEHV-specific antibodies correlate with increased numbers of EEHV-HD fatalities, these results suggest that infection in presence of high maternal antibody levels may protect calves from developing EEHV-HD, while at the same time activating an immune response protective in future encounters with this virus.

Guntawang, T., T. Sittisak, P. Tankaew, C. Thitaram, V. Langkapin, T. Angkawanish, T. Singhla, N. Sthitmatee, W. L. Hsu, R. Thanawongnuwech and K. Pringproa (2022). "Development of Nonstructural Protein-Based Indirect ELISA to Identify Elephant Endotheliotropic Herpesvirus (EEHV) Infection in Asian Elephants (Elephas maximus)." Animals (Basel) **12**(14).

Disease caused by elephant endotheliotropic herpesvirus (EEHV) infection is the most highly fatal hemorrhagic disease in Asian elephant calves worldwide. To date, adult elephants that have been infected with EEHV have predominantly displayed mild clinical signs, while they are believed to serve as EEHV shedders to other elephants. Hence, the diagnostic tools employed for monitoring EEHV-active infection are considered vitally important. In this study, partial EEHV-DNA polymerase (DNApol) nonstructural proteins (NSPs) were used to detect anti-EEHV antibodies through the use of an in-house indirect enzyme-linked immunosorbent assay (ELISA). The results were then compared to those obtained from a PCR test. In this study, a total of 175 serum samples were collected from Asian elephants living in elephant camps located in Chiang Mai and Lampang Provinces, Thailand. The elephants were aged between 2 and 80 years old. The overall percentages of positive samples by the PCR and EEHV-DNApol ELISA tests were 4% (21/175) and 12% (21/175), respectively. The ELISAs demonstrated values of 77.9% (95% posterior probability interval (PPI) = 52.5-95%) sensitivity and 87.7% (PPI = 82.5-91.9%) specificity, respectively. Accordingly, the sera obtained from the elephants exhibiting no clinical signs of EEHV infection, and those who were negative according to PCR tests, revealed a value of 14% seropositivity for EEHV-DNApol. Our results indicate that these asymptomatic, active EEHV-infected elephants could likely serve as a source of EEHV shedding within elephant herds. Consequently, the developed EEHV-DNApol NSPs-based ELISA test employed in the present study may be of use for routine monitoring and identification of EEHV shedders in elephant herds, and could be an alternative diagnostic tool for EEHV detection in Asian elephants.

Costa, T., G. Rocchigiani, F. Zendri, G. Drake, J. Lopez, J. Chantrey and E. Ricci (2022). "Elephant Endotheliotropic Herpesvirus 4 and Clostridium perfringens Type C Fatal Co-Infection in an Adult Asian Elephant (Elephas maximus)." Animals (Basel) **12**(3).

Elephant endotheliotropic herpesvirus hemorrhagic disease (EEHV-HD) is an acute, often fatal, multisystemic hemorrhagic disease and one of the most significant causes of mortality of Asian elephants in captivity. Most fatal cases of EEHV-HD are associated with EEHV1A and EEHV1B in juveniles. This case report describes the clinical and pathological features of a fatal co-infection of Clostridium perfringens type C and EEHV-HD, caused by EEHV4, in an adult female Asian elephant. Although fatal clostridial enterotoxemia has been occasionally reported in elephants, this report highlights the importance of having both EEHV-HD and clostridial enterotoxemia as potential differential diagnoses in cases of widespread tissue necrosis and

internal hemorrhage in elephants, regardless of the animal age group, due to their macroscopic similarities, frequent co-occurrence and cumulative morbid potential.

Common, S. M., Y. Yun, A. Silva-Fletcher, C. Thitaram, T. Janyamethakul, S. Khammesri and F. M. Molenaar (2022). "Developing a non-invasive method of detecting elephant endotheliotropic herpesvirus infections using faecal samples." Vet Rec **190**(2): e833.

BACKGROUND: Elephant endotheliotropic herpesvirus (EEHV)-associated haemorrhagic disease (EEHV-HD) is a leading cause of death in Asian elephant calves across the world. Cases of EEHV-HD have been detected in free-living calves through post-mortem examination (PME) indicating the presence of the virus in the wild. In the absence of a non-invasive sampling method, little research into free-living populations has been possible. This study aimed to provide evidence that faeces can be used as a non-invasive sampling method for the detection of EEHV excretion using quantitative polymerase chain reaction. METHODS: Serial saliva swabs and faecal samples were taken from five captive Asian elephants in Thailand over 12 weeks. To ensure the presence of detectable elephant DNA within the sample, qPCR was run for amplification of the Asian elephant tumour necrosis factor (TNF-α) gene, EEHV1 and EEHV4. RESULTS: Of 28 sample pairs, seven saliva samples were positive for EEHV, of which two had paired positive faecal samples. CONCLUSIONS: This study presents the first evidence that EEHV is excreted in faeces at detectable levels. This method may in future be used for improved understanding of the epidemiology of EEHV in free-living elephant populations, as well as detection of EEHV excretion in captive herds.

Yun, Y., S. Sripiboon, K. Pringproa, P. Chuammitri, V. Punyapornwithaya, K. Boonprasert, P. Tankaew, T. Angkawanish, K. Namwongprom, O. Arjkumpa, J. L. Brown and C. Thitaram (2021). "Clinical characteristics of elephant endotheliotropic herpesvirus (EEHV) cases in Asian elephants (Elephas maximus) in Thailand during 2006-2019." Vet Q **41**(1): 268-279.

BACKGROUND: Elephant endotheliotropic herpesvirus causes a hemorrhagic disease (EEHV-HD) that is a major cause of death in juvenile Asian elephants with EEHV1 and EEHV4 being the most prevalent. AIM: To perform a retrospective clinical data analysis. METHODS: Records of a total of 103 cases in Thailand confirmed by polymerase chain reaction (PCR) on blood and/or tissue samples. RESULTS: The severity of clinical signs varied among EEHV subtypes. EEHV1A was the most prevalent with 58%, followed by EEHV4 with 34%, EEHV1B with 5.8% and EEHV1&4 co-infection with 1.9%. Overall case fatality rate was 66%. When compared among subtypes, 100% case fatality rate was associated with EEHV1&4 co-infection, 83% with EEHV1B, 75% with EEHV1A, and the lowest at 40% for EEHV4. Calves 2- to 4-year old were in the highest age risk group and exhibited more severe clinical signs with the highest mortality. Majority of cases were found in weaned or trained claves and higher number of cases were observed in rainy season. A gender predilection could not be demonstrated. Severely affected

elephants presented with thrombocytopenia, depletion of monocytes, lymphocytes and heterophils, a monocyte:heterophil (M:H) ratio lower than 2.37, hypoproteinemia (both albumin and globulin), severe grade of heterophil toxicity, and low red blood cell counts and pack cell volumes. Survival was not affected by antiviral drug treatment in the severely compromised animals. CONCLUSION: Early detection by laboratory testing and aggressive application of therapies comprising of supportive and anti-viral treatment can improve survival outcomes of this disease.

Tollis, M., E. Ferris, M. S. Campbell, V. K. Harris, S. M. Rupp, T. M. Harrison, W. K. Kiso, D. L. Schmitt, M. M. Garner, C. A. Aktipis, C. C. Maley, A. M. Boddy, M. Yandell, C. Gregg, J. D. Schiffman and L. M. Abegglen (2021). "Elephant Genomes Reveal Accelerated Evolution in Mechanisms Underlying Disease Defenses." Mol Biol Evol **38**(9): 3606-3620.

Disease susceptibility and resistance are important factors for the conservation of endangered species, including elephants. We analyzed pathology data from 26 zoos and report that Asian elephants have increased neoplasia and malignancy prevalence compared with African bush elephants. This is consistent with observed higher susceptibility to tuberculosis and elephant endotheliotropic herpesvirus (EEHV) in Asian elephants. To investigate genetic mechanisms underlying disease resistance, including differential responses between species, among other elephant traits, we sequenced multiple elephant genomes. We report a draft assembly for an Asian elephant, and defined 862 and 1,017 conserved potential regulatory elements in Asian and African bush elephants, respectively. In the genomes of both elephant species, conserved elements were significantly enriched with genes differentially expressed between the species. In Asian elephants, these putative regulatory regions were involved in immunity pathways including tumor-necrosis factor, which plays an important role in EEHV response. Genomic sequences of African bush, forest, and Asian elephant genomes revealed extensive sequence conservation at TP53 retrogene loci across three species, which may be related to TP53 functionality in elephant cancer resistance. Positive selection scans revealed outlier genes related to additional elephant traits. Our study suggests that gene regulation plays an important role in the differential inflammatory response of Asian and African elephants, leading to increased infectious disease and cancer susceptibility in Asian elephants. These genomic discoveries can inform future functional and translational studies aimed at identifying effective treatment approaches for ill elephants, which may improve conservation.

Stremme, C., A. Priadi, G. S. Hayward and A. Zachariah (2021). "IDENTIFICATION OF TWO LETHAL CASES OF ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS HEMORRHAGIC DISEASE IN SUMATRAN ELEPHANT CALVES IN INDONESIA." J Zoo Wildl Med **51**(4): 985-993.

As many as a dozen cases of lethal acute hemorrhagic disease (HD) in young captive-born Sumatran sub-species Asian elephant (Elephas maximus sumatranus roman) calves raised naturally in camps in Sumatra have been

observed in recent years. To address whether these deaths, like many others documented worldwide, might be associated with acute systemic infection by elephant endotheliotropic herpesvirus (EEHV), diagnostic polymerase chain reaction (PCR) tests followed by subtype deoxyribonucleic acid (DNA) sequencing analysis were carried out on pathologic tissue samples from two lethal HD cases that occurred within 6 days of one another in calves at the same camp. Viral DNA from five selected PCR loci was found to be present at high levels in both calves and proved to be the same EEHV1A virus species that has been described most commonly previously in numerous lethal or surviving symptomatic cases in North America, Europe, India, and Thailand. Furthermore, the two cases were identical at all five PCR loci tested (covering a total of 3,050 base pairs) and were therefore likely to have been infected from the same epidemiologic source herdmate. However, the strain involved (which was subtype-D2 in the vGPCR1 locus) differed from all previously characterized EEHV1A strains. In conclusion, these two calves are the first two confirmed HD cases in Sumatra alongside several other suspected HD cases in Sumatra that have succumbed to the same devastating EEHV1A-HD that has afflicted young Asian elephants worldwide over the past 25 yr. Because the progeny of some of the 1,500 remaining red-listed critically endangered Sumatra subspecies elephants are bred naturally in camps from wild parents it seems very likely that the EEHV1A herpesvirus identified here in these HD camp cases is also present in the free-ranging Sumatran elephant population, and this will have to be taken into account in future wildlife management policies and decisions.

Sahoo, N., S. K. Sahu, A. K. Das, D. Mohapatra, S. K. Panda, S. K. Gupta, B. K. Behera, A. Pahari and M. Dash (2021). "ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS HEMORRHAGIC DISEASE OUTBREAK IN AN INDIAN ZOO." J Zoo Wildl Med **52**(4): 1286-1297.

Elephant endotheliotropic herpesvirus hemorrhagic disease (EEHV HD) is an acute viral infection of growing Asian elephants (Elephas maximus). Four apparently healthy subadult Asian elephants aged between 6 and 10 yr at Nandankanan Zoological Park (NKZP), India, died of EEHV HD during August-September 2019. All four elephants were rescued from different reserved forests of Odisha state at less than 1 yr of age and hand reared in the NKZP. Elephants exhibited the clinical signs of lethargy, head swelling, fever, loss of appetite, abdominal distension, scant urination and defecation, signs of colic, lameness, trunk discharge, cyanosis/ulceration of tongue, erratic behavior, and recumbence before death. Period of illness varied between 28 and 42 h. Thrombocytopenia was the common significant hematological observation. No significant biochemical alterations were recorded except for higher creatinine concentrations. Analysis of blood samples in RT-PCR assay using two different sets of primers and probes that targeted terminase gene and major DNA-binding protein gene followed by cPCR and sequencing was positive for EEHV-1A in all four animals. Postmortem examination of all four carcasses showed hemorrhages in internal organs, including the hard palate, heart, lungs, stomach, mesenteric lymph nodes, mesentery, colon serosa,

spleen, liver, kidney, and meninges. Histopathology showed congestion and/or hemorrhages in heart, lung, brain, kidney, and liver. There was presence of intranuclear inclusion bodies in the sinusoidal epithelial cells. The outbreak of EEHV HD that resulted in the acute death of four juvenile captive Asian elephants within <30 d, the first of its kind documented in India, is increasing the fear of similar outbreaks in the future.

Pursell, T., J. L. Spencer Clinton, J. Tan, R. Peng, X. Qin, H. Doddapaneni, V. Menon, Z. Momin, K. Kottapalli, L. Howard, E. Latimer, S. Heaggans, G. S. Hayward and P. D. Ling (2021). "Primary Infection May Be an Underlying Factor Contributing to Lethal Hemorrhagic Disease Caused by Elephant Endotheliotropic Herpesvirus 3 in African Elephants (Loxodonta africana)." Microbiol Spectr **9**(2): e0098321.

Distinct but related species of elephant endotheliotropic herpesviruses (EEHVs) circulate within Asian and African elephant populations. Primary infection with EEHVs endemic among Asian elephants can cause clinical illness and lethal EEHV hemorrhagic disease (EEHV-HD). The degree to which this occurs among African elephants has not been fully established. Recent cases of EEHV-HD caused by the EEHV3 species in African elephants housed in North American zoos has heightened concern about the susceptibility of this elephant species to EEHV-HD. In this study, we utilize the luciferase immunoprecipitation system (LIPS) to generate a serological assay specific for EEHV3 in African elephants by detecting antibodies against the EEHV3 E34 protein. The results showed that the majority of tested elephants from four separate and genetically unrelated herds, including five elephants that survived clinical illness associated with EEHV3, were positive for prior infection with EEHV3. However, African elephants who succumbed to EEHV3-HD were seronegative for EEHV3 prior to lethal infection. This supports the hypothesis that fatal EEHV-HD caused by EEHV3 is associated with primary infection rather than reactivation of latent virus. Lastly, we observed that African elephants, like Asian elephants, acquire abundant anti-EEHV antibodies prenatally and that anti-EEHV3 specific antibodies were either never detected or declined to undetectable levels in those animals that died from lethal disease following EEHV3 infection. IMPORTANCE Prior to 2019, only five cases of clinical disease from EEHV infection among African elephants had been documented. Since 2019, there have been at least seven EEHV-HD cases in North American zoos, resulting in three fatalities, all associated with EEHV3. Evidence is accumulating to suggest that EEHV-associated clinical illness and death among Asian elephants is due to primary infection and may be associated with waning anti-EEHV antibody levels in young elephants. The development of the EEHV3 serological test described in this study enabled us to confirm that similar dynamics may be contributing to EEHV-HD in African elephants. The ability to screen for EEHV immune status in African elephant calves will have a major impact on managing captive African elephant herds and will provide new tools for investigating and understanding EEHV in wild populations.

Prompiram, P., W. Wiriyarat, B. Bhusri, W. Paungpin, W. Jairak, S. Sripiboon and T.

Wongtawan (2021). "The occurrence of elephant endotheliotropic herpesvirus infection in wild and captive Asian elephants in Thailand: Investigation based on viral DNA and host antibody." Vet World **14**(2): 545-550.

BACKGROUND AND AIM: Elephant endotheliotropic herpesvirus (EEHV) is a serious disease, threatening the life of young elephants. Many elephants have been infected with no clinical signs and may serve as carriers spreading this disease. It is important to monitor the disease through clinical signs and molecular diagnosis. In this study we investigated the occurrence of EEHV and the efficiency of different techniques used to monitor EEHV infection in various samples and populations of Asian elephants. MATERIALS AND METHODS: Blood and trunk swabs were collected from live elephants, while visceral organs (lung, digestive tract, spleen, lymph nodes, and kidney) were collected from dead elephants. EEHV was detected by polymerase chain reaction (PCR) in whole blood, trunk swabs, and visceral organs as samples, while elephant anti-EEHV immunoglobulin G (IgG) in serum was detected by enzyme-linked immunosorbent assay (ELISA). A total of 162 samples were analyzed in this study: 129 from healthy, 26 from dead, and 7 from sick elephants. RESULTS: The present study showed that the overall incidence of EEHV was 40.1% (n=65/162). Approximately 46.2% (n=12/26) and 85.7% (n=6/7) of dead and sick elephants were positive for EEHV by PCR, respectively. All sick elephants that were young and affected by EEHV clinical disease tested negative for the IgG antibody ELISA, suggesting primary EEHV infection in this group. In addition, 2.3% (n=3/129) of subclinical infections were detected using PCR, and trunk swab samples showed slightly higher sensitivity (5.3%, n=2/38) to detect EEHV than whole blood (1.2%, n=1/84). As many as, 48.4% (n=44/91) of healthy elephants were EEHV seropositive (ELISA-positive), suggesting that many elephants in Thailand had previously been infected. Overall, 30% of dead wild elephants had been infected with EEHV (n=3/10). Moreover, statistical analysis revealed no significant differences in the EEHV detection rate between different age groups or sexes ($p>0.05$). CONCLUSION: PCR is better than ELISA to detect EEHV active infection in dead/sick elephants and to monitor EEHV in young elephants. ELISA is suitable for detecting previous EEHV infection and carriers, particularly adults. Theoretically, we could use both PCR and ELISA to increase the sensitivity of testing, along with observing abnormal behavior to efficiently monitor this disease. Identification of EEHV carriers within elephant populations is important to prevent transmission to healthy individuals, especially young elephants with high mortality from EEHV. This is the first report from Thailand regarding EEHV infection in wild elephants, showing the importance of preventing disease transmission between captive and wild elephants.

Perrin, K. L., S. S. Nielsen, T. Martinussen and M. F. Bertelsen (2021). "Quantification and risk factor analysis of elephant endotheliotropic herpesvirus-haemorrhagic disease fatalities in Asian elephants Elephas maximus in Europe (1985-2017)." Journal of Zoo and Aquarium Research **9**(1): 8-13.

Perrin, K. L., A. T. Kristensen, M. F. Bertelsen and D. Denk (2021). "Retrospective review of 27 European cases of fatal elephant endotheliotropic herpesvirus-haemorrhagic disease reveals evidence of disseminated intravascular coagulation." Sci Rep **11**(1): 14173.

Elephant endotheliotropic herpesvirus haemorrhagic disease (EEHV-HD) is widely acknowledged as the most common cause of mortality in young Asian elephants (Elephas maximus) in captivity. The objective of the current study was to perform a blinded, retrospective pathology review of European EEHV-HD fatalities, constituting the largest systematic assessment of EEHV-HD pathology to date. Findings between viral genotypes were compared with the aim to investigate if disseminated intravascular coagulation (DIC) could be substantiated as a significant complicating factor, thereby increasing the understanding of disease pathophysiology. Immunohistochemical staining confirmed endothelial cell (EC) damage and the presence of EC intranuclear inclusion bodies, demonstrating a direct viral cytopathic effect. Microthrombi were observed in 63% of cases in several organs, including lungs, which, together with widespread haemorrhage and thrombocytopenia reported in EEHV-HD case reports, supports the presence of overt DIC as a serious haemostatic complication of active EEHV infection. Death was attributed to widespread vascular damage with multi-organ dysfunction, including severe acute myocardial haemorrhage and subsequent cardiac failure. Systemic inflammation observed in the absence of bacterial infection may be caused by cytokine release syndrome. Findings reinforce the necessity to investigate cytokine responses and haemostatic status during symptomatic and asymptomatic EEHV viraemia, to potentially support the use of anti-inflammatory treatment in conjunction with anti-viral therapy and cardiovascular support.

Lee, M. H., S. Nathan, L. Benedict, P. Nagalingam, E. Latimer, T. Hughes, D. Ramirez and J. R. A. Sukor (2021). "The first reported cases of elephant endotheliotropic herpesvirus infectious haemorrhagic disease in Malaysia: case report." Virol J **18**(1): 231.

BACKGROUND: Elephant endotheliotropic herpesvirus haemorrhagic disease (EEHV HD) is the leading cause of death in captive Asian elephant calves in Asia, North America, and Europe with a mortality rate of ~ 65% in calves that are under human care. Although EEHV HD was first found in elephant camps, more recently it was identified in wild populations which poses a greater threat to the elephant population. Deaths due to EEHV HD have been seen in wild elephants, but the in-situ prevalence and mortality rate is unknown. We report the first EEHV HD cases in Malaysia from 3 wild born endangered Bornean elephant calves from Sabah with known typical clinical signs. CASE PRESENTATION: The first calf died within 24 h of the onset of clinical signs; the second calf died within 12 h of the onset of clinical signs. The third calf succumbed within 72 h. Necropsies revealed that all 3 calves had similar presentations of EEHV HD but in the third calf with less severity. We conducted conventional polymerase chain reaction (cPCR) assays and found EEHV DNA at all 7 loci in the 3 calves; it was identified as EEHV1A, the virus

type that has been found in most other reported cases. CONCLUSION: Typical EEHV HD clinical signs and the molecular confirmation of EEHV by cPCR and sequencing point to EEHV as the cause of death. Further genetic investigation of the strain is in progress.

Khammesri, S., Y. Mathura, K. Boonprasert, C. Ampasavate, D. Hongwiset, J. L. Brown and C. Thitaram (2021). "Successful treatment of elephant endotheliotropic herpesvirus infection in an Asian elephant (Elephas maximus) calf by oral acyclovir medication: Case report." J Vet Med Sci **83**(1): 125-129.

Elephant endotheliotropic herpesvirus (EEHV) is a major cause of death in Asian elephant (Elephas maximus) calves. A 2-year, 11-month-old female, captive Asian elephant presented with facial edema and a mild fever. Blood samples were collected and showed EEHV1A positivity with a high viral load by real time PCR. Heterophil toxicity also was reported for the first time in this case. The calf was treated orally with acyclovir, 45 mg/kg tid for 28 days, which reduced the EEHV1A viral load to undetectable levels within 9 days and the calf survived. A successful outcome with oral acyclovir administration provides another and affordable option to treat EEHV hemorrhagic disease in Asian elephants, and one that is easier to administer in untrained calves.

Jesus, S. A., M. G. Doherr and T. B. Hildebrandt (2021). "Elephant Endotheliotropic Herpesvirus Impact in the European Asian Elephant (Elephas maximus) Population: Are Hereditability and Zoo-Associated Factors Linked with Mortality?" Animals (Basel) **11**(10).

EEHV is a ubiquitous virus, which most likely has co-evolved with elephants and is shed by healthy individuals and maintained in the herds. Yet, the factors determining calf susceptibility to the virus remain unknown. Here, we explored the impact of EEHV-HD in the European captive Asian elephant population in a retrospective statistical study spanning the last 35 years. We show that EEHV-HD was implicated in more than half of all deaths recorded in calves older than one months old. Moreover, the median age across EEHV-HD fatalities was significantly lower compared to other death causes. Finally, we investigated if heredity and zoo-associated factors could be linked to a higher susceptibility of calves to this disease. We used a univariable logistic regression model to evaluate if either fathers, mothers, or zoos could, separately, be considered as risk factors to the development of the disease. Afterwards, we used a two multivariable model, combining: (1) fathers and zoos, and (2) mothers and zoos. Overall, we found that two fathers, one mother, and four zoos had three or more times higher risk of their calves becoming sick when compared to all others, pointing us to the presence of a management or environmental element, which can have paternal and maternal influence and leads to calf susceptibility or resistance to EEHV-HD.

Hoornweg, T. E., W. Schaftenaar, G. Maurer, P. B. van den Doel, F. M. Molenaar, A. Chamouard-Galante, F. Vercammen, V. P. M. G. Rutten and C. A. M. de Haan (2021). "Elephant endotheliotropic herpesvirus is omnipresent in elephants in

european zoos and an asian elephant range country." Viruses **13**(2).

Elephant endotheliotropic herpesviruses (EEHVs) may cause acute, often lethal, hemor-rhagic disease (EEHV-HD) in young elephants. Prevalence of EEHV in different elephant populations is still largely unknown. In order to improve diagnostic tools for the detection of EEHV infections and to obtain insight into its spread among elephants, we developed novel ELISAs based on EEHV1A gB and gH/gL. Performance of the ELISAs was assessed using sera from 41 European zoo elephants and 69 semi-captive elephants from Laos, one of the Asian elephant range countries. Sera from all (sub)adult animals tested (≥5 years of age) showed high reactivity with both gB and gH/gL, indicating that EEHV prevalence has been highly underestimated so far. Reactivity towards the antigens was generally lower for sera of juvenile animals (1 > 5 years). Only one (juvenile) animal, which was sampled directly after succumbing to EEHV-HD, was found to be seronegative for EEHV. The two other EEHV-HD cases tested showed low antibody levels, suggesting that all three cases died upon a primary EEHV infection. In conclusion, our study suggests that essentially all (semi-)captive (sub)adult elephants in European zoos and in Laos carry EEHV, and that young elephants with low antibody levels are at risk of dying from EEHV-HD.

Hoornweg, T. E., W. Schaftenaar, G. Maurer, P. B. van den Doel, F. M. Molenaar, A. Chamouard-Galante, F. Vercammen, V. Rutten and C. A. M. de Haan (2021). "Elephant Endotheliotropic Herpesvirus Is Omnipresent in Elephants in European Zoos and an Asian Elephant Range Country." Viruses **13**(2).

Elephant endotheliotropic herpesviruses (EEHVs) may cause acute, often lethal, hemorrhagic disease (EEHV-HD) in young elephants. Prevalence of EEHV in different elephant populations is still largely unknown. In order to improve diagnostic tools for the detection of EEHV infections and to obtain insight into its spread among elephants, we developed novel ELISAs based on EEHV1A gB and gH/gL. Performance of the ELISAs was assessed using sera from 41 European zoo elephants and 69 semi-captive elephants from Laos, one of the Asian elephant range countries. Sera from all (sub)adult animals tested (≥5 years of age) showed high reactivity with both gB and gH/gL, indicating that EEHV prevalence has been highly underestimated so far. Reactivity towards the antigens was generally lower for sera of juvenile animals (1 > 5 years). Only one (juvenile) animal, which was sampled directly after succumbing to EEHV-HD, was found to be seronegative for EEHV. The two other EEHV-HD cases tested showed low antibody levels, suggesting that all three cases died upon a primary EEHV infection. In conclusion, our study suggests that essentially all (semi-)captive (sub)adult elephants in European zoos and in Laos carry EEHV, and that young elephants with low antibody levels are at risk of dying from EEHV-HD.

Edwards, K. L., E. M. Latimer, J. Siegal-Willott, W. Kiso, L. R. Padilla, C. R. Sanchez, D. Schmitt and J. L. Brown (2021). "Patterns of serum immune biomarkers during elephant endotheliotropic herpesvirus viremia in Asian and

African elephants." PLoS ONE **16**(11): e0252175.

Hemorrhagic disease (HD) caused by a group of elephant endotheliotropic herpesviruses (EEHV) is one of the leading causes of death for young elephants in human care. These viruses are widespread and typically persist latently in adult elephants with no negative effects; however, in juvenile Asian and more recently young African elephants, the onset of disease can be rapid and the mortality rate high. Measuring biomarkers associated with the immune response could be beneficial to understanding underlying disease processes, as well as the management of infection and HD. The goal of this study was to measure acute phase proteins and cytokines in serum collected from elephants infected with EEHV (13 Asian and 1 African) and compare concentrations according to presence, severity and outcome of disease. Serum amyloid A (SAA) and haptoglobin (HP) were higher in elephants with EEHV viremia than those without; concentrations increased with increasing viral load, and were higher in fatal cases compared to those that survived. In Asian elephants, SAA was also higher during EEHV1 viremia compared to EEHV5. Cytokine concentrations were typically low, and no statistical differences existed between groups. However, in individuals with detectable levels, longitudinal profiles indicated changes in tumor necrosis factor alpha (TNF-α) and interleukin-2 (IL-2) that may reflect an immune response to EEHV infection. However, the overall low concentrations detected using previously validated assays do not support the presence of a 'cytokine storm' and suggest more work is needed to understand if sub-optimal immune responses could be involved in disease progression. These results highlight the potential benefit of measuring circulating biomarker concentrations, such as APPs and cytokines, to improve our understanding of EEHV viremia and HD, assist with monitoring the progression of disease and determining the impact of interventions.

Boonprasert, K., Y. Yun, W. Kosaruk, P. Towiboon, P. Tankaew, V. Punyapornwithaya, T. Janyamathakul, P. Muanghong, J. L. Brown, C. Thitaram and C. Somgird (2021). "A Longitudinal Study of Hematology and Stress Biomarker Profiles in Young Asian Elephants (Elephas Maximus) in Relation to Elephant Endotheliotropic Herpesvirus (EEHV) in Thailand." Animals (Basel) **11**(9).

Elephant endotheliotropic herpesvirus hemorrhagic disease (EEHV-HD) is a virulent disease that causes severe hemorrhage and sudden death in Asian elephant calves. A change in hematology profiles is one indicator of infection before clinical signs appear; however, to be effective, individual baselines and age-matched reference values are needed. Stress has been speculated to be a factor in clinical EEHV cases, but relationships have not been demonstrated empirically. This study evaluated blood hematology and several stress response markers-salivary cortisol, fecal glucocorticoid metabolites (FGM), salivary Immunoglobulin A (SIgA), and fecal IgA (FIgA) in samples collected for 1 year from three healthy calves with no EEHV history (non-EEHV), and six that had previously been infected, developed clinical signs and survived (prior-EEHV). Hematology values between non-EEHV and prior-EEHV elephants were not different and within published

reference ranges. Concentrations of salivary cortisol, FGM, SIgA, and FIgA also were variable and showed seasonal differences, but no relationships to prior EEHV status. One of the prior EEHV calves became re-infected, developed hemorrhagic disease (HD), and died during the study period. That calf exhibited lymphocytopenia, monocytopenia, and thrombocytopenia. Additionally, all stress biomarker concentrations were lower in the 12 days before viremia was observed. Thus, as in other studies, changes in hematology occur with EEHV infection, while preliminary data in one calf suggests that stress-response measures might also be informative and should be studied further.

Photichai, K., T. Guntawang, T. Sittisak, V. Kochagul, P. Chuammitri, C. Thitaram, H. Thananchai, T. Chewonarin, K. Sringarm and K. Pringproa (2020). "Attempt to Isolate Elephant Endotheliotropic Herpesvirus (EEHV) Using a Continuous Cell Culture System." Animals (Basel) **10**(12).

Elephant endotheliotropic herpesvirus (EEHV) infection is known to cause acute fatal hemorrhagic disease, which has killed many young Asian elephants (Elephas maximus). Until recently, in vitro isolation and propagation of the virus have not been successful. This study aimed to isolate and propagate EEHV using continuous cell lines derived from human and/or animal origins. Human cell lines, including EA. hy926, A549, U937, RKO, SW620, HCT-116 and HT-29, and animal cell lines, including CT26.CL25 and sp2/0-Ag14, were investigated in this study. Mixed frozen tissue samples of the heart, lung, liver, spleen and kidney obtained from fatal EEHV1A- or EEHV4-infected cases were homogenized and used for cell inoculation. At 6, 24, 48 and 72 h post infection (hpi), EEHV-inoculated cells were observed for cytopathic effects (CPEs) or were assessed for EEHV infection by immunoperoxidase monolayer assay (IPMA) or quantitative PCR. The results were then compared to those of the mock-infected controls. Replication of EEHV in the tested cells was further determined by immunohistochemistry of cell pellets using anti-EEHV DNA polymerase antibodies or re-inoculated cells with supernatants obtained from passages 2 or 3 of the culture medium. The results reveal that no CPEs were observed in the tested cells, while immunolabeling for EEHV gB was observed in only U937 human myeloid leukemia cells. However, quantitation values of the EEHV terminase gene, as well as those of the EEHV gB or EEHV DNA polymerase proteins in U937 cells, gradually declined from passage 1 to passage 3. The findings of this study indicate that despite poor adaptation in U937 cells, this cell line displays promise and potential to be used for the isolation of EEHV1 and EEHV4 in vitro.

Oo, Z. M., Y. H. Aung, T. T. Aung, N. San, Z. M. Tun, G. S. Hayward and A. Zachariah (2020). "Elephant Endotheliotropic Herpesvirus Hemorrhagic Disease in Asian Elephant Calves in Logging Camps, Myanmar." Emerg Infect Dis **26**(1): 63-69.

In recent years, an alarming number of cases of lethal acute hemorrhagic disease have occurred in Asian elephant calves raised in logging camps in

Myanmar. To determine whether these deaths were associated with infection by elephant endotheliotropic herpesvirus (EEHV), we conducted diagnostic PCR subtype DNA sequencing analysis on necropsy tissue samples collected from 3 locations. We found that EEHV DNA from 7 PCR loci was present at high levels in all 3 calves and was the same EEHV1A virus type that has been described in North America, Europe, and other parts of Asia. However, when analyzed over 5,610 bp, the strains showed major differences from each other and from all previously characterized EEHV1A strains. We conclude that these 3 elephant calves in Myanmar died from the same herpesvirus disease that has afflicted young Asian elephants in other countries over the past 20 years.

Hengtrakul, P., P. Sudlapa, N. Chaisurat, S. Sodsaengthien, C. Chamnankij, S. Noimoon, C. Punkong, S. Phatthanakunanan, P. Lertwatcharasarakul and S. Sripiboon (2020). "Biological and environmental factors associated with the detection of elephant endotheliotropic herpesvirus in Asian elephants (Elephas maximus) in Thailand." The Journal of veterinary medical science.

Elephant endotheliotropic herpesvirus (EEHV) infection is one of the most common diseases in young elephants, causing severe fatal hemorrhagic disease. Subclinical infection was previously described; however, information about the factors associated with virus shedding and reactivation were scarce. To identify the biological and environmental factors related with EEHV detection, blood and oral swab samples were collected from nine captive Asian elephants in Thailand for one year and tested for EEHV presence using real-time PCR. Data including hematological values, management, environmental temperature, and serum cortisol levels were also recorded and analyzed. Results showed that the viral detection frequency ranged from 0-25%. The highest detection frequency was found in the two youngest elephants, aged less than 15 years. Three types of viruses, EEHV1, EEHV4, and EEHV5, were found in this study, which also detected mixed infection in five elephants. Additionally, the study found that sample type, changes in hematological values, management and health issues, and serum cortisol levels were not associated with herpesvirus detection in the elephants. However, EEHV detection percentage was significantly increased in the summer (mid-Feb to mid-May), possibly due to body fitness reduction from food source limitation and low nutrient content. To obtain a broad aspect of EEHV management, long-term EEHV monitoring is highly recommended in every captive elephant herd.

Guntawang, T., T. Sittisak, S. Srivorakul, V. Kochagul, K. Photichai, C. Thitaram, N. Sthitmatee, W. L. Hsu and K. Pringproa (2020). "In vivo characterization of target cells for acute elephant endotheliotropic herpesvirus (EEHV) infection in Asian elephants (Elephas maximus)." Sci Rep **10**(1): 11402.

Elephant endotheliotropic herpesvirus-hemorrhagic disease (EEHV-HD) is a dangerous viral infectious disease in young Asian elephants. Despite hypotheses underlying pathogenesis of the disease, it is unclear which cell types the virus targets during acute or persistent infections. This study

investigated the tissues and target cells permissive for EEHV infection and replication in vivo. Rabbit polyclonal antibodies against the non-structural proteins of EEHV, DNA polymerase (EEHV DNAPol), were generated and validated. These were used to examine EEHV infection and replication in various tissues of acute EEHV-HD cases and compared to an EEHV-negative control. The results indicated that viral antigens were distributed throughout the epithelia of the alimentary tract and salivary glands, endothelia and smooth muscle cells, and monocytic lineage cells of the EEHV-infected elephants. Moreover, EEHV DNAPol proteins were also found in the bone marrow cells of the EEHV1A-HD and EEHV1A/4-HD cases. This study demonstrated for the first time the target cells that favor in vivo EEHV replication during acute infection, providing a promising foundation for investigating EEHV propagation in vitro.

Grenus, B. G., E. Latimer, A. Cullinane, P. Lyons, G. Creighton and F. B. Nutter (2020). "EVALUATION OF THE EFFICACY OF TWO DIFFERENT SAMPLING SITES FOR THE DETECTION OF ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS (EEHV) IN THREE ASIAN ELEPHANTS (ELEPHAS MAXIMUS) IN IRELAND." J Zoo Wildl Med **51**(2): 303-307.

Elephant endotheliotropic herpesvirus (EEHV) causes a disease that primarily affects juvenile Asian (Elephas maximus) elephants, causing acute hemorrhage and death. Due to the severity of the disease, many zoos have developed EEHV active surveillance programs. Currently, trunk washes are the standard for testing elephants for shedding of EEHV, but it has also been detected from other mucosal surfaces. This study compared the efficacy of oral swabs and trunk washes for the detection of EEHV shedding using previously validated quantitative polymerase chain reaction (qPCR) methods. Oral swab and trunk wash samples from three juvenile elephants at the Dublin Zoo in Ireland were collected in tandem and tested from April to September 2017. Of the 51 paired samples, 21 trunk wash samples were positive for EEHV1, while only 2 of the oral swab samples were positive for EEHV1, suggesting that trunk wash samples are more effective for detecting shedding of EEHV in Asian elephants compared with oral swabs.

Fuery, A., T. Pursell, J. Tan, R. Peng, P. D. Burbelo, G. S. Hayward and P. D. Ling (2020). "Lethal Hemorrhagic Disease and Clinical Illness Associated with Elephant Endotheliotropic Herpesvirus 1 Are Caused by Primary Infection: Implications for the Detection of Diagnostic Proteins." J Virol **94**(3).

Elephant endotheliotropic herpesvirus (EEHV) can cause lethal hemorrhagic disease in juvenile Asian elephants, both in captivity and in the wild. Most deaths associated with the virus 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 often. Whether lethal EEHV infections are due to primary infection or reactivation of latent virus remains unknown, and knowledge of the anti-EEHV antibody levels in young elephants is limited. To close these gaps, we sought to develop a serologic assay capable of distinguishing among

infections with different EEHVs using a luciferase immunoprecipitation system (LIPS) for antibody profiling and a panel of conserved EEHV recombinant proteins and proteins unique to EEHV1. The results showed that elephants dying from EEHV1 hemorrhagic disease or ill from EEHV infection were seronegative for the EEHV species that caused the disease or illness, indicating that the events were associated with primary infection rather than reactivation of latent virus. We also demonstrated that waning of EEHV1-specific antibodies can occur in the first 2 years of life, when a threshold protective level of antibody may be needed to prevent severe EEHV1-related disease. Use of the LIPS assay to identify putative "diagnostic" proteins would be a valuable asset in determining the EEHV immune status of young elephants and responses to candidate EEHV vaccines in the future.IMPORTANCE Whether clinical illness and deaths associated with elephant endotheliotropic herpesvirus (EEHV) infection result from primary infection or reactivation of latent virus is a longstanding question in the field. By applying a relatively new assay, the luciferase immunoprecipitation system (LIPS), combined with the genomic sequences of the viruses, we gained the insights and tools needed to resolve this issue. Our EEHV1-specific LIPS assay should be useful for assessing the vulnerability of elephant calves to infection with different EEHVs and evaluating antibody responses to anti-EEHV vaccines. A significant proportion of the Asian elephant population is under some form of human care. Hence, the ability to screen for EEHV immune status in elephant calves should have a major impact on the management of these animals worldwide.

Wissink-Argilaga, N., A. Dastjerdi and F. M. Molenaar (2019). "USING IN-HOUSE HEMATOLOGY TO DIRECT DECISION-MAKING IN THE SUCCESSFUL TREATMENT AND MONITORING OF A CLINICAL AND SUBSEQUENTLY SUBCLINICAL CASE OF ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS 1B." J Zoo Wildl Med **50**(2): 498-502.

A 3.5-yr-old asymptomatic female Asian elephant (Elephas maximus) with a high load of circulating EEHV1B DNA on qPCR on a routine blood sample, showed progressive depletion of monocytes, lymphocytes, and platelets. Twice daily IV ganciclovir, plasma transfusions, and fluid therapy coincided with a decreasing viral load, which may support potential efficacy of this antiviral drug. An increase in lymphocytes followed initial treatment and preceded the onset of clinical signs. Administration of short-acting glucocorticosteroids for two consecutive days preceded a reduction of lymphocytes, recovery and maturation of monocytes, and gradually decreasing clinical signs, illustrating the potential value of glucocorticosteroids in treatment of clinical EEHV. Three subsequent subclinical episodes with high monocyte and platelet counts did not require intervention. Decision-making was led not just by quantification of viral load and clinical signs, but more specifically by interpretation of the hematological changes using easily accessible, in-house blood smear analysis.

Takehana, K., T. Kinjyo, M. Nemoto and K. Matsuno (2019). "Rapid and sensitive

detection of elephant endotheliotropic herpesvirus 1 (EEHV1) in blood by loop-mediated isothermal amplification (LAMP)." J Vet Med Sci.

Elephant endotheliotropic herpesvirus type 1 (EEHV1) is the most important causative agent of an acute fatal hemorrhagic disease in Asian elephants (Elephas maximus). We employed loop-mediated isothermal amplification (LAMP) to develop a rapid and simple detection method for EEHV1 in blood. When used to test 21 clinical samples collected in Japan, the EEHV1 assay correctly identified one positive and 20 negative clinical samples. It was observed that when samples were spiked with synthetic DNA plasmids including EEHV1 polymerase gene, the detection limit of the LAMP assay was 10(1.2) copies/mul and 100-fold higher than that of conventional PCR. These advantages of the LAMP assay for EEHV1 detection may facilitate better veterinary practices for treating elephants suffering from the acute disease.

Srivorakul, S., T. Guntawang, V. Kochagul, K. Photichai, T. Sittisak, T. Janyamethakul, K. Boonprasert, S. Khammesri, W. Langkaphin, V. Punyapornwithaya, P. Chuammitri, C. Thitaram and K. Pringproa (2019). "Possible roles of monocytes/macrophages in response to elephant endotheliotropic herpesvirus (EEHV) infections in Asian elephants (Elephas maximus)." PLoS ONE **14**(9): e0222158.

Elephant endotheliotropic herpesvirus-hemorrhagic disease (EEHV-HD) is the primary cause of acute, highly fatal, hemorrhagic diseases in young Asian elephants. Although monocytopenia is frequently observed in EEHV-HD cases, the role monocytes play in EEHV-disease pathogenesis is unknown. This study seeks to explain the responses of monocytes/macrophages in the pathogenesis of EEHV-HD. Samples of blood, frozen tissues, and formalin-fixed, paraffin-embedded (FFPE) tissues from EEHV1A-HD, EEHV4-HD, co-infected EEHV1A and 4-HD, and EEHV-negative calves were analyzed. Peripheral blood mononuclear cells (PBMCs) from the persistent EEHV4-infected and EEHV-negative calves were also studied. The results showed increased infiltration of Iba-1-positive macrophages in the inflamed tissues of the internal organs of elephant calves with EEHV-HD. In addition, cellular apoptosis also increased in the tissues of elephants with EEHV-HD, especially in the PBMCs, compared to the EEHV-negative control. In the PBMCs of persistent EEHV4-infected elephants, cytokine mRNA expression was high, particularly up-regulation of TNF-alpha and IFN-gamma. Moreover, viral particles were observed in the cytoplasm of the persistent EEHV4-infected elephant monocytes. Our study demonstrated for the first time that apoptosis of the PBMCs increased in cases of EEHV-HD. Furthermore, this study showed that monocytes may serve as a vehicle for viral dissemination during EEHV infection in Asian elephants.

Pavulraj, S., K. Eschke, A. Prahl, M. Flugger, J. Trimpert, P. B. van den Doel, S. Andreotti, S. Kaessmeyer, N. Osterrieder and W. Azab (2019). "Fatal Elephant Endotheliotropic Herpesvirus Infection of Two Young Asian Elephants." Microorganisms **7**(10).

Elephant endotheliotropic herpesvirus (EEHV) can cause a devastating

haemorrhagic disease in young Asian elephants worldwide. Here, we report the death of two young Asian elephants after suffering from acute haemorrhagic disease due to EEHV-1A infection. We detected widespread distribution of EEHV-1A in various organs and tissues of the infected elephants. Enveloped viral particles accumulated within and around cytoplasmic electron-dense bodies in hepatic endothelial cells were detected. Attempts to isolate the virus on different cell cultures showed limited virus replication; however, late viral protein expression was detected in infected cells. We further showed that glycoprotein B (gB) of EEHV-1A possesses a conserved cleavage site Arg-X-Lys/Arg-Arg that is targeted by the cellular protease furin, similar to other members of the Herpesviridae. We have determined the complete 180 kb genome sequence of EEHV-1A isolated from the liver by next-generation sequencing and de novo assembly. As virus isolation in vitro has been unsuccessful and limited information is available regarding the function of viral proteins, we have attempted to take the initial steps in the development of suitable cell culture system and virus characterization. In addition, the complete genome sequence of an EEHV-1A in Europe will facilitate future studies on the epidemiology and diagnosis of EEHV infection in elephants.

Mahato, G., K. K. Sarma, D. C. Pathak, N. N. Barman, P. Gogoi, M. Dutta and P. Basumatary (2019). "Endotheliotropic herpesvirus infection in Asian elephants (Elephas maximus) of Assam, India." Vet World **12**(11): 1790-1796.

BACKGROUND AND AIM: Elephant endotheliotropic herpesvirus (EEHV) is an emerging disease of elephant. Therefore, a study was conducted to know the actual status of the disease in Assam State of India. MATERIALS AND METHODS: A total of 289 Asian elephants of Assam were screened during 2 years of study from April 2017 to March 2019. The clinical symptoms of diseased as well as gross and histopathological changes of dead elephants were recorded for the diagnosis of the disease. Virus involved in the occurrence of the disease was confirmed by polymerase chain reaction (PCR). RESULTS: In the present study, a total of three elephant calves out of 22 were found positive to EEHV1A. On the other hand, three adult asymptomatic elephants were also found positive for EEHV1 on screening 267 captive Asian elephants of Assam. The amplified PCR product showed band size of 520, 600, and 930 bp. The PCR amplified product with size 600 bp had shown the gene sequence for EEHV1U77/HEL. Gross lesions include congested blood vessels of the liver and intestinal mucosa, foci of petechiae in the spleen, and heart and focal ulceration in the dorsal surface of the tongue. Microscopically, the kidneys showed intertubular edema and focal areas of degeneration associated with coagulative necrosis of the tubular epithelium. The liver showed hydropic degeneration and fatty changes of the hepatocytes. There was a massive proliferation of fibroblasts in the interlobular spaces which penetrated the necrosed areas of the hepatic lobules. CONCLUSION: A total of three wild rescued elephant calves and three asymptomatic adults were found positive for EEHV1A during the 2 years of study. The PCR amplified product with size 600 bp had shown the gene sequence for EEHV1U77/HEL.

Fuery, A., T. Pursell, J. Tan, R. Peng, P. D. Burbelo, G. S. Hayward and P. Ling (2019). "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.

Elephant endotheliotropic herpesvirus (EEHV) can cause lethal hemorrhagic disease in juvenile Asian elephants, both in captivity and in the wild. Most deaths associated with this virus 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 often. Whether lethal EEHV infections are due to primary infection or reactivation of latent virus remains unknown, and knowledge of the anti-EEHV antibody levels in young elephants is limited. To close these gaps, we sought to develop a serologic assay capable of distinguishing among infections with different EEHV types using a luciferase immunoprecipitation system (LIPS) for antibody profiling and a panel of conserved EEHV recombinant proteins and proteins unique to EEHV1. The results show that elephants dying from EEHV1 hemorrhagic disease or ill from EEHV infection were seronegative for the EEHV species that caused this disease or illness, indicating that these events were associated with primary infection rather than reactivation of latent virus. We also demonstrated that waning of EEHV1-specific antibodies can occur in the first 2 years of life, when a threshold protective level of antibody may be needed to prevent severe EEHV1-related disease. Use of the LIPS assay to identify putative "diagnostic" proteins would be a valuable asset in determining the EEHV immune status of young elephants and responses to candidate EEHV vaccines in the future.Importance Whether clinical illness and deaths associated with elephant endotheliotropic herpesvirus (EEHV) infection result from primary infection or reactivation of latent virus is a long standing question in the field. By applying a relatively new assay, the luciferase immunoprecipitation system (LIPS), combined with the genomic sequences of these viruses, we gained the insights and tools needed to resolve this issue. Our EEHV1-specific LIPS assay should be useful for assessing the vulnerability of elephant calves to infection with different EEHV types and evaluating antibody responses to anti-EEHV vaccines. A significant proportion of the Asian elephant population is under some form of human care. Hence, the ability to screen for EEHV immune status in elephant calves should have a major impact on the management of these animals worldwide.

Brown, J. L. (2019). "Update on Comparative Biology of Elephants: Factors Affecting Reproduction, Health and Welfare." Adv Exp Med Biol **1200**: 243-273.

Asian (Elephas maximus) and African (Loxodonta africana) elephants serve as important keystone, umbrella and flagship species. Despite that, population numbers are declining, due mainly to poaching and habitat destruction. Understanding reproductive mechanisms is vital to effective management, particularly insurance populations in captivity, and to that end, long-term biological databases are key to understanding how intrinsic and

extrinsic factors affect reproductive function at individual and population levels. Through decades of hormonal and ultrasonographic monitoring, many unique aspects of zoo elephant reproduction have been identified, including differences in luteal steroidogenic activity, follicular maturation, pituitary gonadotropin secretion, fetal development and reproductive tract anatomy. Reproductive problems also hamper captive propagation efforts, particularly those related to abnormal or lack of ovarian cyclicity. Recent large-scale, multi-institutional studies and use of epidemiological approaches have identified factors important for good welfare and reproduction, which include enrichment, feeding diversity, good elephant-keeper relations, social compatibility, exercise, and not being obese. There are notable differences in reproductive mechanisms between Asian and African elephants, as well as the factors that influence reproduction and welfare, suggesting species-targeted management approaches are needed to maximize fitness. In the first edition, we discussed reproductive function in male and female elephants. Since then, a number of significant advances have been made primarily in female elephants, which will be the focus of this updated review.

Boonprasert, K., V. Punyapornwithaya, P. Tankaew, T. Angkawanish, S. Sriphiboon, C. Titharam, J. L. Brown and C. Somgird (2019). "Survival analysis of confirmed elephant endotheliotropic herpes virus cases in Thailand from 2006 - 2018." PLoS ONE **14**(7): e0219288.

The elephant endotheliotropic herpesvirus (EEHV) has been a known cause of death of young elephants in Thailand for over a decade. In this study, we report on the demography, disease characteristics and mortality of 58 elephants with confirmed EEHV hemorrhagic disease between January 2006 and August 2018 using retrospective data subjected to survival analysis. Median age of EEHV presentation was 29 months, and the mortality rate was 68.97% with a median survival time of 36 h. Most EEHV cases occurred in the north of Thailand, the region where most of the country's captive elephants reside. The hazard ratio analysis identified application of medical procedures and antiviral medications as being significant factors correlated to the risk of death. Our results indicate a need to focus EEHV monitoring efforts on young elephants and to follow current protocols that advise starting treatments before clinical signs appear.

Angkawanish, T., M. Nielen, H. Vernooij, J. L. Brown, P. J. S. van Kooten, P. B. van den Doel, W. Schaftenaar, K. Na Lampang and V. Rutten (2019). "Evidence of high EEHV antibody seroprevalence and spatial variation among captive Asian elephants (Elephas maximus) in Thailand." Virol J **16**(1): 33.

BACKGROUND: Elephant endotheliotropic herpesviruses (EEHV) can cause an acute highly fatal hemorrhagic disease in young Asian elephants (Elephas maximus), both ex situ and in situ. Amongst eight EEHV types described so far, type 1 (subtype 1A and 1B) is the predominant disease-associated type. Little is known about routes of infection and pathogenesis of EEHV, and knowledge of disease prevalence, especially in range countries, is limited. METHODS: A large cross-sectional serological survey was conducted in

captive elephants (n = 994) throughout Thailand using an EEHV-1A glycoprotein B protein antigen specific antibody ELISA. RESULTS: Antibody seroprevalence was 42.3%, with 420 of 994 elephants testing positive. Associations between seropositivity and potential risk factors for EEHV infection were assessed and included: elephant age, sex, camp cluster size, management type (extensive versus intensive), sampling period (wet vs. dry season) and location of camp (region). Univariable regression analysis identified management system and region as risk factors for the presence of EEHV antibodies in elephants, with region being significant in the final multivariable regression model. Prevalence was highest in the North region of the country (49.4%). CONCLUSIONS: This study produced baseline serological data for captive elephants throughout Thailand, and showed a significant EEHV burden likely to be maintained in the captive population.

Zachariah, A., P. K. Sajesh, S. Santhosh, C. Bathrachalam, M. Megha, J. Pandiyan, M. Jishnu, R. S. Kobragade, S. Y. Long, J. C. Zong, E. M. Latimer, S. Y. Heaggans and G. S. Hayward (2018). "Extended genotypic evaluation and comparison of twenty-two cases of lethal EEHV1 hemorrhagic disease in wild and captive Asian elephants in India." PLoS ONE **13**(8): e0202438.

Thirteen new lethal cases of acute hemorrhagic disease (HD) with typical histopathogical features were identified in young Asian elephants (Elephas maximus indicus) in India between 2013 and 2017. Eight occurred amongst free-ranging wild herds, with three more in camp-raised orphans and two in captive-born calves. All were confirmed to have high levels of Elephant Endotheliotropic Herpesvirus type 1A (EEHV1A) DNA detected within gross pathological lesions from necropsy tissue by multi-locus PCR DNA sequencing. The strains involved were all significantly different from one another and from nine previously described cases from Southern India (which included one example of EEHV1B). Overall, eight selected dispersed PCR loci totaling up to 6.1-kb in size were analyzed for most of the 22 cases, with extensive subtype clustering data being obtained at four hypervariable gene loci. In addition to the previously identified U48(gH-TK) and U51(vGPCR1) gene loci, these included two newly identified E5(vGPCR5) and E54(vOX2-1) loci mapping far outside of the classic EEHV1A versus EEHV1B subtype chimeric domains and towards the novel end segments of the genome that had not been evaluated previously. The high levels of genetic divergence and mosaic scrambling observed between adjacent loci match closely to the overall range of divergence found within 45 analyzed North American and European cases, but include some common relatively unique polymorphic features and preferred subtypes that appear to distinguish most but not all Indian strains from both those in Thailand and those outside range countries. Furthermore, more than half of the Indian cases studied here involved calves living within wild herds, whereas nearly all other cases identified in Asia so far represent rescued camp orphans or captive-born calves.

Perrin, K. L., A. K. Krogh, M. Kjelgaard-Hansen, L. Howard, L. Bochsen, W. K. Kiso,

D. Schmitt, A. T. Kristensen and M. F. Bertelsen (2018). "THROMBOELASTOGRAPHY IN THE HEALTHY ASIAN ELEPHANT (ELEPHAS MAXIMUS): REFERENCE INTERVALS AND EFFECTS OF STORAGE." J Zoo Wildl Med **49**(1): 54-63.

Hemorrhagic disease associated with elephant endotheliotropic herpesvirus infection is the most-frequent cause of mortality in captive Asian elephants (Elephas maximus). Survival relies on intensive monitoring of hemostatic status. Thromboelastography (TEG) utilizes whole blood samples containing all the blood components of hemostasis and is therefore a sensitive indicator of the clinical status in the patient. This study was performed to assess the practicability of TEG in Asian elephants in a zoo environment. Citrated stabilized whole blood samples were obtained from 44 healthy Asian elephants. Kaolin-activated TEG was performed on whole blood at 60 min and 24 hr postsampling (to replicate shipment to an external laboratory) as well as on freeze-thawed plasma samples, 12-14 mo postsampling. Reference intervals were calculated for fresh whole blood and freeze-thawed plasma samples. In the 24-hr analysis, storage artifacts, likely due to cellular degeneration, resulted in a hypercoagulable thromboelastogram and thus reduced sensitivity for detecting coagulopathies. Therefore, delayed analysis of whole blood samples is not recommended.

Kochakul, V., K. Boonsri, S. Tiwananthagorn, C. Somgird, C. Thitaram and K. Pringproa (2018). "Development of in situ hybridization for detection of elephant endotheliotropic herpesvirus in Asian elephants." Journal of Veterinary Diagnostic Investigation **30**(4): 628-632.

Kochagul, V., S. Srivorakul, K. Boonsri, C. Somgird, N. Sthitmatee, C. Thitaram and K. Pringproa (2018). "Production of antibody against elephant endotheliotropic herpesvirus (EEHV) unveils tissue tropisms and routes of viral transmission in EEHV-infected Asian elephants." Sci Rep **8**(1): 4675.

Elephant endotheliotropic herpesvirus (EEHV) is one of the most devastating viral infectious diseases in elephants worldwide. To date, it remains unclear how elephants get infected by the virus, where the virus persists, and what mechanisms drive the pathogenesis of the disease. The present study was aimed to develop an antibody against glycoprotein B (gB) of EEHV, investigate the EEHV tissue tropisms, and provide the possible routes of EEHV transmission in Asian elephants. Samples from elephant organs that had died from EEHV1A and EEHV4 infections, peripheral blood mononuclear cells (PBMC) from EEHV4- and non-EEHV-infected calves were used in this study. The results of western immunoblotting indicated that the antibody can be used for detection of gB antigens in both EEHV1A- and EEHV4-infected samples. Immunohistochemical detection indicated that the EEHV gB antigens were distributed mainly in the epithelial cells of the salivary glands, stomach and intestines. Immunofluorescence test of PBMC for EEHV gB in the EEHV4-infected calf indicated that the virus was observed predominantly in the mononuclear phagocytic cells. The findings in the present study unveil tissue tropisms in the EEHV1A- and EEHV4-infected calves and point out that saliva and intestinal content are likely sources for virus transmission in

EEHV-infected Asian elephants.

Fuery, A., A. M. Leen, R. Peng, M. C. Wong, H. Liu and P. D. Ling (2018). "Asian Elephant T Cell Responses to Elephant Endotheliotropic Herpesvirus." J Virol **92**(6).

Elephant endotheliotropic herpesvirus (EEHV) can cause lethal hemorrhagic disease in juvenile Asian elephants, an endangered species. One hypothesis to explain this vulnerability of some juvenile elephants is that they fail to mount an effective T cell response to the virus. To our knowledge, there have been no studies of Asian elephant T cell responses to EEHV. To address this deficiency, we validated the gamma interferon (IFN-gamma) enzyme-linked immunospot assay for tracking antigen-directed T cell activity by monitoring rabies-specific responses in vaccinated elephants. In addition, we generated monoclonal antibodies to Asian elephant CD4 and CD8 to facilitate phenotypic T cell profiling. Using these tools, we screened healthy elephants with a history of EEHV infection for reactivity against nine EEHV proteins whose counterparts in other herpesviruses are known to induce T cell responses in their natural hosts. We identified glycoprotein B (gB) and the putative regulatory protein E40 as the most immunogenic T cell targets (IFN-gamma responses in five of seven elephants), followed by the major capsid protein (IFN-gamma responses in three of seven elephants). We also observed that IFN-gamma responses were largely from CD4(+) T cells. We detected no activity against the predicted major immediate early (E44) and large tegument (E34) proteins, both immunodominant T cell targets in humans latently infected with cytomegalovirus. These studies identified EEHV-specific T cells in Asian elephants for the first time, lending insight into the T cell priming that might be required to protect against EEHV disease, and will guide the design of effective vaccine strategies.IMPORTANCE Endangered Asian elephants are facing many threats, including lethal hemorrhagic disease from elephant endotheliotropic herpesvirus (EEHV). EEHV usually establishes chronic, benign infections in mature Asian elephants but can be lethal to juvenile elephants in captivity and the wild. It is the leading cause of death in captive Asian elephants in North America and Europe. Despite the availability of sensitive tests and protocols for treating EEHV-associated illness, these measures are not always effective. The best line of defense would be a preventative vaccine. We interrogated normal healthy elephants previously infected with EEHV for T cell responses to nine EEHV proteins predicted to induce cellular immune responses. Three proteins elicited IFN-gamma responses, suggesting their potential usefulness as vaccine candidates. Our work is the first to describe T cell responses to a member of the proposed fourth subfamily of mammalian herpesviruses, the Deltaherpesvirinae, within a host species in the clade Afrotheria. An EEHV vaccine would greatly contribute to the health care of Asian and African elephants that are also susceptible to this disease.

Boonsri, K., C. Somgird, P. Noinafai, K. Pringproa, T. Janyamethakul, T. Angkawanish, J. L. Brown, P. Tankaew, S. Srivorakul and C. Thitaram (2018). "ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS ASSOCIATED WITH CLOSTRIDIUM

PERFRINGENS INFECTION IN TWO ASIAN ELEPHANT (ELEPHAS MAXIMUS) CALVES." J Zoo Wildl Med **49**(1): 178-182.

Elephant endotheliotropic herpesvirus (EEHV) is an infection associated with fatal hemorrhagic disease in young Asian elephants (Elephas maximus). This brief communication describes the postmortem evaluation of two Asian elephant calves diagnosed with EEHV4 and EEHV1A in conjunction with Clostridium perfringens infection. Case 1 was a 7-mo-old, male captive-born Asian elephant that developed diarrhea and died 2 days after clinical presentation. Examination of the heart, lungs, liver, and spleen revealed predominantly basophilic intranuclear inclusion bodies in the endothelial cells of the blood vessels. Case 2 was a 3-mo-old, female wild-born Asian elephant that showed signs of lethargy, anorexia, and convulsions and died 6 hr after clinical presentation. No intranuclear inclusion bodies were observed. The heart, lung, liver, and spleen of both calves tested positive for EEHV by polymerase chain reaction. Phylogenetic analysis identified EEHV4 and EEHV1A in Case 1 and 2, respectively. Additionally, liver, spleen, and hemorrhagic intestinal tissue samples tested positive for C. perfringens alpha, beta, and epsilon toxins. This is the first reported case to describe coinfection of EEHV and C. perfringens in Asian elephant calves.

Bauer, K. L., E. Latimer and M. Finnegan (2018). "Long-term, intermittent, low-level elephant endotheliotropic herpesvirus 1A viremia in a captive Asian elephant calf." J Vet Diagn Invest **30**(6): 917-919.

A 2-y-old male Asian elephant (Elephas maximus), with an elevated platelet count (1,100 x 10(9)/L [1,100 x 10(3)/mm(3)]), tested positive for elephant endotheliotropic herpesvirus 1A (EEHV-1A) on conventional PCR (cPCR) of EDTA whole blood. No clinical signs were ever reported and no treatment was administered, but low-level viremia persisted for 2.5 y based on results of cPCR and/or real-time PCR (rtPCR). Sequencing confirmed that the EEHV-1A detected was identical at the beginning through the end of the time period. No other elephants in the herd tested positive for EEHV-1 during this time period. Platelet counts remained elevated throughout the viremia and throughout the animal's life, and direct correlation between the elevated platelet counts and EEHV-1A viremia could not be confirmed. We document long-term, intermittent, low-level viremia of EEHV-1A and provide additional information to consider when determining if treatment is warranted in a case of EEHV infection.

Azab, W., A. M. Damiani, A. Ochs and N. Osterrieder (2018). "Subclinical infection of a young captive Asian elephant with elephant endotheliotropic herpesvirus 1." Arch Virol **163**(2): 495-500.

Elephant endotheliotropic herpesviruses (EEHVs) are a continuous threat for young Asian elephants. We report a laboratory-confirmed infection of a 5-year-old female Asian elephant (AZ_2016) in the Berlin Zoologischer Garten. Initially, high EEHV-1 loads were detected in trunk swabs obtained from the young elephant during routine screening. The animal showed no clinical signs except for slight irritability. EEHV-1 was continuously shed for almost one

year, with fluctuations in viral load from time to time. Our investigations highlight the continuous threat of EEHV-1 to young captive Asian elephants and stress the importance of routine monitoring of captive elephants to allow early detection of infection.

Sripiboon, S., T. Angkawanish, K. Boonprasert, P. Sombutputorn, W. Langkaphin, W. Ditcham and K. Warren (2017). "SUCCESSFUL TREATMENT OF A CLINICAL ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS INFECTION: THE DYNAMICS OF VIRAL LOAD, GENOTYPE ANALYSIS, AND TREATMENT WITH ACYCLOVIR." J Zoo Wildl Med **48**(4): 1254-1259.

This article describes the treatment of clinical elephant endotheliotropic herpesvirus (EEHV) infection in a male Asian elephant (Elephas maximus; approximately 3 yr old), the dynamics of viral load during the active infection, and genetic analysis of the virus. Treatment included injectable acyclovir (12 mg/kg iv, bid), antibiotic, vitamin, and fluids. Quantitative polymerase chain reaction was used to measure the viral levels in blood, which decreased continuously after initiation of intravenous acyclovir. Low levels of virus were detected in the blood for 2 wk, and the virus was undetectable after 1 mo. No complication was observed during the treatment period. This case report suggests that acyclovir, given parenterally, could potentially enhance survival of clinical EEHV-infected individuals.

Luz, S. and L. Howard (2017). Guidelines for Management Elephant Endotheliotopic Herpesvirus in Asia 2nd edition, Wildlife Reserves Singapore Group**:** 19.

Lopez, J., M. S. L. D. Vet, J. Haycock, A. McKenzie, K. Seilern-Moy and A. Dastjerdi (2017). "ASSESSMENT OF A LANCET-AND-SWAB BLOOD SAMPLING TECHNIQUE FOR SURVEILLANCE OF ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS INFECTION." J Zoo Wildl Med **48**(3): 659-667.

Lancing a finger elicits minimal pain in humans and is applied routinely to obtain small volumes of blood for clinical diagnostics. A modified lancet bleeding method and several blood sampling matrices were evaluated in this study for the purpose of routine elephant endotheliotropic herpesvirus (EEHV) surveillance in Asian elephants (Elephas maximus). The procedure enabled weekly sampling from elephants as young as 9 mo of age. The blood sampling matrices were evaluated for their sensitivity measuring beta-actin, tumor necrosis factor alpha, and/or EEHV-1 by quantitative polymerase chain reaction assays. Foam and flocked swabs produced significantly ($P < 0.05$) lower quantitation cycles, ie, increased analytical sensitivity, than filter papers, Whatman(R) FTA cards, or conventional cotton-tipped swabs. The two swab types also demonstrated comparable analytical sensitivity to that of a similar volume of EDTA whole blood for the detection of EEHV-1 DNA. This lancet-and-swab technique proved satisfactory for the detection of EEHV-1 viremia in two Asian elephant calves, and in one instance viremia could be detected 5 days prior to the development of clinical signs. Low blood yield from the lancet application may reduce sensitivity and compromise early detection of viremia. Therefore, standard venipuncture remains the

recommended blood sampling method, and training for consistent and regular vein access should continue to be the priority for collections holding elephants. However, if appropriate measures are taken to collect an optimum blood volume, this lancet-and-swab technique offers a suitable alternative for EEHV surveillance in situations where venipuncture may not be practical.

Bronson, E., M. McClure, J. Sohl, E. Wiedner, S. Cox, E. M. Latimer, V. R. Pearson, G. S. Hayward, A. Fuery and P. D. Ling (2017). "EPIDEMIOLOGIC EVALUATION of ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS 3B INFECTION in AN AFRICAN ELEPHANT (LOXODONTA AFRICANA)." Journal of Zoo and Wildlife Medicine **48**(2): 335-343.

Bhusri, B., P. Suksai, C. Mongkolphan, E. Tiyanun, P. Ratanakorn, K. Chaichoun and L. Sariya (2017). "Detection of elephant endotheliotropic herpesvirus 4 in captive asian elephants (Elephas maximus) in Thailand." Thai Journal of Veterinary Medicine **47**(1): 97-102.

Elephant endotheliotropic herpesviruses (EEHVs) can cause fatal hemorrhagic disease in elephants, especially young captive Asian elephants (Elephas maximus). Currently, seven EEHV types have been reported. In this study, EEHVs were examined in whole-blood samples derived from 56 captive Asian elephants from eight provinces in Thailand by nested PCR using primers specific to the viral DNA polymerase gene in an attempt to monitor EEHV elephant cases. After EEHV testing, one sample (1.78%) was positive and found to be closely related to EEHV4 with 99% amino acid identity. This sample was from a three-year-old female Asian elephant with no clinical signs. These data suggest that asymptomatic EEHV4 infection can occur in Asian elephants.

Barman, N. N., B. Choudhury, V. Kumar, M. Koul, S. M. Gogoi, E. Khatoon, A. Chakroborty, P. Basumatary, B. Barua, T. Rahman, S. K. Das and S. Kumar (2017). "Incidence of elephant endotheliotropic herpesvirus in Asian elephants in India." Vet Microbiol **208**: 159-163.

Elephant endotheliotropic herpesviruses (EEHVs) are the cause of acute hemorrhagic disease in endangered Asian and African elephants. In the present study, we report the incidence of EEHV infection and associated mortality in the captive elephant of Assam, India. Our result showed the gross morphology and histopathological changes of EEHV infection in the elephant. Moreover, the phylogenetic analysis of the polymerase, helicase, and GPCR genes from the infected tissue samples suggested the presence of EEHV1A virus.

Zong, J. C., S. Y. Heaggans, S. Y. Long, E. M. Latimer, S. A. Nofs, E. Bronson, M. Casares, M. D. Fouraker, V. R. Pearson, L. K. Richman and G. S. Hayward (2016). "Detection of quiescent infections with multiple elephant endotheliotropic herpesviruses (EEHVs), including EEHV2, EEHV3, EEHV6, and EEHV7, within lymphoid lung nodules or lung and spleen tissue samples from five asymptomatic adult African elephants." Journal of Virology **90**(6): 3028-3043.

More than 80 cases of lethal hemorrhagic disease associated with elephant endotheliotropic herpesviruses (EEHVs) have been identified in young Asian elephants worldwide. Diagnostic PCR tests detected six types of EEHV in blood of elephants with acute disease, although EEHV1A is the predominant pathogenic type. Previously, the presence of herpesvirus virions within benign lung and skin nodules from healthy African elephants led to suggestions that African elephants may be the source of EEHV disease in Asian elephants. Here, we used direct PCR-basedDNAsequencing to detect EEHV genomes in necropsy tissue from five healthy adult African elephants. Two large lung nodules collected from culled wild South African elephants contained high levels of either EEHV3 alone or both EEHV2 and EEHV3. Similarly, a euthanized U.S. elephant proved to harbor multiple EEHV types distributed nonuniformly across four small lung nodules, including high levels of EEHV6, lower levels of EEHV3 and EEHV2, and a new GC-rich branch type, EEHV7. Several of the same EEHV types were also detected in random lung and spleen samples from two other elephants. Sanger PCR DNAsequence data comprising 100 kb were obtained from a total of 15 different strains identified, with (except for a few hypervariable genes) the EEHV2, EEHV3, and EEHV6 strains all being closely related to known genotypes from cases of acute disease, whereas the seven loci (4.0 kb) obtained from EEHV7 averaged 18% divergence from their nearest relative, EEHV3. Overall, we conclude that these four EEHV species, but probably not EEHV1, occur commonly as quiescent infections in African elephants. © 2016, American Society for Microbiology.

Sripiboon, S., B. Jackson, W. Ditcham, C. Holyoake, I. Robertson, C. Thitaram, P. Tankaew, P. Letwatcharasarakul and K. Warren (2016). "Molecular characterisation and genetic variation of Elephant Endotheliotropic Herpesvirus infection in captive young Asian elephants in Thailand." Infect Genet Evol **44**: 487-494.

Elephant Endotheliotropic Herpesvirus (EEHV) is emerging as a new threat for elephant conservation, since being identified as the cause of severe, often fatal, haemorrhagic disease in young Asian elephants. To describe positive cases and the molecular relatedness of virus detected in elephants in Thailand, we re-examined all available of EEHV samples occurring in young elephants in Thailand between 2006 and 2014 (n=24). Results indicated 75% (18/24) of suspected cases were positive for EEHV by semi-nested PCR. Further gene analysis identified these positive cases as EEHV1A (72%, 13/18 cases), EEHV1B (11%, 2/18) and EEHV4 (17%, 3/18). This study is the first to phylogenetically analyse and provide an overview of most of the known EEHV cases that have occurred in Thailand. Positive individuals ranged in age from one to nine years, with no sex association detected, and occurred across geographical locations throughout the country. All individuals, except one, were captive-born. No history of direct contact among the cases was recorded, and this together with the fact that various subtype clusters of virus were found, implied that none of the positive cases were epidemiologically related. These results concur with the hypothesis that EEHV1 is likely to be an ancient endogenous pathogen in Asian elephants. It

is recommended that active surveillance and routine monitoring for EEHV should be undertaken in all elephant range countries, to gain a better understanding of the epidemiology, transmission and prevention of this disease.

Seilern-Moy, K., K. Darpel, F. Steinbach and A. Dastjerdi (2016). "Distribution and load of elephant endotheliotropic herpesviruses in tissues from associated fatalities of Asian elephants." Virus Res **220**: 91-96.

Elephant Endotheliotropic Herpesviruses (EEHVs) are the cause of a highly fatal haemorrhagic disease in elephants primarily affecting young Asian elephants (Elephas maximus) in both captivity and in the wild. The viruses have emerged as a significant threat to Asian elephant conservation, critically affecting overall sustainability of their population. So far insight into the pathogenesis of EEHV infections has been restricted to examination of EEHV-infected tissues. However, little is known about distribution and burden of the viruses within the organs of fatal cases, crucial elements in the understanding of the virus pathogenesis. This study was therefore undertaken to assess the extent of organ and cell involvement in fatal cases of EEHV-1A, 1B and 5 using a quantitative real-time PCR. EEHV-1 and 5 DNA were detectable in all the tissues examined, albeit with substantial differences in the viral DNA load. The highest EEHV-1A DNA load was observed in the liver, followed by the heart, thymus and tongue. EEHV-1B and 5 showed the highest DNA load in the heart, followed by tongue and liver. This study provides new insights into EEHV pathogenicity and has implications in choice of sample type for disease investigation and virus isolation.

Randima, G. D. D., K. G. D. D. A. Abeysinghe, R. P. G. Vandercone and T. C. Bamunuarachchige (2016). Screening of Asian elephants (Elephas maximus) in captivity at elephant orphanage, Pinnawala and ETH, Udawalawe for Mycobacterium tuberculosis and elephant endotheliotropic herpes virus type1 (EEHV 1) using direct amplification of pathogen DNA from trunk washes. Proc. of the Fourth Intl. Conf. Advances in Bio-Informatics, Bio-Technology and Environmental Engineering.

There are many case reports of elephant pathogenic bacteria and viruses that require quick and sensitive diagnostic techniques due to the impact they generate. Out of these the occurrence of TB in elephants, especially in captivity, leading to zoonotic risk for humans who live at the animal-human interface and the different strains of elephant endotheliotropic herpes virus (EEHV) that pose a threat to Asian elephants are of extreme importance. Hence, this study aims to evaluate the PCR based molecular techniques for the rapid and direct detection of TB in captive elephants by primers targeting gene hsp65 and EEHV 1 strain by primers targeting the terminase gene. Serologically positive captive Asian elephants at Elephant orphanage, Pinnawala were screened for TB by specific primer PCR assay for hsp65 gene of M.tuberculosis using direct DNA isolates from trunk wash samples. Among 21 trunk washes, only a single amplification was observed, with a size closer to 441bp. Sequencing of this resulted a 415bp fragment which was not

responsible for TB. Although, there have been no recorded cases of EEHV in Sri Lanka, many healthy Asian elephants are asymptomatically infected by EEHV1 in the neighboring Indian region. Therefore, asymptomatic Asian elephants in captivity at ETH, Udawalawe were screened for 336bp partial EEHV1 terminase gene using direct DNA isolates from blood, eye swabs and buccal cavity swabs. All tested samples were negative for EEHV1. Since these elephants were closely monitored even after the study and none of them developed classical symptoms of either EEHV or TB, it is difficult to prove the fact that they were originally infected. The nonspecific amplification proves that it is possible to extract microbial DNA from elephant trunk washes.

Pursell, T., J. Tan, R. Peng and P. D. Ling (2016). "Generation and validation of new quantitative real time PCR assays to detect elephant endotheliotropic herpesviruses 1A, 1B, and 4." J Virol Methods **237**: 138-142.

Elephant endotheliotropic herpesviruses (EEHVs) can cause fatal hemorrhagic disease in Asian and African elephants. There are quantitative real time PCR (qPCR) tests that can detect seven known EEHVs (1A, 1B, 2-6) in mucosal secretions, tissue isolates, and blood samples. However, current qPCR tests are unable to distinguish between EEHV 1A and 1B or 3 and 4. To address these inadequacies, new qPCR assays were generated and validated to specifically detect EEHV 1A, 1B, and 4. Each assay demonstrated robust efficiency, a broad linear range, and low intra- and inter-assay variability. Each also proved to be specific for its EEHV target when tested against known banked samples from past EEHV cases. The EEHV1A and 1B assays were then used to characterize an eight-week, low level EEHV1 viremic event in a young Asian elephant. These new tests will allow veterinarians and researchers to pinpoint the specific species causing infection more rapidly. They will also allow veterinarians and elephant keepers to better characterize the EEHV status of each animal within their herd leading to more informed management strategies.

Ling, P. D., S. Y. Long, J. C. Zong, S. Y. Heaggans, X. Qin and G. S. Hayward (2016). "Comparison of the gene coding contents and other unusual features of the GC-rich and AT-rich branch probosciviruses." mSphere **1**(3).

Nearly 100 cases of lethal acute hemorrhagic disease in young Asian elephants have been reported worldwide. All tested cases contained high levels of elephant endotheliotropic herpesvirus (EEHV) DNA in pathological blood or tissue samples. Seven known major types of EEHVs have been partially characterized and shown to all belong to the novel Proboscivirus genus. However, the recently determined 206-kb EEHV4 genome proved to represent the prototype of a GC-rich branch virus that is very distinct from the previously published 180-kb EEHV1A, EEHV1B, and EEHV5A genomes, which all fall within an alternative AT-rich branch. Although EEHV4 retains the large family of 7xTM and vGPCR-like genes, six are unique to either just one or the other branch. While both branches display a highly enriched distribution of A and T tracts in intergenic domains, they are generally much larger within the GC-rich branch. Both branches retain the vGCNT1

acetylglucosamine transferase and at least one vOX-2 gene, but the two branches differ by 25 genes overall, with the AT-rich branch encoding a fucosyl transferase (vFUT9) plus two or three more vOX2 proteins and an immunoglobulin-like gene family that are all absent from the GC-rich branch. Several envelope glycoproteins retain only 15 to 20% protein identity or less across the two branches. Finally, the two plausible predicted transcriptional regulatory proteins display no homology at all to those in the alpha-, beta-, or gammaherpesvirus subfamilies. These results reinforce our previous proposal that the probosciviruses should be designated a new subfamily of mammalian herpesviruses. © 2016 Ling et al.

Kendall, R., L. Howard, N. Masters and R. Grant (2016). "THE IMPACT of ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS on the CAPTIVE ASIAN ELEPHANT (ELEPHAS MAXIMUS) POPULATION of the United Kingdom and Ireland (1995-2013)." Journal of Zoo and Wildlife Medicine **47**(2): 405-418.

Elephant endotheliotropic herpesvirus (EEHV) is one of the most devastating infections and causes of mortality in captive Asian elephant (Elephas maximus) populations. Eight confirmed fatal EEHV cases have occurred since 1995 within the captive Asian elephant population of the United Kingdom and Ireland. This report aims to review the impact of EEHV on the captive Asian elephant population in the United Kingdom and Ireland, document and compare fatal cases, and recommend a framework of monitoring within the United Kingdom and Ireland to increase the success of treatment of EEHV hemorrhagic disease (EEHV HD) in the future. Six zoologic institutions (which include zoos, safari parks, and wildlife parks) that currently house or have previously housed a captive Asian elephant group were included in this report. Medical records and postmortem results were collected from four of these institutions for each confirmed fatal case. EEHV HD was found to be responsible for 29.6% of fatalities in Asian elephants born in captivity in the United Kingdom and Ireland between 1995 and 2013. Following a review of all the cases, it is shown that although clinical signs may be associated with specific EEHV species, the swiftness of disease progression means that most body tissues are impacted 1-6 days following the presentation of visible clinical signs and treatment is less likely to succeed. Therefore, EEHV monitoring should consist of conducting regular polymerase chain reaction analysis of whole blood samples from at-risk, young Asian elephants aged 1-8 yr in order for subclinical viremia to be identified early and treatment to be started before the appearance of visible clinical signs. © Copyright 2016 by American Association of Zoo Veterinarians.

Fuery, A., G. R. Browning, J. Tan, S. Long, G. S. Hayward, S. K. Cox, J. P. Flanagan, M. E. Tocidlowski, L. L. Howard and P. D. Ling (2016). "Clinical Infection of Captive Asian Elephants (Elephas Maximus) with Elephant Endotheliotropic Herpesvirus 4." J Zoo Wildl Med **47**(1): 311-318.

Elephant endotheliotropic herpesvirus (EEHV) can cause lethal hemorrhagic disease in juvenile Asian elephants. A number of EEHV types and subtypes exist, where most deaths have been caused by EEHV1A and EEHV1B. EEHV4

has been attributed to two deaths, but as both diagnoses were made postmortem, EEHV4 disease has not yet been observed and recorded clinically. In this brief communication, two cases of EEHV4 infection in juvenile elephants at the Houston Zoo are described, where both cases were resolved following intensive treatment and administration of famciclovir. A quantitative real-time polymerase chain reaction detected EEHV4 viremia that correlated with clinical signs. High levels of EEHV4 shedding from trunk wash secretions of the first viremic elephant correlated with subsequent infection of the second elephant with EEHV4. It is hoped that the observations made in these cases--and the successful treatment regimen used--will help other institutions identify and treat EEHV4 infection in the future.

Dastjerdi, A., K. Seilern-Moy, K. Darpel, F. Steinbach and F. Molenaar (2016). "Surviving and fatal Elephant Endotheliotropic Herpesvirus-1A infections in juvenile Asian elephants - lessons learned and recommendations on anti-herpesviral therapy." BMC Vet Res **12**(1): 178.

BACKGROUND: Elephant Endotheliotropic Herpesviruses (EEHVs) can cause acute haemorrhagic disease in young Asian elephants (Elephas maximus) and clinical EEHV infections account for the majority of their fatalities. The anti-herpesviral drug famciclovir (FCV) has been used routinely to treat viraemic at-risk elephants, but thus far without proven efficacy. This paper presents clinical and virological investigations of two EEHV-1A infected elephants treated with FCV, and discusses anti-herpesvirus therapies of viraemic elephants. CASES PRESENTATIONS: Two 1.5 year old male Asian elephants at a zoological collection in the UK developed clinical EEHV-1A infections. Case 1 showed signs of myalgia for the duration of 24 hours before returning back to normal. EEHV-1A DNAemia was confirmed on the day of clinical signs and continued to be present for 18 days in total. Trunk shedding of the virus commenced 10 days after detection of initial DNAemia. Case 2 tested positive for EEHV-1A DNAemia in a routine blood screening sample in the absence of clinical signs. The blood viral load increased exponentially leading up to fatal clinical disease seven days after initial detection of DNAemia. Both calves were treated with 15 mg/kg FCV per rectum on detection of DNAemia and penciclovir, the FCV metabolite, could be detected in the blood at assumed therapeutic levels. The early indicators for clinical disease were a marked absolute and relative drop in white blood cells, particularly monocytes prior to the detection of viraemia. The most prognostic haematological parameter at later stages of the disease was the platelet count showing a continuous sharp decline throughout, followed by a dramatic drop at the time of death. CONCLUSIONS: The EEHV-1A viraemic animals investigated here further highlight the ongoing threat posed by these viruses to juvenile Asian elephants. The findings call into question the efficacy of rectal FCV in clinical cases and direct towards the use of alternative anti-herpesvirus drugs and complementary treatments such as plasma infusions if no improvement in either viral load or the above-mentioned blood parameters are observed in the initial days of viraemia

despite anti-herpesvirus therapy.

Long, S. Y., E. M. Latimer and G. S. Hayward (2015). "Review of elephant endotheliotropic herpesviruses and acute hemorrhagic disease." Ilar Journal **56**(3): 283-296.

More than 100 young captive and wild Asian elephants are known to have died from a rapid-onset, acute hemorrhagic disease caused primarily by multiple distinct strains of two closely related chimeric variants of a novel herpesvirus species designated elephant endotheliotropic herpesvirus (EEHV1A and EEHV1B). These and two other species of Probosciviruses (EEHV4 and EEHV5) are evidently ancient and likely nearly ubiquitous asymptomatic infections of adult Asian elephants worldwide that are occasionally shed in trunk wash secretions. Although only a handful of similar cases have been observed in African elephants, they also have proved to harbor their own multiple and distinct species of Probosciviruses-EEHV2, EEHV3, EEHV6, and EEHV7-found in lung and skin nodules or saliva. For reasons that are not yet understood, approximately 20% of Asian elephant calves appear to be susceptible to the disease when primary infections are not controlled by normal innate cellular and humoral immune responses. Sensitive specific polymerase chain reaction (PCR) DNA blood tests have been developed, routine monitoring has been established, the complete large DNA genomes of each of the four Asian EEHV species have now been sequenced, and PCR gene subtyping has provided unambiguous evidence that this is a sporadic rather than epidemic disease that it is not being spread among zoos or other elephant housing facilities. Nevertheless, researchers have not yet been able to propagate EEHV in cell culture, determine whether or not human antiherpesvirus drugs are effective inhibitors, or develop serology assays that can distinguish between antibodies against the multiple different EEHV species.

Lertwatcharasarakul, P., P. Sanyathitiseree, N. Thongtip, P. Charoenphan, B. Boonyasart, N. Maneewan and T. Songserm (2015). "Genetic variant of elephant endotheliotropic herpesvirus detected from captive Asian elephants (Elephas maximus) in Thailand from 2007 to 2013." Thai Journal of Veterinary Medicine **45**(1): 73-79.

The study was aimed at characterizing elephant endotheliotropic herpesvirus (EEHV) that was detected in captive Asian elephants in Thailand from 2007 to 2013. Six tissue samples of dead elephants and two EDTA blood samples of surviving elephants in Thailand showed clinical signs or had lesions of the viral infection. Samples were extracted for DNA amplification using a PCR technique with strain specific primers based on terminase and DNA polymerase genes. Six samples gave positive amplicons for EEHV1 specific primers and two samples gave positive amplicons for EEHV3/4 specific primers. Nucleotide sequencing analysis was assured for strain identification. Five out of the six samples from EEHV1 PCR were positive for the EEHV1A

strain and one sample was positive for the EEHV1B strain. The two samples of EEHV3/4 PCR positive products were revealed to be of the EEHV4 strain based on the sequencing of the partial terminase gene. Three strains of the EEHV including EEHV1A, EEHV1B and EEHV4 have been detected in Asian elephants in Thailand from 2007 to 2013. This study revealed the first EEHV1B isolate that has been detected in a captive Asian elephant in Thailand.

Anonymous (2015). Houston Zoo Asian Elephant EEHV Protocol Houston Zoo**:** 31.

Pandit, A., I. P. dhakal, K. Gairhe, H. B. Rana and D. Karmacharya (2014). "SURVEILLANCE OF THE ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS (EEHV) IN CHITWAN
DISTRICT, NEPAL." International Journal of Recent Scientific Research **5**(10).

Elephant End otheliotropic Herpes Virus (EEHV) has been proved as the cause for the deaths of at least 80 elephant calves worldwide till 1995, when it was first identified for the first time in North America. The study was conducted in 17 elephants (15 elephant calves and 2 adult), maintained at Chit wan Districts namely Elephant Breeding Centre and hattisar of Chit wan National Park and Gaida Wildlife Camp of Sauraha. From the selected elephants, whole blood sample, conjunctival swab and Buffy coat were collected. The samples were stored in deep freeze until taken to laboratory. DNA was extracted off conjunctiva swab, buffy coat and whole blood sample using QIAGEN Dneasy blood and tissue kit and PCR analysis was performed using 2 genes specific to EEHV, Pan EEHV Pol and EEHV1-U38. Gel electrophoresis was done using 2% agarose gel at 110 V. Statistical analysis was done using SPSS 16.0 Version and Microsoft Excel–2007. In the research site, none of the elephants (0%) were infected with EEHV infection at the research period. Though the prevalence rate of EEHV infection is found to be zero in the research site at the research period, there is need for regular monitoring of the disease.

Bouchard, B., B. Xaymountry, N. Thongtip, P. Lertwatcharasarakul and W. Wajjwalku (2014). "First reported case of elephant endotheliotropic herpes virus infection in Laos." Journal of Zoo and Wildlife Medicine **45**(3): 704-707.

The elephant endotheliotropic herpesvirus (EEHV) is now recognized as one of the main causes of death of young Asian elephants (Elephas maximus) in North American zoos. Its impact in wild and domestic elephant populations in Asia is not clearly understood. This article describes the first case of EEHV infection in Lao People's Democratic Republic of a 2.5-yr-old domestic male Asian elephant. Clinical signs and pathological findings reported here are consistent with previous infections in Asian elephant calves. Phylogenetic analyses showed 100% homology with other EEHV-1A strains identified in Asia, Europe, and North America. Contamination of the molecular assays was ruled out, because the DNA polymerase sequence identified in this study differed from the positive control by two base pairs. © 2014 American

Association of Zoo Veterinarians.

Sripiboon, S., P. Tankaew, G. Lungka and C. Thitaram (2013). "The occurrence of elephant endotheliotropic herpesvirus in captive Asian elephants (Elephas maximus): first case of EEHV4 in Asia." J Zoo Wildl Med **44**(1): 100-104.

Elephant endotheliotropic herpesvirus (EEHV) is a type of herpesvirus that causes acute hemorrhagic disease in Asian elephants (Elephas maximus) and is often fatal, especially in calves. This study describes the postmortem evaluation of two captive-born Asian elephants (2 and 3 yr of age, respectively) diagnosed with EEHV in Thailand. Both elephants presented only mild depression, lethargy, and anorexia before death within 24 hr of symptom onset. Necropsies were performed, and tissue samples were tested for EEHV viral presence using polymerase chain reaction. Molecular and phylogenetic evidence illustrated two types of EEHV, which were closely related to EEHV1A in Case 1 and EEHV4 in Case 2. Pathologic findings differed between the cases. More specific organ tropism was found in Case 1, where mainly the cardiovascular system was affected. In contrast, in Case 2, hemorrhages were noted in most organs, including in the gastrointestinal, respiratory, and cardiovascular systems. This report is the first to document EEHV4 in Asia and the second case of this strain to be identified in an elephant worldwide.

Ling, P. D., J. G. Reid, X. Qin, D. M. Muzny, R. Gibbs, J. Petrosino, R. Peng, J. C. Zong, S. Y. Heaggans and G. S. Hayward (2013). "Complete Genome Sequence of Elephant Endotheliotropic Herpesvirus 1A." Genome Announc **1**(2): e0010613.

Elephant endotheliotropic herpesvirus 1A is a member of the Proboscivirus genus and is a major cause of fatal hemorrhagic disease in endangered juvenile Asian elephants worldwide. Here, we report the first complete genome sequence from this genus, obtained directly from necropsy DNA, in which 60 of the 115 predicted genes are not found in any known herpesvirus.

Sariya, L., J. Chatsirivech, P. Suksai, W. Wiriyarat, A. Songjaeng, S. Tangsudjai, O. Kanthasaewee, U. Maikaew and K. Chaichoun (2012). "Development of a SYBR Green I-based real-time PCR for detection of elephant endotheliotropic herpesvirus 1 infection in Asian elephants (Elephas maximus)." Journal of Virological Methods **185**(1): 160-165.

Elephant endotheliotropic herpesvirus 1 (EEHV1) can cause fatal hemorrhagic disease in Asian elephants (Elephas maximus). Several studies have described this virus as a major threat to young Asian elephants. A SYBR Green I-based real-time polymerase chain reaction (PCR) was developed to identify EEHV1 on trunk swabs and necropsied tissues. Two of 29 (6.9%) trunk swab samples from healthy Asian elephants were positive for EEHV1. The viruses were analyzed and classified as EEHV1A based on 231 nucleotides of the terminase gene. Necropsied spleen and heart tissue showed the highest level and second highest levels of DNA virus copy accumulation, respectively. The detection limit of the test was 276. copies/μl of DNA. There was no cross-reaction with other mammalian herpesviruses,

such as herpes simplex virus 1 and equine herpesvirus 2. Inter- and intra-assay showed low coefficients of variation values indicating the reproducibility of the test. The results indicated that the test can be practically used for epidemiological study, clinical diagnosis, and management and control of EEHV1. © 2012 Elsevier B.V..

Brock, A. P., R. Isaza, R. P. Hunter, L. K. Richman, R. J. Montali, D. L. Schmitt, D. E. Koch and W. A. Lindsay (2012). "Estimates of the pharmacokinetics of famciclovir and its active metabolite penciclovir in young Asian elephants (Elephas maximus)." Am J Vet Res **73**(12): 1996-2000.

OBJECTIVE: To determine plasma pharmacokinetics of penciclovir following oral and rectal administration of famciclovir to young Asian elephants (Elephas maximus). ANIMALS: 6 healthy Asian elephants (5 females and 1 male), 4.5 to 9 years old and weighing 1,646 to 2,438 kg. PROCEDURES: Famciclovir was administered orally or rectally in accordance with an incomplete crossover design. Three treatment groups, each comprising 4 elephants, received single doses of famciclovir (5 mg/kg, PO, or 5 or 15 mg/kg, rectally); there was a minimum 12-week washout period between subsequent famciclovir administrations. Serial blood samples were collected after each administration. Samples were analyzed for famciclovir and penciclovir with a validated liquid chromatography-mass spectroscopy assay. RESULTS: Famciclovir was tolerated well for both routes of administration and underwent complete biotransformation to the active metabolite, penciclovir. Mean maximum plasma concentration of penciclovir was 1.3 mug/mL at 1.1 hours after oral administration of 5 mg/kg. Similar results were detected after rectal administration of 5 mg/kg. Mean maximum plasma concentration was 3.6 mug/mL at 0.66 hours after rectal administration of 15 mg/kg; this concentration was similar to results reported for humans receiving 7 mg/kg orally. CONCLUSIONS AND CLINICAL RELEVANCE: Juvenile Asian elephants are susceptible to elephant endotheliotropic herpesvirus. Although most infections are fatal, case reports indicate administration of famciclovir has been associated with survival of 3 elephants. In Asian elephants, a dose of 8 to 15 mg of famciclovir/kg given orally or rectally at least every 8 hours may result in penciclovir concentrations that are considered therapeutic in humans.

Stanton, J. J., J. C. Zong, E. Latimer, J. Tan, A. Herron, G. S. Hayward and P. D. Ling (2011). "Detection of pathogenic elephant endotheliotropic herpesvirus in routine trunk washes from healthy adult Asian elephants (Elephas maximus) by use of a real-time quantitative polymerase chain reaction assay." American Journal of Veterinary Research **71**(8): 925-933.

Objective-To investigate the pathogenesis and transmission of elephant endotheliotropic herpesvirus (EEHV1) by analyzing various elephant fluid samples with a novel EEHV1-specific real-time PCR assay.

Animals-5 apparently healthy captive Asian elephants (Elephas maximus) from the same herd.

Procedures-A real-time PCR assay was developed that specifically detects EEHV1.

The assay was used to evaluate paired whole blood and trunk-wash samples obtained from the 5 elephants during a 15-week period. Deoxyribonucleic acid sequencing and viral gene subtyping analysis were performed on trunk-wash DNA preparations that had positive results for EEHV1. Viral gene subtypes were compared with those associated with past fatal cases of herpesvirus-associated disease within the herd.

Results-The PCR assay detected viral DNA to a level of 1,200 copies/mL of whole blood. It was used to detect EEHV1 in trunk secretions of 3 of the 5 elephants surveyed during the 15-week period. Viral gene subtyping analysis identified 2 distinct elephant herpesviruses, 1 of which was identical to the virus associated with a previous fatal case of herpesvirus-associated disease within the herd.

Conclusions and Clinical Relevance-EEHV1 was shed in the trunk secretions of healthy Asian elephants. Trunk secretions may provide a mode of transmission for this virus. Results of this study may be useful for the diagnosis, treatment, and management of EEHV1-associated disease and the overall management of captive elephant populations.

Hardman, K., A. Dastjerdi, R. Gurrala, A. Routh, M. Banks, F. Steinbach and T. Bouts (2011). "Detection of elephant endotheliotropic herpesvirus type 1 in asymptomatic elephants using TaqMan real-time PCR." Vet Rec **170**(8): 205.

This study assessed the feasibility of identifying asymptomatic viral shedders using a novel TaqMan real-time PCR on trunk washes and swabs from the conjunctiva, palate and vulva of elephants. Six elephants from a UK collection were sampled weekly over a period of 11 weeks for this study. The herd prevalence of elephant endotheliotropic herpesvirus-1 (EEHV-1) was 100 per cent by PCR. The virus DNA was detected in all the sampling sites; however, the prevalence of virus DNA in the conjunctiva swabs was higher. In addition, Asian elephants from two continental European collections were sampled once and one animal tested positive on a trunk wash. The virus from this animal was phylogenetically typed as EEHV-1A based on 231 nucleotides of the terminase gene.

Schaftenaar, W., C. Reid, B. Martina, J. Fickel and A. D. M. E. Osterhaus (2010). "Nonfatal clinical presentation of elephant endotheliotropic herpes virus discovered in a group of captive Asian elephants (Elephas maximus)." Journal of Zoo and Wildlife Medicine **41**(4): 626-632.

Several different strains of elephant endotheliotropic herpes virus-1 (EEHV-1) have been identified via polymerase chain reaction (PCR) techniques in both African and Asian elephants. EEHV-1 has been identified in both cutaneous lesions in healthy African elephants and fatal cases of hemorrhagic syndrome in Asian elephants.6 However, until now, no EEHV-1 strain has been identified or associated with otherwise healthy Asian elephants. This article describes recurrent nonendothelial lesions associated with EEHV-1 infection in a herd of Asian elephants not exhibiting fatal hemorrhagic syndrome. Genotypes of EEHV-1 strains, based on viral DNA polymerase and glycoprotein B, associated with fatal hemorrhagic syndrome, were compared

to those identified in nonendothelial lesions. The same EEHV-1 genotypes were identified in fatal cases and mucosal lesions in otherwise healthy Asian elephants in this herd. Further studies of the Asian elephant immune system and virologic studies to determine the triggers of tissue tropism are needed before any conclusion can be reached. Copyright 2010 by American Association of Zoo Veterinarians.

Wellehan, J. F. X., A. J. Johnson, A. L. Childress, K. E. Harr and R. Isaza (2008). "Six novel gammaherpesviruses of Afrotheria provide insight into the early divergence of the Gammaherpesvirinae." Veterinary Microbiology **127**: 249-257.

Steinmetz, H. W., U. Eulenberger and J. M. Hatt (2008). Daily clinical examinations in a herd of captive asian elephants. Proc American Associaton of Zoo Veterinarians and Assoc of Reptile and Amphibian Veterinarians.

The captive population of Asian elephants (*Elephas maximus*) is not self-sustaining.2 Poor reproduction and high juvenile mortality are key factors in the decreasing population. Infection with endotheliotropic elephant herpes virus (EEHV) is one of the major causes of death in the captive population, and has resulted in the loss of at least 40 captive animals.1 EEHV has been responsible for the peracute death of two juvenile males at Zurich Zoo, Switzerland. Mortality due to peracute infection with EEHV mainly is seen in juveniles. Early detection of characteristic clinical signs of EEHV and immediate initiation of therapy are of crucial importance due to its rapid progression. Based on past fatal EEHV experiences, Zurich Zoo modified its daily clinical health monitoring program to increase staff awareness of EEHV infection. Examinations have been incorporated into the daily routine and include daily evaluation of behaviour, appetite, colour of mucosal membranes and the measurement of body temperature; these examinations are performed by keepers. In our experiences, characteristic signs of acute EEHV infection are lethargy, anorexia, mild colic, and cyanosis of the mucosal membranes. Results of temperature measurements have shown that best estimations of body temperature are done by measurement of the temperature in the centre of a fecal ball 5-9 min after defecation. Mean values of 36.5°C (± 0.2°C SD) are within published reference values, although adult elephants have shown significantly lower body temperature than juveniles. Establishment of individual reference values for each elephant is essential to detect unusual temperature peaks that may indicate possible EEHV viremia. The present study has shown that daily health examinations increase the awareness of keepers for early signs of EEHV infection (e.g., peaks in body temperature and cyanotic mucosal membranes).

ACKNOWLEDGMENTS

The authors thank B. Aeschbach and all elephant keepers for taking special care of our elephants. The work and organization of Ms. G. Hürlimann is gratefully appreciated.

LITERATURE CITED

1. Mikota, S. 2007. Endotheliotropic Herpesvirus (EEHV). http://www.elephantcare.org/herpes.htm. cited: 10.04.2008:
2. Wiese, R. J. 2000. Asian elephants are not self-sustaining in North America. Zoo Biol. 19: 299-309.

Miller, J. and M. McClean (2008). Pharmacokinetics of enrofloxacin in African elephants (Loxodonta africana) after a single rectal dose. Proc American Associaton of Zoo Veterinarians and Assoc of Reptile and Amphibian Veterinarians.

Captive African elephants (*Loxodonta Africana*) are susceptible to many types of gram negative bacterial infections such as *Escherichia coli, Mycoplasma* spp., *Salmonella* spp., *Klebsiella* spp., *Pseudomonas* spp., and *Proteus* spp. Enrofloxacin (Baytril®, Bayer Health Care, Animal Health Division, P.O. Box 390, Shawnee Mission, KS 66201) is a potentially effective antibiotic for treatment of these bacterial infections in elephants. Very limited data exists on the pharmacokinetics of enrofloxacin in elephants2 and most of the dosage regimes for gastrointestinal absorption are based on horse dosages since they share a similar gastrointestinal tract. Three African elephants from Wildlife Safari in Winston, Oregon, two females both 37-yr-old and one male 26-yr-old, were used to determine whether therapeutic levels of enrofloxacin could be achieved thru rectal administration of liquid injectable enrofloxacin (Baytril 100®, 100 mg/ml, Bayer Health Care, Animal Health Division, P.O. Box 390, Shawnee Mission, KS 66201) at a dosage of 2.5 mg/kg. A pretreatment baseline blood sample was collected. Following administration, blood samples were collected at 45 min, 1.5hr, 2.5hr, 5hr, 9hr, 23hr, 36hr to determine plasma enrofloxacin levels. Plasma enrofloxacin levels were measured at North Carolina State University, College of Veterinary Medicine using high performance liquid chromatography (HPLC) analysis. Plasma ciprofloxacin levels were measured concurrently. Results indicate plasma concentrations of enrofloxacin did not reach adequate bacteriocidal levels for any of the the following common bacterial isolates in captive elephants: *Mycoplasma* spp., *Escherichia coli, Salmonella* spp., *Klebsiella* spp., *Pseudomonas* spp., and *Proteus* spp. The study determined that a rectally administered dosage of 2.5 mg/kg of liquid injectable enrofloxacin was insufficient to obtain therapeutic levels in African elephants. The low plasma levels of enrofloxacin in all three elephants may be a result of poor absorption in the distal large intestine. A future study will determine if oral administration will provide a more efficient mode of drug delivery and absorption in African elephants. It is also possible that the current dosage of 2.5 mg/kg is too low to achieve adequate therapeutic levels.

ACKNOWLEDGMENTS

I would like to thank the elephant and veterinary staff at Wildlife Safari for their participation in conducting this study. Thanks to Doctors: Modesto McClean, Jason Bennett, Andi Chariffe, Tessa Lohe, Benji Alacantar. Also thanks to Dinah Wilson, Carol Matthews, Anthony Karels, Mary Iida, Shawn Finnell, Patches Stroud, Katie Alayan.

LITERATURE CITED

1. Haines, G.R., et. al. 2000. Serum concentrations and pharmacokinetics of

enrofloxacin after intravenous and intragastric administration to mares. Can. J.Vet. Res. 64(3):171-177.
2. Sanchez, C.R., et. al. 2005. Pharmacokinetics of a single dose of enrofloxacin administered orally to captive Asian elephants (*Elephas maximus*). Am. J. Vet. Res. 66:1948-1953.

Meyers, D. A., R. Isaza and A. MacNeill (2008). Evaluation of acute phase proteins for diagnosis of inflammation in Asian elephants (Elephas maximus). Proc American Associaton of Zoo Veterinarians and Assoc of Reptile and Amphibian Veterinarians.

In many domestic species, routine hematology assays are useful diagnostic tools to diagnose inflammatory conditions. Unlike other species, these hematologic tests apparently are insensitive indicators of inflammation in elephants.1 We studied a novel group of blood proteins, called acute phase proteins, which increase during inflammatory conditions, for their usefulness in diagnosing elephants with inflammatory diseases. Although these proteins currently are useful in humans and domestic animals, each species has a different set of important proteins that must be individually investigated.2 We tested several acute phase proteins (C-reactive protein, alpha-1 glycoprotein, alpha-1 antitrypsin, serum amyloid A, haptoglobin, fibrinogen, ceruloplasmin, and albumin) as well as complete blood counts, chemistry panels, serum protein electrophoresis, and 3-D gel electrophoresis to determine their usefulness for diagnosing different types of inflammatory conditions in Asian elephants (*Elephas maximus*). Animals with inflammatory conditions were classified as those individuals with known illnesses such as mycobacteriosis, arthritis, nail bed abscesses, and malignant tumors. Control animals were thoseanimals that were suspected to not have any inflammation and be healthy at the time of testing as determined by physical examination and obtaining a thorough medical history.

LITERATURE CITED

1. Lyashchenko, K., R. Greenwald, J. Esfandiari, J. Olsen, R. Ball, G. Dumonceaux, F. Dunker, C. Buckley, M.
Richard, S. Murray, J.B. Payeur, P. Anderson, J.M. Pollock, S. Mikota, M. Miller, D. Sofranko, and W.R.
Waters. 2006. Tuberculosis in Elephants: Antibody responses to defined antigens of *Mycobacterium*
tuberculosis, potential for early diagnosis, and monitoring of treatment. Clin. Vacc. Immunol. 13: 722-732.
2. Murata H., N. Shimada, M. Yoshioka. 2004. Current research on acute phase proteins in veterinary diagnosis:
an overview. Vet J. 168: 28-40.

Maluy, P. and D. Hawkins (2007). Loss of a young Asian elephant to a newly discovered herpesvirus: An historical review of husbandry and behaviour prior to death. International Elephant Conservation and Research Symposium.

Cracknell, J., A. Routh, D. Field, W. Schaftenaar, T. Hildebrandt and S. Hewitt (2007). Elephant Endotheliotrophic Herpes Virus (EEHV) in the United Kingdom: An

update with the development of the national EEHV management programme. Proceedings of 43rd International Symposium on Diseases of Zoo and Wild Animals, IZW, Edinburgh, 16-20th May.

Cracknell, J., A. Routh and D. Field (2007). Education as a tool for disease management: Elephant Endotheliotropic Herpes Virus Case Study in the UK. Proceedings of AAZV.

Cracknell, J. (2007). Elephant Endotheliotrophic Herpes Virus (EEHV) Protocol. UK, United Kingdom Elephant Health Programme; ZSL; BIAZA**:** 49.

Wellehan, J. F. X., A. J. Johnson and R. Isaza (2006). Identification of two novel herpesviruses associated with ocular inflammation in Asian elephants (Elephas maximus). 2006 Proceedings American Association of Zoo Veterinarians.

> Disease caused by a herpesvirus (EEHV) is a serious concern in Asian elephant (Elephas maximus) calves. Herpesviruses are known for latency and life-long infections, with periodic shedding from mild inflammatory lesions in adapted adult hosts, and ocular disease has been seen with other herpesviruses in other species. Ocular inflammation is not uncommonly seen in Asian elephants. Degenerate PCR primers targeting a conserved region of herpesvirus DNA-dependent DNA polymerase were used to amplify products from eye swabs of eight Asian elephants with epiphora, blepharitis, and conjunctivitis. Nucleotide sequencing of the PCR products showed two novel herpesviruses distinct from EEHV. Comparative sequence analysis shows that these viruses are probable members of the subfamily Gammaherpesvirinae. The sequence phylogeny of these viruses has implications for both viral and host evolution. Further understanding and characterization of these viruses is needed to understand their role in elephant health.

Reid, C. E., T. B. Hildebrandt, N. Marx, M. Hunt, N. Thy, J. M. Reynes, W. Schaftenaar and J. Fickel (2006). "Endotheliotropic elephant herpes virus (EEHV) infection. The first PCR-confirmed fatal case in Asia.
." Vet. Q **28**(2): 61-64.

> Since 1995, 4 suspected cases of Endotheliotropic Elephant Herpes Virus (EEHV) infection, i.e. based on clinical presentation, have occurred in Asia without resulting in epidemic outbreaks as expected. In order to confirm the presence of EEHV on the continent of Asia, viral DNA particles from liver samples of a wild-caught 3-year-old elephant found dead at a Cambodian elephant sanctuary and clinically diagnosed with EEHV, were PCR processed using known EEHV strain primers. The presence of EEHV viral nucleic acids was confirmed and the nucleic acids had a 99% sequence similarity to the U.S.A strain (gene bank locus: AF117265) and 97% sequence similarity to the European strain (gene bank locus: AF354746) assigning this case to the EEHV-1 cluster. More than the confirmation of EEHV on the continent of Asia, is the phylogenic relationship to the USA and European strains with no corresponding contact or transport of USA or European elephants to Asia.

Thus, this brings many of the traditional theories into question. Although almost forgotten, this disease is still ramped in captive elephant populations worldwide and continues to devastate particularly the neonatal and weaning-age population. Special attention and continued research are needed specifically in the area of basic virology and epidemiology

McGeoch, D. J., F. J. Rixon and A. J. Davison (2006). "Topics in herpesvirus genomics and evolution." Virus Research **117**(1): 90-104.