

Endotheliotropic Herpes Virus (EEHV) Publications

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Sripiboon, S., et al. (2020). "Subclinical infection of captive Asian elephants (*Elephas maximus*) in Thailand with elephant endotheliotropic herpesvirus." *Arch Virol* **165**(2): 397-401.

Elephant endotheliotropic herpesvirus (EEHV) infection is a conservation threat to the endangered Asian elephant (*Elephas maximus*), causing fatal hemorrhagic disease in juvenile elephants throughout the world, including Thailand. This study revealed a subclinical EEHV1 infection rate of 5.5% in healthy captive Asian elephants in Thailand (n = 362). The virus was detected in all age classes above one year old, in both sexes, and across the country - even in facilities with no history of hemorrhagic disease (EEHV HD). Subclinical EEHV infection in Thailand urgently requires proper health management.

Oo, Z. M., et al. (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.

Jeffrey, A., et al. (2020). "NONINVASIVE SAMPLING FOR DETECTION OF ELEPHANT ENDOTHELIO TROPIC HERPESVIRUS AND GENOMIC DNA IN ASIAN (*ELEPHAS MAXIMUS*) AND AFRICAN (*LOXODONTA AFRICANA*) ELEPHANTS." *J Zoo Wildl Med* **51**(2): 433-437.

Elephant endotheliotropic herpesvirus (EEHV) hemorrhagic disease (EEHV-HD) threatens Asian elephant (*Elephas maximus*) population sustainability in North America. Clusters of cases have also been reported in African elephants (*Loxodonta africana*). Risk to range country elephant populations is unknown. Currently, EEHV detection depends upon sampling elephants trained for invasive blood and trunk wash collection. To evaluate noninvasive sample collection options, paired invasively collected (blood, trunk wash and oral swabs), and noninvasively collected (chewed plant and fecal) samples were compared over 6 wk from 9 Asian elephants and 12 African elephants. EEHV shedding was detected simultaneously in a paired trunk wash and fecal sample from one African elephant. Elephant γ herpesvirus-1 shedding was identified in six chewed plant samples collected from four Asian elephants. Noninvasively collected samples can be used to detect elephant herpesvirus shedding. Longer sampling periods are needed to evaluate the clinical usefulness of noninvasive sampling for EEHV detection.

Guntawang, T., et al. (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., et al. (2020). "EVALUATION OF THE EFFICACY OF TWO DIFFERENT SAMPLING SITES FOR THE DETECTION OF ELEPHANT ENDOTHELIO TROPIC 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., et al. (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., et al. (2019). "USING IN-HOUSE HEMATOLOGY TO DIRECT DECISION-MAKING IN THE SUCCESSFUL TREATMENT AND MONITORING OF A CLINICAL AND SUBSEQUENTLY SUBCLINICAL

CASE OF ELEPHANT ENDOTHELIO TROPIC 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., et al. (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., et al. (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., et al. (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., et al. (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., et al. (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., et al. (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., et al. (2019). "Evidence of high EEHV antibody seroprevalence and spatial variation among captive Asian elephants (*Elephas maximus*) in Thailand." *Virology* **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., et al. (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 histopathological 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., et al. (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., et al. (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., et al. (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., et al. (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., et al. (2018). "ELEPHANT ENDOTHELIO TROPIC 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., et al. (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 10⁹/L [1,100 x 10³/mm³]), 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., et al. (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., et al. (2017). "SUCCESSFUL TREATMENT OF A CLINICAL ELEPHANT ENDOTHELIO TROPIC 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 Endotheliotropic Herpesvirus in Asia 2nd edition, Wildlife Reserves Singapore Group: 19.

Lopez, J., et al. (2017). "ASSESSMENT OF A LANCET-AND-SWAB BLOOD SAMPLING TECHNIQUE FOR SURVEILLANCE OF ELEPHANT ENDOTHELIO TROPIC 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., et al. (2017). "EPIDEMIOLOGIC EVALUATION of ELEPHANT ENDOTHELIO TROPIC HERPESVIRUS 3B INFECTION in AN AFRICAN ELEPHANT (LOXODONTA AFRICANA)." Journal of Zoo and Wildlife Medicine **48**(2): 335-343.

Bhusri, B., et al. (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., et al. (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.

Ackermann, M., et al. (2017). "Identification of shedders of elephant endotheliotropic herpesviruses among Asian elephants (*Elephas maximus*) in Switzerland." PLoS ONE **12**(5): e0176891.

Elephants, particularly Asian (*Elephas maximus*), are threatened by lethal elephant hemorrhagic disease (EHD) due to elephant endotheliotropic herpesviruses (EEHV). At least five of seven known EEHV types have been associated to EHD, with types 1, 4, and 5 predominantly affecting Asian elephants. In Switzerland, at least three Asian elephants have been lost due to EHD but nothing is known about the present EEHV1 circulation. Moreover, the prevalence of other EEHV types has never been assessed. Intermittent shedding of EEHV can be monitored through collecting trunk secretions and analyzing them by PCR methods that discriminate the different EEHV types. To identify EEHV shedders, seven of eight Asian elephants in a Swiss zoo were trained to provide trunk wash samples. These were collected at intervals over a period of four months and tested by PCR for presence of EEHV1 through 6. Moreover, the quality of each sample was assessed by testing for the elephant TNF-alpha gene. Overall, 57% of the samples were valid with five of seven participating elephants identified as EEHV shedders. Two of those shed virus only once, whereas the other three, all closely related among each other, shed virus on multiple occasions. One of the

frequent shedders had been in very close contact to all of the three EHD victims. Therefore, we speculate that this particular animal may represent the virus source in all three cases. However, when subtyping was conducted, the presently circulating virus was identified as EEHV1B, while the virus subtype causing EHD had been 1A in all three cases. In addition to four animals excreting EEHV1, a recently introduced animal was observed to shed EEHV3/4. We suggest that the policy of trunk washing to identify and characterize EEHV-shedders is to be endorsed in zoos with ongoing or planned elephant breeding programs.

Zong, J. C., et al. (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-based DNA sequencing 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 DNA sequence 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., et al. (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., et al. (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.

Pursell, T., et al. (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., et al. (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.

Ling, P. D., et al. (2016). "Complete genome sequence of elephant endotheliotropic herpesvirus 4, the first example of a GC-rich branch proboscivirus." *mSphere* **1**(3).

A novel group of mammalian DNA viruses called elephant endotheliotropic herpesviruses (EEHVs) belonging to the Proboscivirus genus has been associated with nearly 100 cases of highly lethal

acute hemorrhagic disease in young Asian elephants worldwide. The complete 180-kb genomes of prototype strains from three AT-rich branch viruses, EEHV1A, EEHV1B, and EEHV5, have been published. However, less than 6 kb of DNA sequence each from EEHV3, EEHV4, and EEHV7 showed them to be a hugely diverged second major branch with GC-rich characteristics. Here, we determined the complete 206-kb genome of EEHV4(Baylor) directly from trunk wash DNA by nextgeneration sequencing and de novo assembly procedures. Among a total of 119 genes with an overall colinear organization similar to those of the AT-rich EEHVs, major features of EEHV4 include a family of 26 paralogous 7xTM and vGPCR-like genes plus 25 novel or missing genes. The genome also contains an unusual distribution of tracts of 5 to 11 successive A or T nucleotides in intergenic domains between the mostly much higher GC content protein coding regions. Furthermore, an extremely high GC-rich bias in the third wobble position of codons clearly delineates the coding regions for many but not all proteins. There are also two novel captured cellular genes, including a C-type lectin (vECTL) and an O-linked acetylglucosamine transferase (vOGT), as well as an unusually large and complex Ori-Lyt dyad symmetry domain. Finally, 30 kb from a second strain proved to include three small chimeric domains, indicating the existence of distinct EEHV4A and EEHV4B subtypes. © 2016 Ling et al.

Kendall, R., et al. (2016). "THE IMPACT of ELEPHANT ENDOTHELIO TROPIC 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., et al. (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., et al. (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.

Ortega, J., et al. (2015). "Acute death associated with *Citrobacter freundii* infection in an African elephant (*Loxodonta africana*)." Journal of Veterinary Diagnostic Investigation **27**(5): 632-636.

A 21-year-old male African elephant (*Loxodonta africana*) died suddenly with no previous medical history. Grossly, there were severe multifocal epicardial and endocardial hemorrhages of the atria and ventricles, hydropericardium, multifocal pleural hemorrhages, and severe pulmonary congestion and edema. Histologically, there was fibrinoid vasculitis and thrombosis in the heart and lung and myocardial necrosis. *Citrobacter freundii* was isolated in abundance in pure culture from liver and heart samples. Low levels of multiples types of elephant endotheliotropic herpesvirus (EEHV-6, EEHV-2B, and EEHV-3A) were detected in spleen samples, but not in heart samples. The levels of EEHV DNA found were much lower than those usually associated with acute EEHV hemorrhagic disease, and many other genomic loci that would normally be found in such cases were evidently below the level of detection. Therefore, these findings are unlikely to indicate lethal EEHV disease. Polymerase chain reaction for encephalomyocarditis virus (EMCV) and toxicology for oleander (*Nerium oleander*) were negative. Stress, resulting from recent transport, and antimicrobial therapy may have contributed to the death of this animal.

Long, S. Y., et al. (2015). "Review of elephant endotheliotropic herpesviruses and acute hemorrhagic disease." IJAR 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. © The Author 2016. Published by Oxford University Press on behalf of the Institute for Laboratory Animal Research. All rights reserved.

Lertwatcharasarakul, P., et al. (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.

Humphreys, A. F., et al. (2015). "Generation and characterization of antibodies against Asian elephant (*Elephas maximus*) IgG, IgM, and IgA." PLoS ONE **10**(2).

Asian elephant (*Elephas maximus*) immunity is poorly characterized and understood. This gap in knowledge is particularly concerning as Asian elephants are an endangered species threatened by a newly discovered herpesvirus known as elephant endotheliotropic herpesvirus (EEHV), which is the leading cause of death for captive Asian elephants born after 1980 in North America. While reliable diagnostic assays have been developed to detect EEHV DNA, serological assays to evaluate elephant anti-EEHV antibody responses are lacking and will be needed for surveillance and epidemiological studies and also for evaluating potential treatments or vaccines against lethal EEHV infection. Previous studies have shown that Asian elephants produce IgG in serum, but they failed to detect IgM and IgA, further hampering development of informative serological assays for this species. To begin to address this issue, we determined the constant region genomic sequence of Asian elephant IgM and obtained some limited protein sequence information for putative serum IgA. The information was used to generate or identify specific commercial antisera reactive against IgM and IgA isotypes. In addition, we generated a monoclonal antibody against Asian elephant IgG. These three reagents were used to demonstrate that all three immunoglobulin isotypes are found in Asian elephant serum and milk and to detect antibody responses following tetanus toxoid booster vaccination or antibodies against a putative EEHV structural protein. The results indicate that these new reagents will be useful for developing sensitive and specific assays to detect and characterize elephant antibody responses for any pathogen or vaccine, including EEHV. © 2015 Humphreys et al.

Humphreys, A. F., et al. (2015). "Generation and characterization of antibodies against Asian elephant (*Elephas maximus*) IgG, IgM, and IgA." PLoS ONE **10**(2): e0116318.

Asian elephant (*Elephas maximus*) immunity is poorly characterized and understood. This gap in knowledge is particularly concerning as Asian elephants are an endangered species threatened by a newly discovered herpesvirus known as elephant endotheliotropic herpesvirus (EEHV), which is the leading cause of death for captive Asian elephants born after 1980 in North America. While reliable diagnostic assays have been developed to detect EEHV DNA, serological assays to evaluate elephant anti-EEHV antibody responses are lacking and will be needed for surveillance and epidemiological studies and also for evaluating potential treatments or vaccines against lethal EEHV infection. Previous studies have shown that Asian elephants produce IgG in serum, but they failed to detect IgM and IgA, further hampering development of informative serological assays for this species. To begin to address this issue, we determined the constant region genomic sequence of Asian elephant IgM and obtained some limited protein sequence information for putative serum IgA. The information was used to generate or identify specific commercial antisera reactive against IgM and IgA isotypes. In addition, we generated a monoclonal antibody against Asian elephant IgG. These three reagents were used to demonstrate that all three immunoglobulin isotypes are found in Asian elephant serum and milk and to detect antibody responses following tetanus toxoid booster vaccination or antibodies against a putative EEHV structural protein. The results indicate that these new reagents will be useful for developing sensitive and specific assays to detect and characterize elephant antibody responses for any pathogen or vaccine, including EEHV.

Bennett, L., et al. (2015). "Longitudinal study of Asian elephants, *Elephas maximus*, indicates intermittent shedding of elephant endotheliotropic herpesvirus 1 during pregnancy." *Vet Rec Open* **2**(1): e000088.
INTRODUCTION: EEHV-1 is a viral infection of elephants that has been associated with a fatal haemorrhagic syndrome in Asian elephants. Previous studies have suggested that pregnant animals may shed more virus than non-pregnant animals. METHODS: This study examined whether pregnancy affected the frequency or magnitude of shedding of elephant endotheliotropic herpesvirus 1 (EEHV1) using Taq man real-time PCR on trunk washes from four female elephants from a UK collection over three time periods between 2011 and 2014. These periods included pregnancies in two animals (period 1 and period 3). Behavioural observations made by keepers were also assessed. RESULTS: During period 1 there was a high degree of social hierarchical instability which led to a hierarchy change, and was associated with aggressive behaviour. Also during period 1 EEHV-1 shedding was of a higher magnitude and frequency than in the latter two time periods. CONCLUSIONS: These results suggest that there is no clear relationship between shedding and pregnancy, and that behavioural stressors may be related to an increase in EEHV-1 shedding.

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

Zong, J. C., et al. (2014). "Comparative genome analysis of four elephant endotheliotropic herpesviruses, EEHV3, EEHV4, EEHV5, and EEHV6, from cases of hemorrhagic disease or viremia." *J Virol* **88**(23): 13547-13569.

The genomes of three types of novel endotheliotropic herpesviruses (elephant endotheliotropic herpesvirus 1A [EEHV1A], EEHV1B, and EEHV2) associated with lethal hemorrhagic disease in Asian elephants have been previously well characterized and assigned to a new Proboscivirus genus. Here we have generated 112 kb of DNA sequence data from segments of four more types of EEHV by direct targeted PCR from blood samples or necropsy tissue samples from six viremic elephants. Comparative phylogenetic analysis of nearly 30 protein-encoding genes of EEHV5 and EEHV6 show that they diverge uniformly by nearly 20% from their closest relatives, EEHV2 and EEHV1A, respectively, and are likely to have similar overall gene content and genome organization. In contrast, seven EEHV3 and EEHV4 genes analyzed differ from those of all other EEHVs by 37% and have a G+C content of 63% compared to just 42% for the others. Three strains of EEHV5 analyzed clustered into two partially chimeric subgroups EEHV5A and EEHV5B that diverge by 19% within three small noncontiguous segments totaling 6.2 kb. We conclude that all six EEHV types should be designated as independent species within a proposed new fourth Deltaherpesvirinae subfamily of mammalian herpesviruses. These virus types likely initially diverged close to 100

million years ago when the ancestors of modern elephants split from all other placental mammals and then evolved into two major branches with high- or low-G+C content about 35 million years ago. Later additional branching events subsequently generated three paired sister taxon lineages of which EEHV1 plus EEHV6, EEHV5 plus EEHV2, and EEHV4 plus EEHV3 may represent Asian and African elephant versions, respectively. **IMPORTANCE:** One of the factors threatening the long-term survival of endangered Asian elephants in both wild range countries and in captive breeding populations in zoos is a highly lethal hemorrhagic herpesvirus disease that has killed at least 70 young Asian elephants worldwide. The genomes of the first three types of EEHVs (or probosciviruses) identified have been partially characterized in the preceding accompanying paper (L. K. Richman, J.-C. Zong, E. M. Latimer, J. Lock, R. C. Fleischer, S. Y. Heaggans, and G. S. Hayward, *J. Virol.* 88:13523-13546, 2014, <http://dx.doi.org/10.1128/JVI.01673-14>). Here we have used PCR DNA sequence analysis from multiple segments of DNA amplified directly from blood or necropsy tissue samples of six more selected cases of hemorrhagic disease to partially characterize four other types of EEHVs from either Asian or African elephants. We propose that all six types and two chimeric subtypes of EEHV belong to multiple lineages of both AT-rich and GC-rich branches within a new subfamily to be named the Deltaherpesvirinae, which evolved separately from all other mammalian herpesviruses about 100 million years ago.

Wilkie, G. S., et al. (2014). "First fatality associated with elephant endotheliotropic herpesvirus 5 in an asian elephant: Pathological findings and complete viral genome sequence." *Scientific Reports* **4**.
Infections of Asian elephants (*Elephas maximus*) with elephant endotheliotropic herpesvirus (EEHV) can cause a rapid, highly lethal, hemorrhagic disease, which primarily affects juvenile animals up to the age of four years. So far, the majority of deaths have been attributed to infections with genotype EEHV1 or, more rarely, EEHV3 and EEHV4. Here, we report the pathological characteristics of the first fatality linked to EEHV5 infection, and describe the complete viral DNA sequence. Gross post-mortem and histological findings were indistinguishable from lethal cases previously attributed to other EEHV genotypes, and the presence of characteristic herpesviral inclusions in capillary endothelial cells at several sites was consistent with the diagnosis of acute EEHV infection. Molecular analysis confirmed the presence of EEHV5 DNA and was followed by sequencing of the viral genome directly from post-mortem material. The genome is 180,800 bp in size and contains 120 predicted protein-coding genes, five of which are fragmented and presumably nonfunctional. The seven families of paralogous genes recognized in EEHV1 are also represented in EEHV5. The overall degree of divergence (37%) between the EEHV5 and EEHV1 genomes, and phylogenetic analysis of eight conserved genes, support the proposed classification of EEHV5 into a new species (Elephantid herpesvirus 5).

Stanton, J. J., et al. (2014). "Detection of elephant endotheliotropic herpesvirus infection among healthy Asian elephants (*Elephas maximus*) in South India." *Journal of Wildlife Diseases* **50**(2): 279-287.
Elephant endotheliotropic herpesviruses (EEHVs) can cause fatal hemorrhagic disease in Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. Of the seven known EEHV species, EEHV1 is recognized as the most common cause of hemorrhagic disease among Asian elephants in human care worldwide. Recent data collected from ex situ Asian elephants located in multiple North American and European institutions suggest that subclinical EEHV1 infection is common in this population of elephants. Although fatal EEHV1-associated hemorrhagic disease has been reported in range countries, data are lacking regarding the prevalence of subclinical EEHV infections among in situ Asian elephants. We used previously validated EEHV-specific quantitative real-time PCR assays to detect subclinical EEHV infection in three regionally distinct Asian elephant cohorts, totaling 46 in situ elephants in South India, during October and November 2011. Using DNA prepared from trunk washes, we detected EEHV1, EEHV3/4, and EEHV5 at frequencies of 7, 9, and 20% respectively. None of the trunk washes was positive for EEHV2 or 6. At least one EEHV species was detectable in 35% (16/46) of the samples that were screened. These data suggest that subclinical EEHV infection among in situ Asian elephants occurs and that Asian elephants may be natural hosts for EEHV1, EEHV3 or 4, and EEHV5, but not EEHV2 and EEHV6. The methodology described in this study provides a foundation for further studies to determine prevalences of EEHV

infection in Asian elephants throughout the world. © Wildlife Disease Association 2014.

Richman, L. K., et al. (2014). "Elephant endotheliotropic herpesviruses EEHV1A, EEHV1B, and EEHV2 from cases of hemorrhagic disease are highly diverged from other mammalian herpesviruses and may form a new subfamily." *J Virol* **88**(23): 13523-13546.

A family of novel endotheliotropic herpesviruses (EEHVs) assigned to the genus Proboscivirus have been identified as the cause of fatal hemorrhagic disease in 70 young Asian elephants worldwide. Although EEHV cannot be grown in cell culture, we have determined a total of 378 kb of viral genomic DNA sequence directly from clinical tissue samples from six lethal cases and two survivors. Overall, the data obtained encompass 57 genes, including orthologues of 32 core genes common to all herpesviruses, 14 genes found in some other herpesviruses, plus 10 novel genes, including a single large putative transcriptional regulatory protein (ORF-L). On the basis of differences in gene content and organization plus phylogenetic analyses of conserved core proteins that have just 20% to 50% or less identity to orthologues in other herpesviruses, we propose that EEHV1A, EEHV1B, and EEHV2 could be considered a new Deltaherpesvirinae subfamily of mammalian herpesviruses that evolved as an intermediate branch between the Betaherpesvirinae and Gammaherpesvirinae. Unlike cytomegaloviruses, EEHV genomes encode ribonucleotide kinase B subunit (RRB), thymidine kinase (TK), and UL9-like origin binding protein (OBP) proteins and have an alphaherpesvirus-like dyad symmetry Ori-Lyt domain. They also differ from all known betaherpesviruses by having a 40-kb large-scale inversion of core gene blocks I, II, and III. EEHV1 and EEHV2 DNA differ uniformly by more than 25%, but EEHV1 clusters into two major subgroups designated EEHV1A and EEHV1B with ancient partially chimeric features. Whereas large segments are nearly identical, three nonadjacent loci totaling 15 kb diverge by between 21 and 37%. One strain of EEHV1B analyzed is interpreted to be a modern partial recombinant with EEHV1A. IMPORTANCE: Asian elephants are an endangered species whose survival is under extreme pressure in wild range countries and whose captive breeding populations in zoos are not self-sustaining. In 1999, a novel class of herpesviruses called EEHVs was discovered. These viruses have caused a rapidly lethal hemorrhagic disease in 20% of all captive Asian elephant calves born in zoos in the United States and Europe since 1980. The disease is increasingly being recognized in Asian range countries as well. These viruses cannot be grown in cell culture, but by direct PCR DNA sequence analysis from segments totaling 15 to 30% of the genomes from blood or necropsy tissue from eight different cases, we have determined that they fall into multiple types and chimeric subtypes of a novel Proboscivirus genus, and we propose that they should also be classified as the first examples of a new mammalian herpesvirus subfamily named the Deltaherpesvirinae.

Pellett, P. E. (2014). "Trunkloads of viruses." *J Virol* **88**(23): 13520-13522.

Elephant populations are under intense pressure internationally from habitat destruction and poaching for ivory and meat. They also face pressure from infectious agents, including elephant endotheliotropic herpesvirus 1 (EEHV1), which kills ~20% of Asian elephants (*Elephas maximus*) born in zoos and causes disease in the wild. EEHV1 is one of at least six distinct EEHV in a phylogenetic lineage that appears to represent an ancient but newly recognized subfamily (the Deltaherpesvirinae) in the family Herpesviridae.

Pandit, A., et al. (2014). "SURVEILLANCE OF THE ELEPHANT ENDOTHELIO TROPIC 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.

Furuse, Y., et al. (2014). "Analysis of viral microRNA expression by elephant endotheliotropic herpesvirus 1." *Virology* **454-455**: 102-108.

Elephant endotheliotropic herpesvirus 1 (EEHV1), a member of the Betaherpesvirinae subfamily, has recently emerged as an important viral pathogen of Asian elephants that can cause a severe, often fatal, hemorrhagic disease. EEHV1 does not replicate in culture and little is currently known about the molecular biology of this emerging pathogen, with the notable exception of its genomic DNA sequence. Here, we have used small RNA deep sequencing to determine whether EEHV1, like other human and murine betaherpesviruses, expresses viral microRNAs in infected tissues in vivo. Our data provide evidence supporting the existence of at least three novel viral microRNAs encoded by EEHV1 and one of these, miR-E3-5p, is shown to repress target mRNA expression. Moreover, miR-E3-5p expression was readily detectable in tissue samples derived from two infected elephants, including in whole blood. These data shed new light on the biology of EEHV1 and identify small RNAs that have the potential to be useful in the diagnosis of sub-clinical infections in captive Asian and African elephants.

Bouchard, B., et al. (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.

Zachariah, A., et al. (2013). "Fatal herpesvirus hemorrhagic disease in wild and orphan asian elephants in southern India." *J Wildl Dis* **49**(2): 381-393.

Up to 65% of deaths of young Asian elephants (*Elephas maximus*) between 3 mo and 15 yr of age in Europe and North America over the past 20 yr have been attributed to hemorrhagic disease associated with a novel DNA virus called elephant endotheliotropic herpesvirus (EEHV). To evaluate the potential role of EEHV in suspected cases of a similar lethal acute hemorrhagic disease occurring in southern India, we studied pathologic tissue samples collected from field necropsies. Nine cases among both orphaned camp and wild Asian elephants were identified by diagnostic PCR. These were subjected to detailed gene subtype DNA sequencing at multiple PCR loci, which revealed seven distinct strains of EEHV1A and one of EEHV1B. Two orphan calves that died within 3 days of one another at the same training camp had identical EEHV1A DNA sequences, indicating a common epidemiologic source. However, the high level of EEHV1 subtype genetic diversity found among the other Indian strains matches that among over 30 EEHV1 strains that have been evaluated from Europe and North America. These results argue against the previous suggestions that this is just a disease of captive elephants and that the EEHV1 virus has crossed recently from African elephant (*Loxodonta africana*) hosts to Asian elephants. Instead, both the virus and the disease are evidently widespread in Asia and, despite the disease severity, Asian elephants appear to be the ancient endogenous hosts of both EEHV1A and EEHV1B.

Wilkie, G. S., et al. (2013). "Complete genome sequences of elephant endotheliotropic herpesviruses 1a

and 1b determined directly from fatal cases." Journal of Virology **87**(12): 6700-6712.

A highly lethal hemorrhagic disease associated with infection by elephant endotheliotropic herpesvirus (EEHV) poses a severe threat to Asian elephant husbandry. We have used high-throughput methods to sequence the genomes of the two genotypes that are involved in most fatalities, namely, EEHV1A and EEHV1B (species Elephantid herpesvirus 1, genus Proboscivirus, subfamily Betaherpesvirinae, family Herpesviridae). The sequences were determined from postmortem tissue samples, despite the data containing tiny proportions of viral reads among reads from a host for which the genome sequence was not available. The EEHV1A genome is 180,421 bp in size and consists of a unique sequence (174,601 bp) flanked by a terminal direct repeat (2,910 bp). The genome contains 116 predicted protein-coding genes, of which six are fragmented, and seven paralogous gene families are present. The EEHV1B genome is very similar to that of EEHV1A in structure, size, and gene layout. Half of the EEHV1A genes lack orthologs in other members of subfamily Betaherpesvirinae, such as human cytomegalovirus (genus Cytomegalovirus) and human herpesvirus 6A (genus Roseolovirus). Notable among these are 23 genes encoding type 3 membrane proteins containing seven transmembrane domains (the 7TM family) and seven genes encoding related type 2 membrane proteins (the EE50 family). The EE50 family appears to be under intense evolutionary selection, as it is highly diverged between the two genotypes, exhibits evidence of sequence duplications or deletions, and contains several fragmented genes. The availability of the genome sequences will facilitate future research on the epidemiology, pathogenesis, diagnosis, and treatment of EEHV-associated disease. © 2013, American Society for Microbiology.

Stanton, J. J., et al. (2013). "Kinetics of viral loads and genotypic analysis of elephant endotheliotropic herpesvirus-1 infection in captive Asian elephants (*Elephas maximus*)." J Zoo Wildl Med **44**(1): 42-54.

Elephant endotheliotropic herpesviruses (EEHVs) can cause fatal hemorrhagic disease in juvenile Asian elephants (*Elephas maximus*); however, sporadic shedding of virus in trunk washes collected from healthy elephants also has been detected. Data regarding the relationship of viral loads in blood compared with trunk washes are lacking, and questions about whether elephants can undergo multiple infections with EEHVs have not been addressed previously. Real-time quantitative polymerase chain reaction was used to determine the kinetics of EEHV1 loads, and genotypic analysis was performed on EEHV1 DNA detected in various fluid samples obtained from five Asian elephants that survived detectable EEHV1 DNAemia on at least two separate occasions. In three elephants displaying clinical signs of illness, preclinical EEHV1 DNAemia was detectable, and peak whole-blood viral loads occurred 3-8 days after the onset of clinical signs. In two elephants with EEHV1 DNAemia that persisted for 7-21 days, no clinical signs of illness were observed. Detection of EEHV1 DNA in trunk washes peaked approximately 21 days after DNAemia, and viral genotypes detected during DNAemia matched those detected in subsequent trunk washes from the same elephant. In each of the five elephants, two distinct EEHV1 genotypes were identified in whole blood and trunk washes at different time points. In each case, these genotypes represented both an EEHV1A and an EEHV1B subtype. These data suggest that knowledge of viral loads could be useful for the management of elephants before or during clinical illness. Furthermore, sequential infection with both EEHV1 subtypes occurs in Asian elephants, suggesting that they do not elicit cross-protective sterilizing immunity. These data will be useful to individuals involved in the husbandry and clinical care of Asian elephants.

Stanton, J. J., et al. (2013). "Acute phase protein expression during elephant endotheliotropic Herpesvirus-1 Viremia in Asian elephants (*Elephas Maximus*)." Journal of Zoo and Wildlife Medicine **44**(3): 605-612.

Infection of Asian elephants (*Elephas maximus*) with elephant endotheliotropic herpesvirus (EEHV) can be associated with rapid, lethal hemorrhagic disease and has been documented in elephant herds in human care and in the wild. Recent reports describe real-time quantitative polymerase chain reaction (qPCR) assays used to monitor clinically ill elephants and also to detect subclinical EEHV1 infection in apparently healthy Asian elephants. Acute phase proteins have been demonstrated to increase with a variety of infectious etiologies in domesticated mammals but have

not yet been described in elephants. In addition, the immune response of Asian elephants to EEHV1 infection has not been described. In this study, whole blood and trunk wash samples representing repeated measures from eight elephants were examined for the presence of EEHV1 using a qPCR assay. Elephants were classified into groups, as follows: whole blood negative and positive and trunk wash negative and positive. Serum amyloid A (SAA) and haptoglobin (HP) levels were compared between these groups. A significant difference in SAA was observed with nearly a threefold higher mean value during periods of viremia ($P = 0.011$). Higher values of SAA were associated with $>10,000$ virus genome copies/ml EEHV1 in whole blood. There were no significant differences in HP levels, although some individual animals did exhibit increased levels with infection. These data indicate that an inflammatory process is stimulated during EEHV1 viremia. Acute phase protein quantitation may aid in monitoring the health status of Asian elephants. © American Association of Zoo Veterinarians.

Sripiboon, S., et al. (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.

Miller, M. and F. Olea-Popelka (2013). "One Health in the shrinking world: Experiences with tuberculosis at the human-livestock-wildlife interface." Comparative Immunology, Microbiology and Infectious Diseases **36**(3): 263-268.

Tuberculosis (TB) is a global anthroponotic infection that has raised awareness of the impact of disease at the human-livestock-wildlife interface. There are examples of transmission from livestock resulting in establishment of reservoirs in wildlife populations, and exposures from interactions between humans and wildlife that have resulted in disease outbreaks. A One Health approach is crucial to managing and protecting the health of humans, livestock, wildlife and the environment. Although still in its infancy in many areas of the world, the use of transdisciplinary teams to address wildlife-human-livestock boundary diseases will broaden the scope of options for solutions. This paper reviews some less commonly known examples of threats and outcomes using lessons learned from tuberculosis. © 2012 Elsevier Ltd.

Ling, P. D., et al. (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.

Gall, A. and A. Palser (2013). "An elephantine viral problem." Nat Rev Microbiol **11**(8): 512.

This month's Genome Watch highlights how deep sequencing was used to generate the first full genomes of herpesviruses associated with a fatal disease in elephants.

Atkins, L., et al. (2013). "Elephant endotheliotropic herpesvirus 5, a newly recognized elephant herpesvirus associated with clinical and subclinical infections in captive Asian elephants (*Elephas*

maximus)." J Zoo Wildl Med **44**(1): 136-143.

Elephant endotheliotropic herpesviruses (EEHVs) can cause acute hemorrhagic disease with high mortality rates in Asian elephants (*Elephas maximus*). Recently, a new EEHV type known as EEHV5 has been described, but its prevalence and clinical significance remain unknown. In this report, an outbreak of EEHV5 infection in a herd of captive Asian elephants in a zoo was characterized. In February 2011, a 42-yr-old wild-born female Asian elephant presented with bilaterally swollen temporal glands, oral mucosal hyperemia, vesicles on the tongue, and generalized lethargy. The elephant had a leukopenia and thrombocytopenia. She was treated with flunixin meglumine, famciclovir, and fluids. Clinical signs of illness resolved gradually over 2 wk, and the white blood cell count and platelets rebounded to higher-than-normal values. EEHV5 viremia was detectable starting 1 wk before presentation and peaked at the onset of clinical illness. EEHV5 shedding in trunk secretions peaked after viremia resolved and continued for more than 2 mo. EEHV5 trunk shedding from a female herd mate without any detectable viremia was detected prior to onset of clinical disease in the 42-yr-old elephant, indicating reactivation rather than primary infection in this elephant. Subsequent EEHV5 viremia and trunk shedding was documented in the other five elephants in the herd, who remained asymptomatic, except for 1 day of temporal gland swelling in an otherwise-healthy 1-yr-old calf. Unexpectedly, the two elephants most recently introduced into the herd 40 mo previously shed a distinctive EEHV5 strain from that seen in the other five elephants. This is the first report to document the kinetics of EEHV5 infection in captive Asian elephants and to provide evidence that this virus can cause illness in some animals.

Stanton, J. J., et al. (2012). "Development and validation of quantitative real-time polymerase chain reaction assays to detect elephant endotheliotropic herpesviruses-2, 3, 4, 5, and 6." J Virol Methods **186**(1-2): 73-77.

Elephant endotheliotropic herpesviruses (EEHVs) can cause lethal hemorrhagic disease in both African and Asian elephants. At least seven EEHV types have been described, and sensitive real-time PCR tests have been developed for EEHV1A and 1B, which are associated with the majority of characterized Asian elephant deaths. Despite growing knowledge of the different EEHV types, the prevalence of each type within African and Asian elephants remains to be determined and there is considerable need for diagnostic tests to detect and discriminate between each EEHV species for clinical management of African and Asian elephants that develop illness from one or more of these viruses. To begin to address these issues, we developed real-time PCR assays for EEHV2, 3, 4, 5, and 6. Overall, each assay had robust PCR efficiency, a dynamic linear range over 5log(10) concentrations, a limit of detection of 10 copies/test reaction with 100% sensitivity, and low intra- and inter-assay variability. Each assay proved to be specific for the EEHV targets for which it was designed, with the exception of EEHV3 and EEHV4, which was expected because of greater DNA sequence similarity between these two EEHV species than the others. These new tools will be useful for conducting surveys of EEHV prevalence within captive and range country elephants, for diagnostic testing of elephants with suspected EEHV-associated disease, and for managing the treatment of elephants with EEHV-induced illness.

Sariya, L., et al. (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/ μ l 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..

Hayward, G. S. (2012). "Editorial: Conservation: Clarifying the risk from herpesvirus to captive Asian elephants." Veterinary Record **170**(8): 202-203.

Denk, D., et al. (2012). "Fatal elephant endotheliotropic herpesvirus type 5 infection in a captive Asian elephant." Vet Rec **171**(15): 380-381.

Brock, A. P., et al. (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 µg/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 µg/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., et al. (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.

Nolen, R. S. (2011). "Herpesvirus claims another elephant as search for answers continues." J Am Vet Med

Assoc **239**(2): 166-168.

Masters, N. J., et al. (2011). "Detection of EGHV-5 in a self-limiting papillomalike lesion in the trunk of an Asian elephant (*Elephas maximus*)." Veterinary Record **169**(8).

Latimer, E., et al. (2011). "Detection and evaluation of novel herpesviruses in routine and pathological samples from Asian and African elephants: identification of two new probosciviruses (EEHV5 and EEHV6) and two new gammaherpesviruses (EGHV3B and EGHV5)." Vet Microbiol **147**(1-2): 28-41.

Systemic infections with elephant endotheliotropic herpesviruses (EEHV) cause a rapid onset acute hemorrhagic disease with an 85% mortality rate. More than 60 cases have been confirmed worldwide occurring predominantly in juvenile Asian elephants. Originally, three virus types EEHV1A, EEHV1B and EEHV2 were identified, all members of the Proboscivirus genus within the Betaherpesvirinae. However, four elephant gammaherpesviruses (EGHV) have also been found by DNA PCR approaches in eye and genital secretions of asymptomatic animals, and two more versions of the probosciviruses, EEHV3 and EEHV4, were recently detected in acute hemorrhagic disease cases. To ask whether even more species of elephant herpesviruses may exist, we have developed several new diagnostic DNA PCR assays using multiple round primers in the DNA POL region. These have been used routinely for nearly three years to screen samples submitted to the Elephant Herpesvirus Laboratory for diagnosis of possible cases of EEHV disease in blood and necropsy tissue, as well as in biopsies of other suspicious lesions or growths. Several more cases of EEHV1-associated hemorrhagic disease were confirmed, but in addition, we describe here eleven examples of other known and novel herpesviruses detected and evaluated with these reagents. They include the prototypes of four new elephant herpesviruses, two more within the proboscivirus group EEHV5 and EEHV6, plus two more gammaherpesviruses EGHV3B and EGHV5. We also report initial semi-quantitative PCR assays demonstrating very high viral loads in the blood of the EEHV3 and EEHV4-associated hemorrhagic disease cases.

Hardman, K., et al. (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.

(2011). "With few resources, researchers work to contain fatal elephant virus." Am J Vet Res **72**(8): 1006.

Zong, J. C., et al. (2010). Analysis of species and strain differences among elephant endotheliotropic herpesviruses by gene subtyping: absence of epidemiological connections among 20 cases of EEHV1 associated with acute hemorrhagic disease in captive Asian elephants. Proceedings 2010 IEF Symposium.

Stanton, J. J., et al. (2010). "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." Am J Vet Res **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.

Schaftenaar, W., et al. (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.

Garner, M. M., et al. (2009). "Clinico-pathologic features of fatal disease attributed to new variants of endotheliotropic herpesviruses in two Asian elephants (*Elephas maximus*)." Veterinary Pathology **46**(1): 97-104.

The first herpesviruses described in association with serious elephant disease were referred to as endotheliotropic herpesviruses (EEHV) because of their ability to infect capillary endothelial cells and cause potentially fatal disease. Two related viruses, EEHV1 and EEHV2, have been described based on genetic composition. This report describes the similarities and differences in clinicopathologic features of 2 cases of fatal endotheliotropic herpesvirus infections in Asian elephants caused by a previously unrecognized virus within the betaherpesvirus subfamily. EEHV3 is markedly divergent from the 2 previously studied fatal probosciviruses, based on polymerase chain reaction sequence analysis of 2 segments of the viral genome. In addition to ascites, widespread visceral edema, petechiae, and capillary damage previously reported, important findings with EEHV3 infection were the presence of grossly visible renal medullary hemorrhage, a tropism for larger veins and arteries in various tissues, relatively high density of renal herpetic inclusions, and involvement of the retinal vessels. These findings indicate a less selective organ tropism, and this may confer a higher degree of virulence for EEHV3.

Zong, J. C., et al. (2008). Pathogenesis and molecular epidemiology of fatal elephant endotheliotropic disease associated with the expanding Proboscivirus genus of the betaherpesvirinae. Proceedings 2007 IEF Symposium.

Wellehan, J. F. X., et al. (2008). "Six novel gammaherpesviruses of Afrotheria provide insight into the early divergence of the Gammaherpesvirinae." Veterinary Microbiology **127**: 249-257.

Steinmetz, H. W., et al. (2008). Daily clinical examinations in a herd of captive asian elephants. Proc American Association 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 (\pm 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.

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Miller, J. and M. McClean (2008). Pharmacokinetics of enrofloxacin in African elephants (*Loxodonta africana*) after a single rectal dose. *Proc American Association 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.

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Meyers, D. A., et al. (2008). Evaluation of acute phase proteins for diagnosis of inflammation in Asian elephants (*Elephas maximus*). Proc American Association 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 those animals 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.

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Cracknell, J., et al. (2007). Elephant Endotheliotropic 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., et al. (2007). Education as a tool for disease management: Elephant Endotheliotropic Herpes Virus Case Study in the UK. Proceedings of AAZV.

Cracknell, J. (2007). Elephant Endotheliotropic Herpes Virus (EEHV)

Protocol. UK, United Kingdom Elephant Health Programme; ZSL; BIAZA: 49.

Wellehan, J. F. X., et al. (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., et al. (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 phylogenetic 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., et al. (2006). "Topics in herpesvirus genomics and evolution." Virus Research **117**(1): 90-104.

Hildebrandt, T. B., et al. (2006). Ultrasonographic assessment and ultrasound-guided biopsy of the retropharyngeal lymph nodes in elephants. 2006 Proceedings American Association of Zoo Veterinarians.

So far there are no valid diagnostic tools available for identifying latent carriers of endotheliotropic elephant herpes virus (EEHV). For this reason, the lateral retropharyngeal lymph node complex (LARELYNOC) of elephants, identified during postmortem studies as target organ for EEHV and suitable for transcutaneous biopsy, was grossly described. Transcutaneous ultrasound (3.5 MHz) was applied behind the ear region to identify the LARELYNOC containing up to four single lymph nodes on each side. The lymph node tissue is situated 20-50 mm below the skin surface. An ultrasonographic assessment of the LARELYNOC and two biopsies were performed on 39 healthy Asian elephants (*Elephas maximus*). Samples were tested for EEHV via PCR. Whole blood samples were also collected and tested for active EEHV infection. Lymph nodes were ultrasonographically classified as active (calculated mean volume=17.4 ± 6.9 cm³, P>0.001), inactive (calculated mean volume=3.1 ± 0.6 cm³, P<0.001), or chronic active (calculated mean volume=10.6 ± 1.0 cm³, P<0.05). Histology confirmed not only the presence of lymph tissue but also the ultrasonographically diagnosed reactivity status of the lymph node biopsies. Although all samples including whole blood were found to be negative for the EEHV DNA particles, the successful

development of this procedure in elephants could prove beneficial for the screening of not only latent EEHV infections but might also be a less dangerous alternative method for the diagnosis of zoonotic infections such as tuberculosis.

Ehlers, B., et al. (2006). "Endotheliotropic elephant herpesvirus, the first betaherpesvirus with a thymidine kinase gene
411." *J. Gen. Virol* **87**(Pt 10): 2781-2789.

Endotheliotropic elephant herpesvirus (elephantid herpesvirus 1; EIHV-1) is apathogenic for African elephants (*Loxodonta africana*), but causes fatal haemorrhagic disease in Asian elephants (*Elephas maximus*). This is thought to occur through transmission from African elephants in places where both species are housed, such as zoological gardens. The virus has caused considerable losses in North American and European zoological gardens and thus severely impedes breeding of the endangered Asian elephant. Previously, the ultrastructural and genetic characterization of EIHV-1 from a male Asian elephant that died from the disease at the Berlin zoological gardens in 1998 have been reported. Here, a partial characterization of the EIHV-1 genome is presented. A 60 kbp locus, spanning 34 open reading frames, was analysed. Most of the detected genes were found to be conserved among the herpesviruses and showed an overall arrangement most similar to that of betaherpesviruses, in particular Human herpesvirus 6 and Human herpesvirus 7. Most importantly, in addition to a protein kinase gene that is homologous to the human cytomegalovirus UL97 gene, a thymidine kinase (TK) gene was found, which is generally missing in betaherpesvirus genomes. Thus, EIHV-1 is the only known betaherpesvirus to encode a TK gene. This peculiarity might contribute to the fulminant pathogenicity of EIHV-1, but also provide a crucial enzymic activity for developing an efficient antiviral therapy with currently available nucleoside analogues

Hildebrandt, T. B., et al. (2005). "Ultrasonographic assessment and ultrasound-guided biopsy of the retropharyngeal lymph nodes in Asian elephants (*Elephas maximus*)
552." *Vet. Rec* **157**(18): 544-548.

Endotheliotropic herpesvirus causes a fatal disease in young Asian elephants, but there are no methods for identifying latent carriers of the virus. During the postmortem study of one female African elephant and three male and two female Asian elephants, a lymph node located bilaterally caudoventral to the parotid gland, approximately 1.5 to 5 cm below the skin, was identified as suitable for transcutaneous ultrasound-guided biopsy. An ultrasonographic assessment and two biopsies were performed on 39 Asian elephants, and these lymph nodes were classified ultrasonographically as active, inactive or chronically active. The calculated mean (se) volume of 10 active lymph nodes was 17.4 (6.9) cm³, and that of three chronically active lymph nodes was 10.6 (1.0) cm³, whereas the mean volume of 17 inactive lymph nodes was 3.1 (0.6) cm³. The presence of lymph node tissue in samples obtained by ultrasound-guided biopsy from three animals that were maintained under conditions that allowed for additional sampling was confirmed histologically. The dna extracted from the lymphoid tissue and the whole blood of all the elephants was negative for endotheliotropic herpesvirus by PCR.

Isaza, R., et al. (2003). Famciclovir pharmacokinetics in young Asian elephants (*Elephas maximus*). Proc. American Assoc. of Zoo Veterinarians.

Asian elephants (*Elephas maximus*) are susceptible to a unique infection caused by elephant endotheliotropic herpesvirus (EEHV).^{3,4} Worldwide, between the years 1983 and 2000, there have been 26 confirmed deaths from this virus in Asian elephants.² Although most cases have been fatal, treatment with famciclovir (Famvir, SmithKline Beecham Pharmaceuticals, Philadelphia, PA 19101 USA) has been associated with survival in three cases of six cases of EEHV infection proven by PCR.^{2,5,6} Dose selections for surviving elephants (5.5 - 8.0 mg/kg, p.o. every 8 hr) were made without the benefit of elephant pharmacokinetics and were a direct extrapolation from recommended human dosages (7 mg/kg, p.o. every 8 hr).^{5,6} In this study, famciclovir was administered both orally and rectally in healthy young Asian elephants. The doses tested in this study were 5 mg/kg orally, 5 mg/kg rectally, and 15 mg/kg rectally. Blood samples were analyzed for famciclovir and penciclovir using a validated LC/MS assay. Famciclovir was absorbed well by

both routes and underwent rapid biotransformation to the active compound penciclovir. None of the plasma samples had detectable famciclovir. Pharmacokinetic parameters for penciclovir were determined using non-compartmental analysis. After a single oral dose of 5 mg/kg the C_{max} was 1.3 µg/mL with a T_{max} at 1.1 h. After a rectal dose of 5 mg/kg the C_{max} was 1.2 µg/mL with a T_{max} at 0.34 hr. After a rectal dose of 15 mg the t_{1/2} was 2.6 h, with a C_{max} of 3.6 µg/mL at T_{max} 0.66 h. These results were similar to those reported in humans where an oral dose of 500 mg (7 mg/kg) had a t_{1/2} of about 2 h with a C_{max} of 3.3 µg/mL. A dose range of 8 -15 mg/kg given orally or rectally every 8 hours should produce penciclovir concentrations in Asian elephants that are considered therapeutic in humans.

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Fickel, J., et al. (2003). "Comparison of glycoprotein B (gB) variants of the elephant endotheliotropic herpesvirus (EEHV) isolated from Asian elephants (*Elephas maximus*)." *Vet Microbiol* **91**(1): 11-21. The recently described elephant endotheliotropic herpesviruses (EEHV) have been associated with the deaths of numerous captive elephants. A proposed tool for the detection of EEHV infection in elephants is the PCR-based screening for EEHV-DNA in whole blood samples. Unfortunately, this detection method has only been successful in post-mortem analyses or in animals already displaying clinical signs of EEHV disease, thus rendering this method unsuitable for identification of carrier elephants. Here, we focus on glycoprotein B (gB) for serologic assay development, since gB is an envelope protein known to induce a neutralising antibody response in other herpesvirus infections. We sequenced the entire gB gene from five Asian elephants with EEHV, representing four different gB variants. Computer-aided methods were used to predict functionally important regions within EEHVgB. An extra-cytoplasmic region of 153 amino acids was predicted to be under positive selection and may potentially contain antigenic determinants that will be useful for future serologic assay development.

Schaftenaar, W., et al. (2001). Successful treatment of a subadult Asian elephant bull (*Elephas maximus*) infected with elephant herpes virus. Proc. of the International Symposium for diseases of Zoo and Wildlife Animals (Rotterdam).

Ryan, S. J. and S. D. Thompson (2001). "Disease risk and inter-institutional transfer of specimens in cooperative breeding programs: Herpes and the elephant species survival plans." *Zoo Biology* **20**: 89-101. Managers of cooperative breeding programs and re-introduction projects are increasingly concerned with the risk of disease transmission when specimens are transferred among facilities or between facilities and the natural environment. We used data maintained in North American studbooks to estimate the potential risks of disease transmission by direct and indirect contact of specimens in the American Zoo and Aquarium Association's Elephant Species Survival Plan.

Histological evidence for a novel herpesvirus disease transmitted between and within elephant species housed in North American facilities prompted an examination of the scope of possible transmission routes within the captive population. We found that, compared with other species managed through Species Survival Plans, elephants experience relatively few transfers between zoos. Nevertheless, the number of direct contacts with other elephants born during the study period of 1983-1996 (excluding stillbirths) was much higher than we had anticipated (\bar{x} = 25 +/- 27; N = 59) and the number of potential indirect contacts was surprisingly large (\bar{x} = 143 +/- 92; N = 59). Although these high rates of potential contacts complicate exact identification of infection pathways for herpesvirus, we were able to propose potential routes of transmission for the histologically identified cases. Furthermore, the extraction of data from studbooks allowed us to readily identify other specimens that did not succumb to the disease despite similar exposure. Moreover, we were able to identify other possible cases to recommend for histological examination. Herein we reveal the possibilities of multiple disease transmission pathways and demonstrate how complex the patterns of transmission can be, confounded by the unknown latency of this novel herpesvirus. This emphasizes the need for zoo veterinarians and cooperative breeding programs to consider the full potential for disease transmission associated with each and every inter-zoo transfer of specimens.

Richman, L. K. and R. J. Montali (2001). Elephant herpesvirus infections. Infectious Diseases of Wild Mammals, 3rd edition. E. S. Williams and I. K. Barker. Ames, Iowa, USA, Iowa State University Press: 170-178.

Ratanakorn, P. (2001). Elephant Health Problems and Management in Cambodia, Lao and Thailand. A Research Update on Elephants and Rhinos; Proceedings of the International Elephant and Rhino Research Symposium, Vienna, June 7-11, 2001, Schuling Verlag.

Montali, R. J., et al. (2001). Management Aspects of Herpesvirus Infections and Tuberculosis in Elephants. A Research Update on Elephants and Rhinos; Proceedings of the International Elephant and Rhino Research Symposium, Vienna, June 7-11, 2001, Schuling Verlag.

Elephant endotheliotropic herpesvirus (EEHV) infections and tuberculosis have emerged as causes of illness and mortality in captive elephants. Twenty-six confirmed EEHV cases are documented. Since 1995, 7 have occurred in North America, 10 in Europe and 2 in Asia. A PCR test was used to detect the virus in symptomatic animals; a serological test to identify carrier elephants is under development. The African elephant is a potential source of the EEHV that is lethal for Asian elephants. Fatal infections have also occurred in Asian elephants without African elephant contacts. Three of 6 elephants recovered after treatment with antiviral famciclovir; however, more research is needed to improve the usefulness of this drug. Asian elephants that are less than 10-years old and have been moved to another facility and/or have had contact with African elephants are at increased risk for contracting EEHV. Animals traveling between facilities with a history of EEHV cases may be at greater risk. All young elephants should be monitored daily for anorexia, lethargy, body swellings and blue discoloration (bruising) of the tongue, and be trained for blood sampling and potential oral and rectal treatment with famciclovir.

Since 1996, *Mycobacterium tuberculosis* has affected about 3% of Asian elephants in North America. Most were from 5 U.S. States with some contacts between private herds. Mandatory annual testing for tuberculosis by trunk wash cultures was established in 1998, and 22 culture-positive *M. tuberculosis* elephants were identified between 1996-2001. Fifteen were treated with anti-tuberculosis drugs and 7 that died or were euthanized were proven to have tuberculosis at necropsy. Antemortem sera was available from 4/7 4 (75%) were strongly ELISA positive. Tuberculosis is uncommon in African elephants but was recently associated with *M. bovis* in the U.S. and *M. tuberculosis* in Germany. Conversely, *M. bovis* tuberculosis, apparently unrecognized in Asian elephants, recently occurred in Germany. Management issues of elephant tuberculosis will be discussed relative to its complex epidemiology and clinical-pathological correlations.

Hildebrandt, T. B., et al. (2001). Survey on presence of the endotheliotropic elephant herpesvirus (EEHV)

in Thai camp elephants. Proceedings American Association of Zoo Veterinarians, American Association of Wildlife Veterinarians, Association of Reptilian and Amphibian Veterinarians and the National Association of Zoo and Wildlife Veterinarians Joint Conference 2001, American Association of Zoo Veterinarians.

Fickel, J., et al. (2001). "A variant of the endotheliotropic herpesvirus in Asian elephants (*Elephas maximus*) in European zoos." Veterinary Microbiology **82**(2): 103-109.

Newly discovered, lethal elephant endotheliotropic herpesviruses (EEHV) have been identified in both Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. Carried by otherwise healthy African elephants, they can be fatal, mainly for young Asian elephants. Since zoos often harbour both elephant species, we conducted a survey on the presence of EEHV in elephants (Asian elephants, n=57; African elephants, n=17) from 12 zoos and 3 circuses in Europe (Germany, Switzerland and the Netherlands), and 1 zoo in Israel [date not given]. Six of the 57 Asian elephants were positive for EEHV. Five elephants died of the infection, while one survived. EEHV was not detected in any of the 17 African elephants. All EEHV that affected the Asian elephants belonged to the EEHV1 group. We described the detection and the partial sequencing of an endotheliotropic herpesvirus variant (named EEHV1b) in Asian elephants, being either an EEHV endogenous to Asian elephants or indicating different sources (African elephants) of infection.

Ehlers, B., et al. (2001). "Genetic and ultrastructural characterization of a European isolate of the fatal endotheliotropic elephant herpesvirus." J Gen Virol **82 (Pt 3)**: 475-482.

A male Asian elephant (*Elephas maximus*) died at the Berlin zoological gardens in August 1998 of systemic infection with the novel endotheliotropic elephant herpesvirus (EIHV-1). This virus causes a fatal haemorrhagic disease in Asian elephants, the so-called endothelial inclusion body disease, as reported from North American zoological gardens. In the present work, EIHV-1 was visualized ultrastructurally in affected organ material. Furthermore, a gene block comprising the complete glycoprotein B (gB) and DNA polymerase (DPOL) genes as well as two partial genes was amplified by PCR-based genome walking and sequenced. The gene content and arrangement were similar to those of members of the Betaherpesvirinae. However, phylogenetic analysis with gB and DPOL consistently revealed a very distant relationship to the betaherpesviruses. Therefore, EIHV-1 may be a member of a new genus or even a new herpesvirus subfamily. The sequence information generated was used to set up a nested-PCR assay for diagnosis of suspected cases of endothelial inclusion body disease. Furthermore, it will aid in the development of antibody-based detection methods and of vaccination strategies against this fatal herpesvirus infection in the endangered Asian elephant.

Schmitt, D. L., et al. (2000). "Use of famciclovir for the treatment of endotheliotropic herpesvirus infections in Asian elephants (*Elephas maximus*)." Journal of Zoo and Wildlife Medicine **31**(4): 518-522.

Two juvenile Asian elephants (*E. maximus*) presented with an acute onset of facial oedema and lethargy. Examination of the oral cavity of each animal revealed cyanosis of the tip and distal margins of the tongue suggestive of endothelial inclusion body disease (EIBD) of elephants. Whole-blood samples were obtained, and polymerase chain reaction tests confirmed the presence of elephant herpesvirus. The animals were administered famciclovir (Flamvir; 500 mg/70 kg body weight, with a loading dose of 1000 mg/70 kg body weight) a potent human anti-herpesvirus drug, in the course of their disease, and recovery followed a treatment regime of 3-4 wk. These are the first known cases of elephants surviving EIBD.

Richman, L. K., et al. (2000). "Review of a newly recognized disease of elephants caused by endotheliotropic herpesviruses." Zoo Biology **19**(5): 383-392.

There are two newly recognized herpesviruses that cause a fatal disease syndrome in elephants. They are known as the elephant endotheliotropic herpesviruses, of which one is fatal for Asian elephants (*Elephas maximus*) and the other for African elephants (*Loxodonta africana*) The disease syndrome affects predominantly young elephants and has been described in North America, Europe, and Israel. The predominant clinical signs for both species include lethargy, oedematous swellings of the head, neck, and thoracic limbs, oral ulceration, cyanosis of the tongue, and death

of most elephants in 1-7 days. Three affected young Asian elephants recovered after a 3-4-week course of therapy with the anti-herpesvirus drug famciclovir. Additional reported herpesvirus-associated lesions in otherwise healthy elephants include localized skin papillomas in African elephants, proliferative vulval lymphoid patches in African elephants, and pulmonary nodules in African elephants. Recent findings suggest that these localized herpesvirus-associated lesions in healthy African elephants may be one source of the herpesvirus that causes disseminated disease and death in the Asian species and the African species. These findings have implications for management practices in facilities keeping both African and Asian elephants and in protecting natural elephant habitats from virulent forms of the virus.

Richman, L. K., et al. (2000). "Clinical and pathological findings of a newly recognized disease of elephants caused by endotheliotropic herpesviruses." Journal of Wildlife Diseases **36**(1): 1-12.

The unique clinical and pathological findings in nine Asian elephants (*Elephas maximus*) and two African elephants (*Loxodonta africana*) from North American Zoos with a highly fatal disease caused by novel endotheliotropic herpesviruses are described. Consensus primer polymerase chain reaction combined with sequencing yielded molecular evidence that confirmed the presence of 2 novel but related herpesviruses associated with the disease, one in Asian elephants and the other in African elephants. Disease onset was acute, with lethargy, edema of the head and thoracic limbs, oral ulceration and cyanosis of the tongue followed by death of most animals in 1 to 7 days. Pertinent laboratory findings in 2 of 3 clinically evaluated animals included lymphocytopenia and thrombocytopenia. Two affected young Asian elephants recovered after a 3- to 4-week course of therapy with famciclovir. PM examination in the fatal cases revealed pericardial effusion and extensive petechial hemorrhages in the heart and throughout the peritoneal cavity, hepatomegaly, cyanosis of the tongue, intestinal hemorrhage and ulceration. Histologically, there were extensive microhemorrhages and edema throughout the myocardium and mild, subacute myocarditis. Similar hemorrhagic lesions with inflammation were evident in the tongue, liver and large intestine. Lesions in these target organs were accompanied by amphophilic to basophilic intranuclear viral inclusion bodies in capillary endothelial cells. Transmission electron microscopy of the endothelial inclusion bodies revealed 80 to 92 nm diameter viral capsids consistent with herpesvirus morphology. The short course of the herpesvirus infections, with sudden deaths in all but the 2 surviving elephants, was ascribed to acute cardiac failure attributed to herpesvirus-induced capillary injury with extensive myocardial hemorrhage and edema.

Fickel, J., et al. (2000). Survey on the occurrence of the endotheliotropic elephant herpesvirus (EEHV) in Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants in European zoos. European Association of Zoo and Wildlife Veterinarians Third Scientific Meeting, Paris, France, May 31-June 4, 2000.

Richman, L. K., et al. (1999). Status of a new, fatal herpesvirus disease in elephants in North America and Europe. Verhandlungsbericht des 39 International Symposium uben Erkrankungen der Zoo und Wildtiere, Wien. 39:17-21. Verh.ber Erkr. Zootiere 39.

Richman, L. K., et al. (1999). "Novel endotheliotropic herpesviruses fatal for Asian and African elephants." Science **283**(5405): 1171-1176.

A highly fatal haemorrhagic disease was identified in 10 young Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants at zoos in the USA between 1983 and 1997. In the affected animals there was ultrastructural evidence for herpesvirus-like particles in endothelial cells of the heart, liver, and tongue. Consensus primer polymerase chain reaction combined with sequencing yielded molecular evidence that confirmed the presence of 2 novel but related herpesviruses associated with the disease, one in Asian elephants and another in African elephants. Otherwise healthy African elephants with external herpetic lesions yielded herpesvirus sequences identical to that found in Asian elephants with endothelial disease. It is suggested that the Asian elephant deaths were caused by cross-species infection with a herpesvirus that is naturally latent in, but normally not lethal to, African elephants. A reciprocal relationship may exist for the African elephant disease.

Richman, L. K., et al. (1999). Clinical and pathologic aspects of a fatal herpesvirus disease in Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. Proceedings of the American Association of Zoo Veterinarians.

Mikota, S. K. (1999). "Diseases of the Elephant: A Review." Verh. ber. Erkr. Zootiere **39**: 1-15.

Ferber, D. (1999). "Virus suspect identified in elephant deaths." Science **283**(5405): 1093-1094.

Burkhardt, S., et al. (1999). Elephant herpes virus - a problem for breeding and housing of elephants. Berliner und Munchener Tierarztliche Wochenschrift
Elefantenherpesvirus - ein Problem fur die Zucht und Haltung von Elefanten. **112**: 174-179.

Herpesvirus infections which take a fatal turn on African elephants as well as on Asian elephants seem to occur increasingly not only in the USA but also in European stocks. The endotheliotropic herpesvirus causes a rapidly progressing and severe disease which makes any therapeutical effort unsuccessful and finally results in death of the animal, especially in young Asian elephants. As all attempts to culture the virus failed up to now, molecular biological procedures have to be used more often for diagnostic purpose together with the common methods of pathology, virology, and electronmicroscopical evaluation. This is a report on the case of 'KIBA', an eleven year old male elephant at the Zoological Garden Berlin, infected with the endotheliotropic elephants herpesvirus. 'KIBA' was born at the Zoo in Houston, Texas, and raised within his herd. Upon arriving in Berlin in November 1997 he adapted to the new premises and climate and new social circumstances without any problems. In June 1998 he already serviced three females of his new herd several times. In August 1998 he died after passing a peracute progression of the disease after residing in Berlin for only 9 months. The dissection of the animal revealed some evidence on an agent damaging the endothelium. Major signs indicating this agent were bleedings in several serous membranes, mucosa and on the right atrium, as well as other parts of the myocardium. Furthermore there have been ulcerations at various localizations of the whole digestive tract. Slightly basophilic intranuclear inclusion bodies have been found histologically in endothelial cells of different organ samples. An examination of altered organ-material by electronmicroscopy made some herpesvirus-like particles visible. A virological investigation first revealed evidence of giant cell formations with solitary basophilic intranuclear inclusion bodies in different cell cultures, however, without any distinct cytopathogenic effect. Supported by molecular biological procedures the infection of 'KIBA' could be verified as the elephants herpesvirus. By means of PCR and subsequent sequence analysis a DNA-sequence typical for the elephants herpesvirus could be obtained which showed an identity of 97% with the terminase sequence of the elephant herpesvirus described by American authors. The deduced amino acid-sequences were 100% identical. To the terminase of the human cytomegalovirus, the elephant sequence had an identity of 53% (similarity: 74%). Based on the cooperation of ILAT, Institute of Veterinary-Pathology/Free University Berlin, Robert-Koch-Institut Berlin, and Zoological Garden Berlin, the cause of 'KIBA's' death could be discovered immediately. Possible implications of this case especially on breeding and keeping elephants are discussed briefly.

Schmitt, D. L. and D. A. Hardy (1998). "Use of famciclovir for the treatment of herpesvirus in an Asian elephant." Journal of the Elephant Managers' Association **9**: 103-104.

Bhat, M. N., et al. (1997). "Serological evidence of bovine herpesviruses 1 and 2 in Asian elephants." Journal of Wildlife Diseases **33**(4): 919-920.

Antibodies were detected against bovine herpesviruses 1 (BHV 1) and 2 (BHV 2) in Asian elephants (*Elephas maximus*) using the passive haemagglutination (PHA) test. The study was conducted during May to December 1994 using sera collected from zoos and national parks in India. Four (4%) of 109 elephant sera had PHA titres ranging from 1:8 to 1:32 against BHV 1. 25 (23%) of the 109 elephant sera had PHA titres ranging from 1:8 to 1:64 against BHV 2. It is concluded that Asian elephants appear to be better reservoirs for herpesviruses which are serologically related to

BHV 2.

Barnard, B. J. H. (1997). "Antibodies against some viruses of domestic animals in southern African wild animals." Onderstepoort Journal of Veterinary Research **64**(2): 95-110.

Twenty-four species of South African wild animals were tested for the presence of antibodies to the viruses of 16 common diseases of domestic animals around 1993-5. Positive results were obtained for African horse sickness, equine encephalomyelitis virus, equine herpesvirus-1, bovine herpesvirus-1, Allerton disease (Herpes mammillitis; bovine herpesvirus 2), lumpy skin disease, parainfluenza, encephalomyocarditis, bluetongue, Wesselsbron disease, bovine ephemeral fever, and Akabane disease complex. No antibodies could be demonstrated against the viruses of equine influenza, equine infectious anaemia, equine viral arteritis or Rift Valley fever. The negative results support observations that the latter diseases, with the exception of equine viral arteritis, are absent in South Africa. The number of animal species found positive for a specific virus, ranged from 0-16. No antibodies were found in crocodiles or warthogs, whereas antibodies against Wesselsbron and bovine herpesvirus-1 were present in 16 species. Antibodies against viruses of horses were found almost exclusively in zebras and, although elephants reacted to African horse sickness, no neutralizing antibodies against it could be demonstrated in their sera. Zebras were also found to be positive for Wesselsbron and Akabane, which are usually regarded as viruses of ruminants. Antibodies against most viruses were encountered in all vegetation zones in South Africa, but most viruses were more prevalent in the high-rainfall zone in KwaZulu-Natal.

Richman, L. K., et al. (1996). Endothelial inclusion body disease: a newly recognized fatal herpes-like infection in Asian elephants. Proceedings American Association of Zoo Veterinarians.

Cambre, R. C. and W. W. Buick (1996). "Special challenges of maintaining wild animals in captivity in North America." Rev Sci Tech **15**(1): 251-266.

The maintenance of wild animals in captivity in North America is regulated by a number of different laws and government agencies in each country. Member institutions of zoo and aquarium associations in Canada, the United States of America and Mexico experience an extra tier of regulation in the form of industry standards, which are sometimes stricter than those imposed by government. Climate, natural disasters and harmful pest species all contribute to the challenge of keeping animals in certain locales. Vigilance against zoonotic disease transmission is maintained through industry and government-mandated sanitation standards, which are fortified by reporting regulations of local, regional and Federal health agencies. Current controversies in the keeping of particular taxa in North America include the threat to non-human primate breeding programmes precipitated by strict new import regulations, the fear of herpesvirus B infection, and commercial airline transport bans. Successive human fatalities among elephant handlers have prompted the industry and governments to re-examine the manner in which these potentially dangerous creatures are maintained in captivity.

Ossent, P., et al. (1990). "Acute and fatal herpesvirus infection in a young Asian elephant (*Elephas maximus*)." Vet. Pathol **27**: 131-133.

Infections with herpesvirus may cause papillomatous lesions in the Asian and African elephant. In both species, the virus has been reported to localize only in the skin. Disseminated nodules of epithelial cells were found in the lungs of a high percentage of wild African elephants. In these cases, the proliferated cells contained intranuclear inclusion bodies in which herpesvirus particles were observed by electron microscopy. The virus in those cases caused no illness. This report documents the necropsy findings of a juvenile Asian elephant dying peracutely from massive generalized hemorrhage due to lesions in the endothelial cells of the capillaries. The cell nuclei frequently contained inclusion bodies in which herpesvirus particles were demonstrated. This has not been described in elephants before.

Metzler, A. E., et al. (1990). "Serological evidence of herpesvirus infection in captive Asian elephants (*Elephas maximus*)." J. Wildl. Dis **26**(1): 41-49.

In mid 1988 a 3-yr-old Asian elephant (*Elephas maximus*) from a circus in Switzerland died following generalized manifestation of a herpesvirus infection. In an effort to determine prevalence of infection with the herpesvirus, and due to lack of a corresponding virus isolate, it was decided to evaluate contact animals and elephants from a second herd for antibody to bovine herpesvirus 1 (BHV1) and bovine herpesvirus 2 (BHV2). Of 15 sera tested four displayed low neutralizing antibody titers to BHV2. None of the sera neutralized BHV1. However, as evidenced by protein A-mediated immunoprecipitation of metabolically radio-labeled virus-infected and mock-infected cell antigens, followed by separation of precipitation products in SDS-polyacrylamide gels, the 15 sera precipitated multiple antigens from both viruses. Similar results were obtained when using BHV4 antigens. The extent of reaction was most distinct with respect to BHV2 antigens, less prominent with BHV1 antigens, and least with BHV4 antigens. The respective protein patterns, although less marked, matched well with those obtained with bovine reference sera. Additional evaluation of sera from six elephants from two zoos in the Federal Republic of Germany gave essentially identical results. It was concluded that at least one herpesvirus, immunologically related to BHV2, may be widely distributed among captive Asian elephants, and that this virus apparently does not cause overt disease in the majority of animals

Pilaski, J., et al. (1988). Two virus diseases of different aetiology in Asian elephant (*Elephas maximus*) in small traveling circus. Erkr. Zootiere
Swei Viruserkrankungen unterschiedlicher Aetiologie bei Asiatischen Elefanten (*Elephas maximus*) in einem kleinen Wanderzirkus. **30**: 263-269.

Pilaski, J., et al. (1987). Herpes virus infection in an Asian elephant (*Elephas maximus*). Erkr. Zootiere
Eine Herpesvirus-Infektion bei einem Asiatischen Elefanten (*Elephas maximus*). **29**: 179-184.

Jacobson, E. R., et al. (1986). "Cutaneous papillomas associated with a herpesvirus-like infection in a herd of captive African elephants." J. Am. Vet. Med. Assoc **189**(9): 1075-1078.

Proliferative cutaneous lesions developed in a herd of captive African elephants (33 from an animal importer in Texas, and 63 young elephants collected in Zimbabwe). Group-1 elephants were purchased 8 months before the arrival of the group-2 elephants. On arrival, 7 group-1 elephants had raised nodular fibrous growths, located predominantly on their trunks. Lesions were not observed in the group-2 elephants until approximately 3 months after they were acquired. Lesions on group-2 elephants began as small focal proliferative growths that regressed or that progressed into large nodular fibrous growths that were similar in appearance to those seen in the group-1 elephants. Lesions at various stages of development were biopsied and examined. Histologically, early lesions were inverted papillomas, with hyperplastic and hypertrophic epithelial cells containing amphoteric intranuclear inclusions in the lesion center. Older, large, nodular fibrous growths were ulcerated and were composed predominantly of a thickened dermis containing fibroblasts, collagen, and a mixed inflammatory cell infiltrate; inclusions were not observed in adjacent epidermal cells. Using a peroxidase-antiperoxidase technique, we did not detect group-specific papillomavirus antigens. Southern blot hybridization analysis of DNA from lesion specimens did not indicate papillomavirus-specific genomes. Electron-microscopically, inclusions consisted of aggregates of virus particles. The particles had electron-dense and electron-lucent cores and were 95 to 103 nm in diameter. Virions developed envelopes from nuclear membranes. Mature particles were seen within the cytoplasm and filled the intercellular spaces. On the basis of size, location, conformation, and envelopment, the particles most closely resembled those of herpesviruses.

Plowright, W. (1981). Herpesvirus of wild ungulates, including malignant catarrhal fever virus. Infectious diseases of wild mammals. J. W. Davis, L. H. Karstad and D. O. Trainer. Ames, Iowa, Iowa State University Press.

McCully, R. M., et al. (1971). "Herpes nodules in the lung of the African elephant [*Loxodonta africana* (Blumenbach, 1797)]." Onderstepoort J. Vet. Res **38**(4): 225-236.

Lymphoid nodules associated with Cowdry Type A intranuclear inclusions in epithelial and syncytial

cells were found in the lungs of 74% of 50 African elephants in the Kruger National Park. Subsequent studies proved these were caused by a herpes virus (Erasmus, McCully, Pienaar, Young, Pieterse & Els, 1971). The disease appears to be subclinical or latent. This virus, in common with other herpes viruses, might be more pathogenic in some other host. The pathogenesis of the lymphoid nodules and the various stages of their formation are given and the detailed characteristics are illustrated.

Erasmus, B. J., et al. (1971). The isolation of a herpes virus from the African elephant [*Loxodonta africana* (Blumenbach, 1797)].

Same case as McCully, 1969, 1971; Some references cite this article as J. Gen. Virol. "in press" 1971, however the article was apparently never actually published.

Basson, P. A., et al. (1971). "Some parasitic and other natural diseases of the African elephant in the Kruger National Park." Onderstepoort J. Vet. Res **38**(4): 239-254.

McCully, R. M., et al. (1969). "Herpes nodules in elephants." J. S. Afr. Vet. Med. Assoc **40**: 422.

Full text: The lungs of the majority of 50 elephants examined recently in the Kruger National Park had several lymphoid nodules varying in size from 3 to 30 mm (Fig. 1 arrow). The alveolar lining cells within these nodules were metaplastic and contained Type A intranuclear inclusions (Fig. 2). Electronmicroscopy revealed the presence of a virus in the epithelial cells. It was subsequently successfully isolated in tissue culture and characterized as a herpes virus.