

# INTERNATIONAL ELEPHANT FOUNDATION

## Elephant Endotheliotropic Herpesvirus

### Progress Report 3-2009

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*Elephant hemorrhagic disease caused by some species and strains of elephant endotheliotropic herpesviruses (EEHV) is a very serious problem worldwide for successful breeding of future generations of endangered Asian elephants. The lethal form of this rapid onset disease targets vascular endothelial cells and leads to hemorrhaging in all major organs. Active acute infection can be detected by a set of PCR DNA blood tests in whole blood and sometimes serum and in necropsy tissues. Over 50 cases have been confirmed in North America and Europe, with an over 80% fatality rate. The survivors lose all trace of the virus in the blood within a*

*few weeks and are not known to have transmitted their virus to any other animals.*

The National Elephant Herpesvirus Laboratory (NEHL) at the Smithsonian National Zoological Park (NZIP) in Washington is currently the only laboratory in North America solely committed to the research and diagnostics of EEHV. The NEHL funded in large part by the International Elephant Foundation and managed by Erin Latimer and Laura Richman carries out diagnostic PCR blood tests for suspected cases of EEHV worldwide. They are assisted by Jianchou Zong and Sarah Heaggans in Gary Hayward's Laboratory at the Johns Hopkins University in Baltimore, who carry out gene subtyping and genome analysis and are engaged in developing specific PCR assays for improved more sensitive detection of and research on all of the EEHVs. Samples from elephants exhibiting signs of illness, lethargy or are just a "little off", as there are differing/lack of obvious symptoms of EEHV, are provided to the NEHL to determine if EEHV is the cause of the symptoms and if so, the shedding characteristics of the viruses. Saliva, urine, trunk washes, and swabs of lesions and eyes are now commonly requested from sick elephants, in addition to the usual whole blood. Repeat testing of archival samples using the primers for the newly discovered viruses also occurs at the NEHL to determine if the new viruses were involved in the previous cases.

The NEHL has also developed a serum antibody detection assay for serological surveys to evaluate the extent of previous or latent infections by EEHV1. This assay for antibodies to the particular glycoprotein B variant encoded by EEHV1A is now used to provide epidemiologic data on virus transmission patterns within a herd by predicting previous exposure that could result in virus shedding and transmission to non-immune elephants. It is thought that when an elephant naive to EEHV is exposed to an elephant shedding EEHV it will either seroconvert with unapparent/mild illness or develop disseminated EEHV disease. An elephant that makes antibodies to EEHV1 has probably been exposed to the virus at some point during its life and

should be protected from getting the acute form of the disease with the same subtype of EEHV, but becomes a silent carrier.

Misleading statements from some non-experts would have the public believe that: (1) this is just a disease of zoos and captivity; (2) that certain zoos are contaminated hotspots for the virus and spread it others; and (3) that suspending captive breeding programs will somehow help to solve the problem. Also the public often assumes that because of the name “herpes” this is a sexually transmitted disease like the well-known human neurotrophic alphaherpesvirus HSV2. However, none of those above four statements are correct. It is exactly the mission of zoo’s and related conservation facilities to study and educate the public about wildlife, including the natural diseases that afflict endangered species such as Asian elephants, whose survival in the wild is seriously threatened because of the stresses related to ivory poaching, deforestation, habitat fragmentation, accidents, and increasingly frequent and violent human-elephant conflicts, all caused by encroaching human activities.

More than a dozen cases of lethal hemorrhagic disease have been identified in the past few years in three different countries in Asia, including in both captive and wild Asian elephant calves. This is exactly the same disease as seen in North America and Europe and is caused by one of the same species (EEHV1A). Secondly, genetic fingerprinting (or gene subtyping) shows that all of the last 23 confirmed cases in the past 15 years in North America have been caused by different strains or species of EEHV. Five different species of the virus (EEHV1A, EEHV1B, EEHV2, EEHV3 and EEHV4) have all caused lethal disease, but EEHV1A has been by far the most prevalent. There is no linear chain of transmission among the cases and in only one instance has the same identical strain (of EEHV1B) been found in two animals infected at the same time at the same facility. Therefore the disease is sporadic not epidemic or zoonotic.

There is nothing unexpected about elephants having herpesviruses. All mammals, including mice, cats, horses, dolphins and whales, as well as many other animals (fish, birds and reptiles) have their own versions of them. Even humans have eight different species of herpesviruses, although only one (HSV2) sometimes rates as an STD, but some of them occasionally cause cancer and lethal disease, especially in AIDS and organ transplant patients. In natural conditions in the wild, primary asymptomatic infections with endogenous herpesviruses should be nearly universal in early infancy. In general, these infections are transmitted in saliva or other body fluids at a very young age, and do not cause clinically significant disease. In captivity, these endogenous viruses tend to be lost so that natural immunity does not develop and when infections do occur they are much later than usual and often with the wrong species or subspecies of EEHV.



We now know of eleven different species of herpesviruses carried by elephants: there are also five different species of gamma herpesviruses (EGHV), that are frequently found in eye and genital secretions as well as in oral and genital mucosal lesions of healthy Asian and African elephants, but these do not cause any other symptoms or known disease. The only surprise is the severity of the disease caused by primary infection by EEHV1A and EEHV1B in particular, which have been responsible for 90% of the known cases of elephant hemorrhagic disease, predominantly in Asian elephant calves between one and eight years of age. A great deal more laboratory and field scientific research is required to understand why, and to learn how to control it effectively or to develop a vaccine.

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Since lethal hemorrhagic disease has been identified in wild Asian elephants, we know that EEHV is not just a disease of the captive Asian elephant in Western countries. EEHV has been identified in elephant populations in India, Thailand and Cambodia and through our work and support, researchers in range countries are participating in the annual EEHV workshops, and our associate in Kerala, Dr Arun Zachariah, has already begun to run EEHV assays. Dr Arun Zachariah's laboratory was provided equipment, reagents and training in June 2008 through the International Elephant Foundation, Erin Latimer and Laura Richman of the Smithsonian National Zoo, and Gary Hayward through the National Institute of Health, John Hopkins University. Dr. Zachariah has obtained post-mortem evidence for widespread hemorrhagic pathologic changes in up to ten cases including both captive (camp elephants) and wild Asian elephant calves (eight within the past year). Several of these elephants had morphological evidence for typical herpesvirus nuclear inclusion bodies in microvascular endothelial cells visible by microscopy of histological slides and preliminary PCR evidence has been obtained by the Kerala laboratory that eight of Dr. Zachariah's necropsy cases had high levels of EEHV1 DNA in their tissues. He has alerted local field veterinarians, mahouts, and other Wildlife Service personnel to make reports on any cases of sudden illness and death to help him obtain the necessary timely blood and necropsy samples to aid in the evaluation of the impact of EEHV in India.

Serologic assays have not yet been performed on free-ranging Asian elephants, nor on wild or captive African elephants. Similarly, we also do not yet know whether there is just a single strain of EEHV1A circulating in India (which could have been introduced via the African elephants at the zoo in Mysore for example) or whether there are multiple strains causing disease, which would instead imply either that EEHV1s were introduced to Asia when mixed elephant populations came into contact through warfare associated with the Greek and Persian Empires for example, or that they are in fact natural endogenous viruses in the area. To both evaluate the extent of latent infection in range countries and determine whether EEHV1A is an

endogenous virus of Asian or of African elephants in the wild, these EEHV1A serology assays must now be undertaken. The results will be critical to determine whether this is an introduced exotic epidemic disease that has crossed species barriers or one that has arisen because of disruption and fragmentation of habitat and the introduction of other unknown factors that contribute to enhanced severity of an ancient low grade infection.



**Dr. Laura Richman**



**Dr Gary Hayward**



**Dr. Gary Hayward and Erin Latimer**



**Dr. Arun Zachariah**

The NEHL and the IEF have been approached by several Asian colleagues requesting help in setting up EEHV labs in their home countries. Each has requested scientific and technical aid, as well as assistance in equipping the laboratories and as we have described, this work has already

begun in Southern India. The laboratory in Thailand is fully equipped but is in need of staff training. This lab when fully operational will be able to diagnose cases and determine seropositivity from the surrounding countries of Burma, Laos, Cambodia, and Malaysia as well as Thailand. The Sri Lankan laboratory will do the same for the Sri Lankan elephants. While there have been no reports of EEHV disease in Sri Lanka as yet, confirmation of a lack of EEHV disease would be equally important. It is hoped that results in range countries paired with ongoing information being gathered in the United States and Europe, will allow researchers to trace global movements of EEHV species and strains and determine the effect that EEHV disease may be having currently on Asian elephants in range countries and ultimately in the future prevent deaths by elucidating the source and transmission of the virus.

### 2009 – 2010 Project Goals:

- To assess the levels of EEHV associated hemorrhagic disease, identify the species and strains involved, and understand the source and pattern of transmission in free-ranging elephants in Asian countries as well as those managed in captive situations worldwide. For serology, the immediate top priority is to determine whether silent EEHV1 infections are prevalent within Asia. Therefore, an initial survey is planned of 24 freshly collected serum samples from the Kerala area (mostly from camp animals at this stage). A similar survey would be the goal in the first year for Thailand and later also in Sri Lanka. One possible outcome if this is an exotic disease introduced from outside Asia would be that no seropositive samples would be identified even in India. Another likely outcome would be that there are some seropositives in India but not at the other two sites. Alternatively, if EEHV1 is endogenous to or well-established in Asia, we will find a high proportion of seropositives, especially in free-range wild animals. If the latter, we would also have to be aware of the possibility of serological cross-reaction with a different but closely related latent endogenous EEHV species. An assessment as to whether to then extend the studies in the future to a similar sized second group focused on wild serum samples, will depend on the outcome of the first batch. Ultimately, the results will permit judgments about whether it will be valuable to maintain long-term surveillance for evidence of the virus if there are no seropositive animals, or alternatively what can be learned from future carefully designed statistically-based epidemiological studies about the patterns and spread of infection if there are seropositive elephants.
- We are aware of the difficulties (both physical and bureaucratic) involved in transporting Asian elephant serum samples to the US, so it is critical for the range countries to have the capability to do in-country serology research on EEHV, as well as being able to carry out at



least the initial stages of PCR DNA testing themselves. Therefore we will help establish EEHV diagnostic and research laboratories in Asian elephant range countries in addition to furthering the capacity of the recently established laboratory in India by forming collaborations with committed in-country investigators, and providing them with start-up reagents and technical training. We are already actively involved in this work and are committed to developing a network of concerned veterinarians and virologist researchers dedicated to the collecting and sharing of all information and findings that could lead to vaccines or other approaches to controlling this devastating disease in endangered elephant populations worldwide. Ultimately, the local labs will be able to study the potential impact of the disease on the wild elephant populations in Asia.

- Provide further training and assistance to Dr. Arun Zachariah and his collaborators in Kerala, India, for both diagnostic EEHV PCR and ELISA assays on necropsy samples, blood, and serum collected there. Also provide an opportunity for Dr. Zachariah and his technician to further refine their expertise and participate directly in all stages of the project and analysis by visiting our laboratories in the U.S.
- Make operational a regional EEHV PCR and ELISA diagnostic laboratory in Thailand where key local academic participants are already identified and equipment installed. We will provide testing protocols and start-up reagents for the EEHV ELISA and the EEHV PCR tests (will include labeled detection antibodies and positive controls for the ELISA) to the identified laboratory and provide on-site training and technical expertise in all of the tests, as needed.
- Confirm the EEHV1A diagnosis in India and carry out “gene subtyping” on all positive DNA samples from a set of 24 collected so far in Kerala to determine whether these cases are all caused by the same identical strain and so likely to represent the spread of a single recent exogenous introduction or instead represent a variety of different closely related strains that are endogenous to this part of India or some combination of the two. The samples include eight different organ samples from two orphans at Kodanad Elephant Orphanage, one or two organs each from another seven suspected hemorrhagic disease necropsies, blood from the orphans’ herdmates, plus several unrelated negative autopsy controls. This work will be carried out in our combined laboratories here at the Smithsonian National Zoo/Johns Hopkins or by arranging for us to use the core facilities of the Madras Veterinary School at Chennai in Tamilnadu. The extensive EEHV DNA sequencing analysis envisaged is not routine and far exceeds the level needed for diagnostic PCR DNA testing. It requires many dozens of specific PCR primers and second and third round PCR amplification procedures, including a variety of controls and duplicated samples to avoid errors and contamination artifacts. We have developed all of the necessary reagents at Johns Hopkins and already have a great deal of experience in this type of work for human herpesviruses as well as with all six EEHV species from captive U.S. elephants. We are also continually seeking ways to enhance the sensitivity and improve the comparative evaluation of the information gained. In addition, we have the resources to also evaluate a more sensitive three-round PCR level to determine whether there is any evidence for low level infection by any of the other EEHV species in the otherwise EEHV1-negative samples already collected in India.



**Kodanad Orphanage and Training Center**

### **Conclusion**

Unfortunately, for wild-life diseases, there is no organized equivalent of the National Institutes of Health, which funds scientific research by large teams of Virology and Immunology researchers studying human viral diseases and immune mechanisms. Instead, progress in understanding diseases and immunity in elephants depends largely on donations, a small number of other wild-life research organizations, and by the volunteered time of a dedicated group of expert professionals (Virologists, Veterinarians and Zoo Staff) who are committed to do all they can to protect and preserve future generations of Asian elephants.

**The International Elephant Foundation is proud to be a leader in this effort.**

