ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS RESEARCH

(Elephas maximus and Loxodonta africana)

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INTRODUCTION

Infectious disease is one of the factors threatening the long-term survival of Asian and African elephants. EEHV can be a fatal disease of elephants in human care and in the wild, and is one of the many conditions which can impact the overall health and survivability of elephants. Young elephants are most vulnerable to EEHV, making it a particularly devastating disease. Reproductive failures and early deaths of juvenile elephants in North America and Europe have been attributed to EEHV, and EEHV has been confirmed as the cause of death in up to ten wild elephants in India, Thailand and Cambodia including both orphaned and free-ranging calves [Reid et al 2006; Zachariah et al, IEF Conservation & Research Symposium conference abstracts Bangkok 2008, Pretoria 2010]. It is not known if there have been widespread outbreaks in Asia; however the impact of EEHV may now be exacerbated by increased fragmentation of elephant populations. Little is known regarding basic epidemiology of this virus, such as transmission patterns, incubation period, site, and cell tropism for viral latency.

EEHV is associated with a group of unique herpesviruses (8 species or sub-species - EEHV1A, EEHV1B, EEHV2, EEHV3, EEHV4, EEHV5, EEHV6 and EEHV7 - of which 5 have caused fatal disease [Ossent 1990, Richman 1999; Richman, 2000; Garner, 2009; Latimer 2010]. These herpesviruses affect primarily young elephants (<10 years of age) and can have a fatal outcome. The onset of the disease may be very rapid with few prodromal signs and peracute death within hours to 7 days. Clinical signs are often vague and can include lethargy, lameness, colic, anemia, thrombocytopenia, edematous swellings of the head and thoracic limbs, oral ulceration and cyanosis of the tongue. Necropsy findings are consistent with vasculitis and include extensive cardiac and serosal hemorrhages and edema, hydropericardium, cyanosis of the tongue and oral and intestinal ulcers. Histological features are microhemorrhages with very mild inflammation in the heart, liver and tongue accompanied by intranuclear inclusion bodies in the capillary endothelium. Transmission electron microscopy of the inclusion bodies shows 80-90 nm diameter viral capsids consistent with herpesvirus morphology.

As of August 2010, there have been 39 known clinical cases in North America since 1977 with 29 deaths (27 in Asian elephants). EEHV1A is the most common type (21 deaths in North America) and there are significant differences even among the 21 EEHV1As. There have been four deaths worldwide from EEHV1B, two deaths from EEHV2, and one death each from EEHV3, EEHV4 [Latimer, 2010]. Diagnosis of EEHV is made by detecting herpesvirus DNA in EDTA whole blood using polymerase chain reaction (PCR). Of 20 sick calves that were treated with famciclovir, eight survived. Ganciclovir has also been more recently used successfully.

Serological tests have been developed to detect antibodies to EEHV1A in Asian elephants. However, diagnostic tests are confounded by the inability as yet to cultivate any of these viruses *in vitro*. At present about 10% of the Asian elephants tested in the US have given consistently positive serological results; these animals are predominantly greater than 30 years old and were wild-born. Therefore, it is likely that many of the wild-born elephants in the North American population were carrying EEHV1 strains upon importation, suggesting that there may be asymptomatic carriers among North American elephants. The serological status of North American African elephants has yet to be investigated. A pilot trunk wash study has shown low

level viral DNA shedding in two asymptomatic adults with the same strain of EEHV1 that caused the death of a calf two years earlier at the same facility [Stanton, 2010].

Herpesviruses have been evolving within most mammalian host species for over 300 million years, where they usually establish a stable host-parasite relationship that only rarely leads to serious or fatal disease. Many animals, including humans, carry several species of herpesviruses throughout their lives and never become clinically ill. Once inside a host animal, herpesviruses establish a latent (or hidden) phase after causing mild symptoms or asymptomatic infection. The virus then persists in the body, undetected by diagnostic tests or the body's immune system. For transmission to a new host, all herpesviruses need to have a mechanism by which they occasionally reactivate and shed infectious particles from localized skin lesions or in saliva or other body fluids. Different herpesvirus families establish latency in different cell types or organs and have different mechanisms for reactivation. For reasons not completely understood, some primary or reactivated herpesvirus infections lead to massive viremia, where virus particles circulate through the bloodstream, infect multiple organs and cause serious or lethal systemic disease.

Under normal conditions, primary asymptomatic infections with endogenous herpesviruses should be nearly universal in early infancy in the natural well-adapted host species. While serious disease is not normal in the natural host species for most herpesviruses, serious disease can occur if the host species is immunosuppressed, fighting other concurrent infections, or in rare situations when a virus comes into contact with and is able to infect an animal that is not the normal host species. Healthy adult African elephants carry EEHV2, EEHV3 and EEHV6 in lymphoid lung nodules, where it can be detected because of localized reactivation in epithelial cells. A few African elephant calves have also been reported to have EEHV1 in skin nodules. Although studies have not been performed to verify this hypothesis, it is likely that many healthy wild-born Asian elephants are asymptomatically infected as well. There is no treatment for latent herpesviruses in animals or humans: however anti-viral drugs can suppress viral replication and cell damage when virus is circulating. It is believed that early detection of EEHV and immediate intervention with supportive care are critical to the success of treating an elephant affected by EEHV. Antiviral medications may also play an important role in treatment. Timely intervention with the human anti-viral drug famciclovir is credited with contributing to the survival of eight Asian elephant calves with confirmed EEHV disease. No animals are known to have survived systemic EEHV disease without treatment; however, treatment does not guarantee recovery.

EEHV infections in elephant populations in human care may be a potential useful predictor for EEHV's impact on the increasing small, isolated wild elephant populations in Asia. Plans to develop additional trunk-wash and serological assays specific for each of the other seven EEHV species based on the limited DNA sequence available have been initiated.

EEHV SUMMARY POINTS

- EEHV infection can be a fatal disease of African and Asian elephants and has been found in captive and wild Asian elephants.
- EEHV affects mainly young elephants (<10 years of age, peak between 1 and 3 years).
- Clinical signs are often vague and may include lethargy, lameness, colic, anemia, thrombocytopenia, edematous swellings of the head and thoracic limbs, oral ulceration and cyanosis of the tongue. Signs may progress to death within hours or days.
- Necropsy findings may include extensive cardiac and serosal hemorrhages and edema, hydropericardium, cyanosis of the tongue, oral and intestinal ulcers, and lymphoid nodules (3-30 mm) in lungs, skin and vestibule.
- Histological features are microhemorrhages in the heart, liver and tongue accompanied by intranuclear inclusion bodies in the capillary endothelium.
- 39 known clinical cases in North America since 1977 with 29 deaths (27 in Asian elephants). EEHV1A is the most common type (21 deaths in North America) and there are significant differences even among the 21 EEHV1As. There have been four deaths worldwide from EEHV1B, two deaths from EEHV2, and one death each from EEHV3, EEHV4 [Latimer, 2010].
- Diagnosis and status of EEHV in clinical cases is made by detecting herpesvirus DNA in EDTA whole blood and sometimes serum, using polymerase chain reaction (PCR).
- It is believed that early detection of EEHV and immediate intervention with supportive care and antiviral therapy are critical to the success of treating an elephant affected by EEHV.
- Famciclovir and ganciclovir have been used for successful treatment in elephants.
- Recent evidence shows that there are asymptomatic carriers among North American Asian elephants.
- Serological tests have been developed to detect antibodies to EEHV1A in Asian elephants. At present about 10% of the Asian elephants tested in the US have given consistently positive serological results; these animals are predominantly greater than 30 years old and were wild-born. The serological status of North American African elephants has yet to be investigated.
- Studies suggest that it is likely that many wild-born elephants in the North American population were carrying EEHV1 strains upon importation
- There is no evidence of shedding of virus in semen or transmission of EEHV through breeding, natural or artificial insemination, or through transport.

ALL ELEPHANT HOLDING FACILITIES MUST HELP FIND ANSWERS TO EEHV

The knowledge we have gained and will continue to gain from the elephants held in North America is highly significant for the protection of elephant populations worldwide. There is still much that needs to be done to enable us to be able to prevent and treat this deadly disease in elephants.

In particular, we need each facility to:

1) **Review all protocols with keepers and vets annually;**
2) **Familiarize keepers and vets with EEHV, its symptoms, and research sample needs from healthy, sick and recently deceased elephants;**
3) **Provide samples from each of your living elephants for ongoing research projects;**
4) **Contact research groups at the first sign of any elephant injury or illness, regardless of how insignificant it might appear or whether or not a diagnosis of another issue has been made;**
5) **Refer back to the protocols to determine samples needed from elephants under veterinary care;**
6) **Contact research groups if an elephant is to be euthanized;**
7) **Contact research groups immediately upon all elephant deaths;**
8) **Develop an institutional EEHV diagnostic and therapeutic plan, especially for breeding facilities or those with young animals;**

FREQUENTLY ASKED QUESTIONS ABOUT EEHV

In the wild, elephants face extreme pressure from human-elephant conflict, habitat loss and poaching. In North America, elephants are important conservation ambassadors for their species and ecosystems. Seeing, hearing, and even smelling these magnificent animals up close is critical to helping visitors make an emotional connection to the natural world of elephants and take action to help protect their future. We need elephants in human care if we are to save them.

There are many questions about this complex group of viruses. We hope these questions and answers help you better understand as well as explain to others these viruses and the diseases they can cause.

What do we know about elephant herpesviruses?

To date, scientists have identified 14 genetically different elephant herpesvirus types, five of which are known to cause hemorrhagic disease. The viruses found in symptomatic elephants at different zoos and other institutions are genetically distinct, which means that they are not all the same strain spread by the transfers of elephants between and among zoos.

Herpesviruses are widespread in all mammal species, including humans. While species-specific, they share common features. Once inside a host, the virus can go into a latent (hidden) phase after causing only mild symptoms or no signs of disease at all. Scientists do not yet know where in the body EEHV resides in the latent phase.

For reasons unknown, primary or reactivated latent elephant herpesvirus infections can sometimes circulate uncontrolled throughout the bloodstream, causing disease. This is the only time when a herpesvirus can be readily detected in blood samples. As yet, reliable tests are not available to detect a latent (hidden) infection. Most elephants are able to fight the virus and survive when it comes out of latency. Calves appear to be most susceptible to EEHV disease after they have been weaned, at a time when they are not protected by their mother's antibodies.

Does EEHV affect elephants only in zoos?

We know that EEHV is not just a disease of the captive Asian elephant in western countries. According to an International Elephant Foundation progress report of spring 2009 by EEHV experts, more than a dozen cases of EEHV have been identified in elephant populations in India, Thailand and Cambodia – including several wild as well as orphaned Asian elephant calves that have died within the past few years. Moreover, these deaths only represent the cases in which necropsies were conducted in sufficient time to detect it.

Current research indicates that the elephant-specific herpesvirus may have been in elephant populations for tens of millions of years, just as human herpesviruses have been in human populations. Since this is a naturally occurring disease, every elephant – in the wild and in human care – probably carries one or more forms of elephant herpesvirus within them.

If elephants in both zoo and wild populations probably have one or more herpesvirus, why do some get ill and others don't?

Many animals and humans carry herpesviruses throughout their lives and never become ill. What researchers don't know is what triggers the virus to become active and where exactly in the body the virus

hides in its latent phase. We don't know why some animals become ill and others don't. It's important to understand that it's not about *who has* the virus, but *who gets ill* and *when*.

Can elephants transmit EEHV to other elephants?

There is not enough research to confirm how EEHV itself is transmitted, but that is normally how viruses spread. Viral shedding occurs when it comes out of latency and most human herpesviruses are transmitted predominantly in saliva. Until recently EEHV could only be detected when active through a blood test, but new studies now suggest that some healthy Asian elephants periodically shed low levels of EEHV1 in secretions from the trunk (which may or may not be infectious). What we do know is that many elephants – in the wild and in human care – probably carry one or more forms of latent herpesvirus within them.

Can the elephant herpesvirus be transmitted through semen?

- There is no evidence of shedding of virus into semen or transmission of EEHV through natural breeding or artificial insemination.
- There is no evidence to suggest that EEHV is being transmitted between elephants through transport and breeding activities. At present no two facilities have been found to have disease caused by the same strain of EEHV1, they are all different.

Is a facility contaminated once an outbreak of EEHV has occurred?

Like all mammals and humans, elephants carry a variety of different herpesviruses throughout their lives. Some cause mild disease and some cause severe disease or death. This is how herpesviruses operate. Claims that certain zoos are contaminated once an animal becomes ill from EEHV are unfounded and based on a lack of understanding of how the viruses live within their hosts. Having a herpesvirus is the norm, not the exception. Like all viruses, herpesviruses cannot live very long outside the body, so a herpes outbreak does not "contaminate" a facility.

Is there a cure for EEHV?

There is no cure for herpesviruses in animals ***or*** in humans. Based on what we are learning from our ongoing research and from elephant care institutions that have experienced an EEHV outbreak, the treatment protocols continue to improve, and detection and treatment recommendations continue to evolve.

Current treatments suppress EEHV and elephants can potentially recover if treatment starts early. Of the elephants that have been treated, the success rate with anti-viral therapy against EEHV has been about 40 percent. Veterinarians and scientists continue to collaborate to better understand this disease and develop more effective treatment options. To date, anti-viral drugs have been used successfully in treating eight Asian elephants in North America.

Shouldn't zoos discontinue breeding elephants if calves are at risk for EEHV?

Stopping breeding in zoos will severely impede the progress that is being made in studying EEHV and finding a cure. Running away from captive breeding is not the way to solve the disease. When an outbreak of equine herpes occurred in 2005 in horses, the industry did not shut down. Instead it funded research that resulted in treatment, prevention and control of that disease. When black-footed ferrets were nearly driven to extinction in the 1980s from canine distemper, we did not stop breeding them. U.S. Fish and Wildlife Service, AZA institutions, private landowners, conservation organizations, and other groups

collaborated on a rescue and recovery program. An effective vaccine was developed and the species bounced back from the brink of extinction.

But why take the risk of exposing another calf to EEHV?

While we have no guarantees as to the fate of a future elephant calf, we have operated for many years under the conservative assumption that all elephants could have one or more latent (hidden) herpesviruses. The risk is no higher or lower for an elephant born in the wild or at a zoo or sanctuary. We will continue to gather the evolving research and use the latest information to guide our decisions in caring for elephants.

Is further research being done to learn more about EEHV?

Multiple research teams worldwide are dedicated to investigating this set of diseases, to understanding how to protect elephants in human care and in the wild, and to solving the mystery of how EEHV is spread and developing an effective vaccine for the virus.

The collaborative work to better understand EEHV may have important implications for wild elephants in the future. Wildlife biologists may one day need to draw upon the growing body of work and knowledge generated by the international elephant community to contribute to the long-term survival of the species both wild populations and those in human care.