ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS (EEHV) RESEARCH AND TISSUE REQUEST PROTOCOL

(Elephas maximus and Loxodonta africana)

Updated June 2015
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>3</td>
</tr>
<tr>
<td>Elephant Endotheliotropic Herpesvirus Alert</td>
<td>3</td>
</tr>
<tr>
<td>EEHV Summary Points</td>
<td>5</td>
</tr>
<tr>
<td>References</td>
<td>7</td>
</tr>
<tr>
<td>All Facilities Must Help Find Answers to EEHV</td>
<td>8</td>
</tr>
<tr>
<td>Checklist for EEHV Samples</td>
<td>9</td>
</tr>
<tr>
<td>EEHV Necropsy Protocol</td>
<td>11</td>
</tr>
<tr>
<td>Research Requests and Contact Information</td>
<td>12</td>
</tr>
<tr>
<td>Logistics and Necropsy Tips</td>
<td>15</td>
</tr>
<tr>
<td>Elephant Necropsy Protocol Gross Examination Worksheet</td>
<td>17</td>
</tr>
<tr>
<td>Tissue Checklist</td>
<td>20</td>
</tr>
<tr>
<td>Consent Form for Use of Samples by AZA Elephant TAG/SSP</td>
<td>21</td>
</tr>
<tr>
<td>Frequently Asked Questions about EEHV</td>
<td>22</td>
</tr>
</tbody>
</table>
INTRODUCTION

This protocol is a collaborative effort of the Association of Zoos & Aquariums (AZA) Elephant Taxon Advisory Group/Species Survival Plan (TAG/SSP), the International Elephant Foundation (IEF), and the EEHV Advisory Group. Its purpose is to provide a format for the systematic collection of information and samples that will add to our knowledge of Elephant Endotheliotropic Herpesvirus (EEHV) and contribute to the diagnosis and treatment of EEHV Hemorrhagic Disease (EEHV HD). All North American institutions caring for elephants will receive a copy of this protocol. More information on the viruses can be found at eehvinfo.org. The password for the “Member Area” can be gotten by emailing EEHVinfo@si.edu. Please include in the email your affiliation and connection to elephants (researcher, elephant manager, veterinarian, etc.).

ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS ALERT

Infectious disease is one of the factors threatening the long-term survival of Asian and African elephants. EEHV Hemorrhagic Disease (EEHV HD) 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. It is thought that the EEHVs are endogenous viruses of both African and Asian elephants, with different subtypes being found in the two elephant species and different viral subtypes causing acute illness and/or fatal disease in the two elephant species. Young elephants are most vulnerable to EEHV HD, making it a particularly devastating disease. Reproductive failures and early deaths of juvenile elephants in North America and Europe have been attributed to EEHV HD, and EEHV HD has been confirmed as the cause of death in up to 20 wild elephants in Cambodia, India, Thailand, Sumatra, and Myanmar, including both orphaned and free-ranging calves [Reid et al 2006; Zachariah et al, IEF Conservation &
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 all but EEHV7 have caused fatal disease [Ossent 1990, Richman 1999; Richman, 2000; Garner, 2009; Latimer 2011; Denk, 2012]. These herpesviruses affect primarily young elephants (<8 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 systemic microhemorrhages accompanied by intranuclear inclusion bodies in the capillary endothelium, sometimes with very mild inflammation in the heart, liver, kidney and tongue, and in some cases vascular fibrinoid necrosis. Transmission electron microscopy of the inclusion bodies shows 80-90 nm diameter viral capsids consistent with herpesvirus morphology.

There have been more than 40 known clinical cases in North America since 1977 with over 30 deaths (the majority in Asian elephants). EEHV1A is the most common type and there are significant genetic differences even among the over 20 EEHV1As identified. There have also been deaths worldwide from EEHV1B, EEHV2, EEHV4, EEHV3 and EEHV5 [Latimer, 2010; Denk, 2012]. 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 prior to 2010, eight survived. Since 2010, there have been no deaths from EEHV in North America; there have been several cases of clinical illness from EEHV HD disease which responded to early detection, supportive therapy, and famciclovir or ganciclovir treatment.

Serological tests are being developed to detect antibodies to EEHV in Asian and African elephants. However, diagnostic tests are confounded by the inability as yet to cultivate any of these viruses in vitro. At present 10% - 30% 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. It is likely that many of the wild-born elephants in the North American population were carrying EEHV1 strains upon importation. The serological status of North American African elephants has yet to be investigated. Based on multiple analyses of EEHV shedding in trunk washes, it is believed that many if not most captive and wild elephants are latently infected by one or more of the EEHVs (Stanton, 2010; Hardman, 2012; Stanton, 2014).

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 signs or subclinical 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 subclinical 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, has concurrent infections with other agents, 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, EEHV6, and EEHV7 in lymphoid lung nodules, where it can be detected because of localized reactivation in epithelial cells. Although studies have not been performed to verify this hypothesis, it is possible that many healthy wild-born Asian elephants are subclinically infected as well. There is no treatment for latent herpesviruses in any species; however, anti-viral drugs can suppress viral replication and cell damage during periods of viremia and productive infection. 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 HD. 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 HD disease. No animals are known to have survived systemic EEHV HD disease without treatment; however, treatment does not guarantee recovery.

EEHV infections in elephant populations in human care may be a potentially useful predictor for EEHV’s impact on the increasingly smaller, isolated wild elephant populations in Asia. Plans have been initiated to develop molecular and serological assays specific for each of the other seven EEHV species based on the available DNA sequences.

**EEHV SUMMARY POINTS**

- EEHV HD 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 (<8 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 systemic microhemorrhages accompanied by intranuclear inclusion bodies in the capillary endothelium, sometimes accompanied by mild neutrophilic inflammation or vascular necrosis.
• More than 40 known clinical cases in North America since 1977 with over 30 deaths (the majority in Asian elephants). EEHV1A is the most common type and there are significant genetic differences even among the over 20 EEHV1As identified. There have also been deaths worldwide from EEHV1B, EEHV2, EEHV3, EEHV4 and EEHV5 [Latimer, 2010; Denk, 2012].
• 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 HD.
• Famiclovir and ganciclovir have been used for successful treatment in elephants, although their efficacy is unknown. The success of the treatment also may have been due to concurrent supportive care.
• Recent evidence shows that there are subclinically infected carriers among North American Asian elephants.
• Serological tests are being developed to detect antibodies to EEHV in Asian elephants. At present 10-30% 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 EEHV strains upon importation.
• There is no evidence that virus is shed in semen, or that transmission of EEHV occurs during breeding, natural or artificial insemination, or during transport. Therefore, the AZA Elephant TAG/SSP recommends that institutions continue to exchange elephants and elephant semen as specified in the breeding recommendations.
References


ALL 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 very important 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 this protocol with keepers and vets annually;
2) Familiarize keepers and vets with EEHV, its signs, and research needs for 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;
5) Refer back to this protocol for standard and ancillary procedures and sample collection;
6) Contact research groups if an elephant is to be euthanized;
7) Contact research groups immediately upon all elephant deaths;
8) Identify a necropsy team for elephants that are morbid, dead or planned euthanasias, and provide the team with a copy of this protocol;
9) Develop an institutional EEHV diagnostic and therapeutic plan, especially for breeding facilities or those with young animals; examples can be found at eehvinfo.org on the Elephant Management and Training page.
10) Keep Elephant TAG/SSP chair and advisors informed of cases/suspects, deaths and planned euthanasias; and
11) Contact the Elephant TAG/SSP chair and advisors if there are any questions or if more information is needed.
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<thead>
<tr>
<th>Event</th>
<th>National Elephant Herpes Lab</th>
<th>Johns Hopkins University</th>
<th>Baylor College of Medicine</th>
<th>Fox Chase Cancer Center</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Birth of Asian calf</strong></td>
<td>Placenta, EDTA WB and serum from mother and calf (one-time collection in the first month, if possible) *</td>
<td>Umbilical cord and blood</td>
<td>1-2 ml serum</td>
<td>Umbilical cord and amniotic sac</td>
</tr>
<tr>
<td><strong>Birth of African calf</strong></td>
<td>Placenta, EDTA WB and serum from mother and calf (one-time collection in the first month, if possible) *</td>
<td>Umbilical cord and blood</td>
<td></td>
<td>Umbilical cord and amniotic sac</td>
</tr>
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<td><strong>Unconfirmed illness</strong> **</td>
<td>**</td>
<td>For diagnosis: EDTA WB, serum, plus swabs/biopsies if lesions present.</td>
<td></td>
<td>Fresh saliva swab and fresh whole blood</td>
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<td><strong>Confirmed illness prior to treatment</strong></td>
<td>EDTA WB, serum to monitor viral load</td>
<td>2-4 ml EDTA WB and 20-100 ml serum, to be shipped unfrozen for viral genome sequencing and virus cell culture.</td>
<td>2-4 ml EDTA WB and 10-100 ml serum, frozen and sent on dry ice to Baylor College of Med and Houston Zoo, for viral sequencing</td>
<td>Fresh saliva swab and fresh whole blood</td>
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<tr>
<td><strong>Confirmed illness after treatment</strong></td>
<td>EDTA WB, serum, from case to monitor viral load</td>
<td>1-2 ml EDTA WB and 1-2 ml serum</td>
<td>1-2 ml EDTA WB and 1-2 ml serum</td>
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<td><strong>Necropsy tissue from EEEHV death in Asian or African elephant</strong></td>
<td>Tongue, heart, liver, spleen, intestine, WB, serum, any tissue with a lot of hemorrhages. Any kind of nodules – skin, vestibular, lung etc. Hilar, mandibular, thoracic and mesenteric lymph</td>
<td>EDTA WB, heart, liver, lung, spleen, tongue tissue samples unfrozen on ice for virus cell culture.</td>
<td>EDTA WB, heart, liver, lung, spleen, tongue tissue samples unfrozen on ice for virus cell culture.</td>
<td>Heart, spleen, , lymph node, , trigeminal ganglion, whole brain</td>
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<tr>
<td><strong>Necropsy tissue from non-EEHV death in Asian or African elephant</strong></td>
<td>Tongue, heart, liver, spleen, WB, serum. Any kind of nodules – skin, vestibular etc. Hilar, mandibular, thoracic and mesenteric lymph nodes.</td>
<td>Latent EEEHV detection: Look hard for hidden palpable “herpes” lung lymphoid nodules (likely few, white to gray, smooth or spongy texture, small 2-10 mm in diameter-will likely need to bread loaf the lung to locate). If multiple nodules please keep separate. Also if no nodules, two different random tissue samples from lung, spleen and lymph nodes only - prefer fresh rather than frozen for maximum diagnostic sensitivity.</td>
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KEY

* Please use your routine husbandry/veterinary procedures when samples are collected. Please wear appropriate vinyl/nitrile/latex gloves when collecting. We request that elephants not be restrained by physical or chemical restraint to collect research samples (behavioral restraint is OK). Please limit your venipuncture attempts to three attempts per weekly session.

** anytime elephant exhibits any abnormal behavior, something as minor as not napping as long as normal to lameness, stiffness, lethargy, inappetence and the more common symptoms of EEHV
EEHV NECROPSY PROTOCOL

We hope that institutions will not have to face the immense task of performing an elephant necropsy, but if this occurs, it should be viewed as an important learning opportunity. **Collection and review of the requested data and samples is our best means of defeating EEHV.** With the increased availability of digital cameras, it is strongly recommended that photographs of both normal and pathologic structures be recorded for future reference.

Specific information about EEHV sample and data collection during an elephant necropsy is included in this protocol. Please send the completed forms to Dr. Michele Miller (contact information below).

Broader necropsy information and requests for samples for other research projects, in addition to EEHV, are contained in a separate document, Elephant Necropsy Protocol, available online at [www.elephanttag.org/Professional/ElephNecropsy_2010.pdf](http://www.elephanttag.org/Professional/ElephNecropsy_2010.pdf). All elephant vets, pathologists and caretakers should be acquainted with the protocols in both documents (Elephant Necropsy Protocol and Elephant Endotheliotropic Herpesvirus (EEHV) Research And Tissue Request Protocol) and should have the necessary equipment ready to facilitate sample collection. A team should be designated in advance for data and sample collection to save valuable time. A list of researchers interested in participating in elephant necropsies is included in the Elephant Necropsy Protocol.

Post-mortem examination of an elephant can be a daunting task, but with proper personnel, planning, and experience, it can be done safely and efficiently. If at all possible, institutions should make preparations or contingency plans for the movement, necropsy, and disposal of an elephant ahead of time to avoid the stress of planning following the death of the animal. The information gained from an elephant necropsy is potentially hugely valuable to institutions, the AZA, and to elephants both in human care and in the wild.

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RESEARCH REQUESTS AND CONTACT INFORMATION

1. National Elephant Herpes Lab

Erin Latimer  
Smithsonian’s National Zoo  
Department of Pathology  
3001 Connecticut Ave. NW  
Washington, D.C.  20008  
Work:  202-633-4252  
Cell:  703-855-9611  
Email:  latimere@si.edu

1. Serum – 2 mls; transfer to plastic screw-top tube and store at -80C or non-defrosting freezer until shipped. Ship samples overnight with ice packs or dry ice. 2. Whole blood – 1-2 mls in EDTA tube, then transfer to plastic screw-cap for storage at -80C freezer until shipped (for EEHV detection). 3. Placenta – freeze 1 inch³ piece in liquid nitrogen or dry ice, then store at -80C freezer until shipped. Also, serum and whole blood from dam and baby. 4. Suspected herpetic lesions – wet a cotton swab with small amount of sterile saline, swab lesion and place in sterile 15 ml plastic test tube; store at -80C until shipped. 5. Necropsy tissues (heart, liver, tongue, spleen, intestine, any tissue with hemorrhages, any kind of nodules – skin, lung, vestibular) – aseptically place 3x3 cm piece of tissue in small Ziploc or WhirlPak bag. Label with type of tissue, elephant ID, date. Use separate bag for each tissue; store at -80C until shipped. Shipping – FedEx overnight; email tracking number to latimere@si.edu. EEHV Lab will pay for shipping of samples after a birth; contact lab for account information.

2. John Hopkins School of Medicine

Gary Hayward  
Johns Hopkins School of Medicine, Viral Oncology Program  
3M09, Bunting-Blaustein Cancer Research Building,  
1650 Orleans St,  
Baltimore, MD 21287 (Fedex)  
PH 410-955-8684 Fax 410-955-8685 (work)  
PH 410-821-8197 (Home/weekend)  
Email: ghayward@jhmi.edu  
Contact lab for FedEx information

1. Necropsy of All Asymptomatic Wild-Born Adult Asian and African Elephants: Lung Nodules and EEHV Latency:  
(a) Multiple small pieces of as fresh as possible unfrozen lung tissue from breadloaved bronchiolar area. Transport on ice in 40ml sterile plastic tubes. Please also collect multiple "palpable" lung nodules if present. Different nodules from the same animal have proven to contain different EEHV species. Small nodules are white and ovoid with smooth surfaces and 2-3mm in size embedded within the parenchyma. Larger ones may be gray/white and spongy. We have also been able to detect virus at low levels even in nearby non-nodular bronchiolar tissue--perhaps because there are "micro-nodules". Fresh tissue sent on ice packs is preferred. But if tissues have been frozen before transport, do not thaw. Send frozen tissue with ice packs in an insulated Styrofoam container.  
(b) Fresh lung necropsy tissue (several small pieces) from upper airway for epithelial cell culture. Transport unfrozen with ice packs in 40 ml plastic tubes containing PBS plus antibiotics.

2. Fresh Unfrozen Blood and Serum from All Live Confirmed EEHV Viremia Positive Animals: Virus Culture and EEHV Whole Genome DNA Sequencing.  
(a) Blood and Serum: Fresh heart blood (2ml to 4ml) and serum (20ml to 200ml in butterfly catheter: ie as much serum as possible please) transported on ice/refrigerated packs. In late stage untreated viremic cases and non-responders a great deal of virus is released as cell-free virions into the serum. This has not been the case in early stage acute disease or in drug-treated survivors. Cell-free virus is needed for attempts at deep sequencing of intact...
EEHV genomes. Unfrozen blood samples will be used for PBMC fractionation and virus cell culture attempts in primary elephant vascular endothelial cells. **Preferably please collect and ship a first set of samples obtained before or at the same time that FCV/GCV treatment is initiated.** A second sample at 24 or 48 hours (and subsequent ones if desired) after treatment will allow us to evaluate the effectiveness of the medication in terms of increase or decrease of viral load.

### 3. Necropsy of EEHV-Positive Hemorrhagic Disease Case. Virus Culture and EEHV Whole Genome DNA Sequencing.

(a) Blood and Serum (as above). **As much serum as possible please----may be frozen in this case or on ice.**

(b) Fresh unfrozen tissue containing foci of hemorrhage (eg heart, lung, spleen, liver, kidney or tongue) for further attempts at virus cell culture (culture has been so far unsuccessful). Ship directly on ice in 40 ml sterile plastic tubes or with small volume of transport medium (PBS plus Pen/Str/Fungizone or similar)

### 4. Birth of Asian or African Elephant Calf. For Primary Endothelial Cell, Epithelial Cell and Lymphocyte Cell Cultures.

[All samples for culture are shared with Virginia Pearson, Visiting Scientist at Fox Chase Cancer Center](a) Fresh unfrozen umbilical cord (several 8 to 12 in. segments) in 1x or 2x 500ml wide-mouth bottles provided with sterile PBS plus antibiotics (wash thoroughly with contents of second bottle). Transport on ice/refrigerator packs ASAP with return collect FedEx pre-package that will be provided.

(b) Fresh unfrozen whole cord blood (4-10 ml if possible) in EDTA tubes for PBMC fractionation. Transport on ice/refrigeration packs provided.

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### 3. Baylor University

Paul Ling, PhD  
Department of Molecular Virology & Microbiology  
Baylor College of Medicine  
Mail Stop BCM-385  
One Baylor Plaza  
Houston, TX  77030  
Work: (713) 253-9282 (Jeff Stanton)  
Email: Jstanton@bcm.edu; pling@bcm.edu

**Trunk Wash Collection Protocol for EEHV Screens:** Generally follow standard trunk wash collection protocol for *M. tuberculosis* culture: Instill 50mL sterile saline solution into the nares. Elevate the trunk 2-3ft for 30-60 seconds. Have elephant blow into a clean container – use new ziplock/plastic bags for each sample to reduce the chance of cross contamination. Transfer sample to a 50mL conical tube – the more mucous and exfoliated respiratory epithelial cells the better (SNOT IS GOOD). Chill the sample on ice until it can be centrifuged. Centrifuge at 1500Xg for 10min @ 4°C or room temperature. Gently poor off and discard the supernatant. Ideally the cell pellet is stored @ -80°C and shipped on dry ice, however it is acceptable to store cell pellets @ -20°C and ship on ice.

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### 4. Fox Chase Cancer Center

Virginia R. Pearson, Visiting Scientist  
Rall Laboratory  
Fox Chase Cancer Center  
333 Cottman Ave,  
Philadelphia, PA 19111  
virginiarpearson@gmail.com
1) From healthy **African and Asian elephants** primary cell culture: Birth of Asian or African Elephant Calf. For Primary Endothelial Cell, Epithelial Cell and Lymphocyte Cell Cultures.

(a) **Fresh unfrozen umbilical cord** (several 8 to 12 in. segments) in 1x or 2x 500ml wide-mouth bottles provided with sterile PBS plus antibiotics (wash outside thoroughly with contents of second bottle). Transport on ice/refrigerator packs ASAP with return collect Fedex pre-package that will be provided.

(b) **Fresh unfrozen whole cord blood** (4-10 ml if possible) in EDTA tubes for PBMC fractionation. Transport on ice/refrigeration packs.

(c) **Amniotic sac unfrozen** (fetal, still or live birth) 3x 3sq" pieces and 6" section of umbilical cord connecting to placenta - include blood vessels lying over and 1” sq piece of placenta

d) Saliva from newborn and mother, and remainder of herd

2) The following necropsy tissues for epithelial cell culture and virus latency investigation. All FRESH NOT FROZEN tissues must be harvested as soon as possible after death and shipped within 24 hours. From sudden death or suspected cases of EEHV for viral latency and transmission study:

1. **Aorta** - 2 x 6-inch pieces to fit 50ml tube, include section where aorta connects to heart

2. **Heart** - 4 x 1sq" pieces from outside wall and inside cavity

3. **Salivary gland** - intact

4. **Trigeminal ganglion** - 6” section toward top of trunk if possible.

5. **Lymph gland** - whole

6. **whole brain**

NOTIFY before shipment and as early as possible about pending euthanasia, expected or sudden deaths to receive shipping reagents. WASH all tissues gently with sterile PBS unless otherwise noted, DO NOT WASH inside of blood vessels; SUBMERGE all tissues in fresh sterile PBS containing Pen/Strep/Fungizone; USE 50ml tubes; KEEP on wet ice until packed for shipping. SHIP on wet ice (DO NOT FREEZE TISSUES) overnight for next morning delivery to:

Virginia Pearson
701 West Gravers Lane,
Philadelphia, PA 19118
home 215-247-1287
LOGISTICS AND NECROPSY TIPS

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

The necropsy of an elephant should proceed in the same manner as the necropsy of any smaller mammalian species. Although the size and scope of an elephant necropsy may seem intimidating, the procedure can be accomplished in 8-10 hours (sometimes less) by a team of dedicated prosectors and assistants. The necropsy should be performed with the elephant in left lateral recumbency. An external examination is performed to evaluate body condition and lesions. The oral cavity should be closely examined for evidence of lesions suggestive of endotheliotropic herpesvirus infection.

Assigning specific tasks to team members will help the necropsy proceed in an orderly manner. For example, a team may be assigned to each of these areas: head, forelegs, hind legs, abdominal region. One person should oversee the collection, labeling, and processing of research materials and any communication concerning research requests. It may be helpful to designate a media spokesperson. One of the most important tasks to be assigned is the task of knife sharpener. One person with knife sharpening experience should be assigned to be continually sharpening knives and cycling sharpened knives to prosectors, and it is best to have approximately 20 suitable necropsy knives available for the procedure. The knives should be new or relatively new, not serrated, and straight shafted with a sharp point, rather than having a curved shaft. The shaft of the knife should be at least 5-6 inches long (no pocket knives). One person should be designated as the photographer, preferably not a prosector. One station should be set up and staffed by one person dedicated to tissue collection from organs removed from the carcass. All containers for histology, archives and special studies should be labeled in advance and within reach of the station prosector. A first aid kit should be available in the event a prosector is cut during the necropsy.

Whole blood and serum samples from sick or dead elephants should be obtained for diagnostic testing in any suspected case of herpesvirus infection.

Small numbers of white to gray nodules with a spongy texture (3-30 mm in cross sectional diameter) in lungs have been found in a high fraction of African elephants culled in the wild and the few that have been examined so far proved to contain high levels of EEHV2, EEHV3 and/or EEHV6 (subclinical or latent infection). These lung nodules have also been reported in Asian elephants and a thorough search for lung nodules by slicing through the lung at regular intervals (“breadloafing”) with palpation at necropsy should facilitate collection of such nodules in both Asian and African elephants. The nodules may be very small and rare within the lung, or could be obvious and more numerous and are now expected to be found in most otherwise healthy elephants. In the absence of obviously visible nodules we are also requesting random lower bronchiolar tissue samples.

Similarly, raised skin nodules with darker fibrous centers have been found occasionally in otherwise healthy juvenile African elephants and in one outbreak of skin lesions in Florida. A
third type of lesion has been associated with EEHV1: variably sized, red ulcers or vesicles in the distal vestibulum of the genital tract of female African elephants. More samples of all of these types of lesions (lung and skin nodules, vestibular ulcers/vesicles) are required from both captive and wild Asian and African elephants to evaluate the natural history of the EEHVs. Please search carefully for and collect “benign herpes” lung nodules especially in all elephant necropsies.
ELEPHANT NECROPSY PROTOCOL GROSS EXAMINATION WORKSHEET

Institution/Owner__________________________________________________________

Address__________________________________________________________________

Species__________________ISIS#_______________Studbook#_______________

Name_____________________________

Birth date/Age_________________________Sex__________Weight (Kg)_____________ Actual □ Estimate □

Death date________________________Death location______________________________________

Necropsy date_____________________Necropsy location_______________________________________

Post mortem interval______________________

Captive Born □□□ Wild Caught □

History (clinical signs, circumstances of death, clinical lab work, diet & housing)
_____________________________________________________________________________________________
_____________________________________________________________________________________________
_____________________________________________________________________________________________
_____________________________________________________________________________________________

GROSS EXAMINATION
(If no abnormalities noted, mark normal or not examined (NE); use additional sheets if needed)

General Exam (physical and nutritional condition, skin, body orifices, superficial lymph nodes). Skin nodules have been associated with EEHV in African elephants* (samples for fresh/frozen/formalin should be saved).
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Musculoskeletal System (bones, marrow, joints, muscles)
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* Skin nodules have been associated with Elephant Endotheliotropic Herpesvirus (EEHV) in African elephants. Samples for fresh/frozen/formalin should be saved for future research.
Body Cavities (fat stores, pleura, thymus, lymph nodes)

Spleen

Respiratory System (trunk passages, pharynx, larynx, trachea, bronchi, lungs, regional lymph nodes; submit lung lesions for TB culture. Bronchial lymph nodes should be cultured for TB even if normal in appearance). Lymphoid nodules in lungs may be associated with EEHV infections* (samples for fresh/frozen/formalin should be saved).

Cardiovascular System (heart, pericardial sac, great vessels, myocardium, valves, chambers) Be sure to closely examine abdominal aorta for subtle or obvious aneurysms)

Digestive System (mouth, teeth, tongue, esophagus, stomach, small intestine, cecum, large intestine, rectum, liver, pancreas, mesenteric lymph nodes)

Urinary System (kidneys, ureters, bladder, urethra)
Reproductive System (testes/ovaries, uterus & cervix, penis/vagina, urogenital canal, prostate, seminal vesicles, bulbo-urethral gland, mammary gland, placenta). **Uterine masses/tumors are common in Asian elephants and multiple tumor types may be present.**

Endocrine System (thyroids, parathyroids, adrenals, pituitary)

Central Nervous System (brain, meninges, spinal cord)

Sensory Organs (eyes, ears)

Additional Comments or Observations:

Prosector: __________ Date: __________

Summarize Preliminary Diagnoses:

Laboratory Studies: Please attach results of cytology, fluid analysis, urinalysis, serum chemistries, bacteriology, mycology, virology, parasitology, x-ray, photographs, or other data collected.
TISSUE CHECKLIST

Freeze 3-5 cm blocks of tissue from lesions and major organs (e.g., lung, liver, kidney, spleen) in small plastic bags. Freezing at -70 degrees Celsius in an ultra-low freezer is preferred. If this is unavailable, freezing at conventional temperatures is acceptable (use a freezer without an automatic defrost cycle if possible).

Any lesions noted in the lungs should be submitted to NVSL or other qualified mycobacterial laboratory for mycobacterial culture (i.e., National Jewish Diagnostic Lab, Colorado). Bronchial lymph nodes should be cultured for TB even if normal in appearance. Preserve as many of the tissues listed below as possible in 10% buffered formalin at a ratio of approximately 1 part tissue to 10 parts solution. Tissues should be no thicker than 0.5 to 1.0 cm. Fix diced (1x1 mm) pieces of kidney, liver, spleen and lung in a suitable EM fixative if possible - glutaraldehyde base e.g., Trump-McDowell fixative. NOTE: There is generally no need to fix and label each tissue separately. Take 2 sets of fixed tissue. Bank one set. Send tissues required for diagnosis to the primary pathologist and request a duplicate set of slides for the SSP pathologist, Dr. Scott Terrell who should be contacted for further instructions. Also, freeze post mortem serum (from heart), urine and any abnormal fluid accumulations. Consult Elephant Research and Tissue Request Protocol for specific project sample requests. Consult for specific sample requests, which sometimes includes fresh chilled but unfrozen tissue from necropsy for cell culture, Johns Hopkins and Princeton University.

- Adrenal
- Blood *
- Bone with marrow
- Bulbo-urethral gland
- Brain
- Cecum
- Diaphragm
- Esophagus
- Eye
- Hepatic bile duct
- Heart/aorta
- Hemal node
- Kidney
- Large intestine
- Liver
- Lung
- Parathyroid
- Mammary gland
- Muscle
- Nerve (sciatic)
- Ovary/testis
- Epididymus
- Pancreas
- Lymph nodes (tracheobronchial, submandibular, tonsillar, mesenteric)
- Penis
- Pituitary
- Prostate
- Salivary gland
- Temporal gland
- Skin
- Small intestine
- Spinal cord
- Spleen
- Stomach
- Thyroid gland
- Thymus
- Tongue
- Trachea
- Trunk cross section
- Seminal vesicles
- Ureter
- Urinary bladder
- Vaginal/urogenital canal
- Uterus/cervix
- Adrenal
- Kidney
- Penis
- Thymus
- Blood *
- Large intestine
- Prostate
- Tongue
- Bone with marrow
- Liver
- Salivary gland
- Trachea
- Bulbo-urethral gland
- Lung
- Temporal gland
- Trunk cross section
- Brain
- Parathyroid
- Seminal vesicles
- Cecum
- Mammary gland
- Ureter
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- Muscle
- Thymus
- Diaphragm
- Nerve (sciatic)
- Ureter
- Esophagus
- Parathyroid
- Vaginal/urogenital canal
- Eye
- Mammary gland
- Uterus/cervix
- Hemal node
- Epididymus
- Vaginal/urogenital canal
- Kidney
- Pancreas
- Thymus

* Collect post mortem blood, separate serum and freeze for retrospective studies.

Primary Pathologist (Name): ____________________________________________________________
Lab ______________________________________________________________________________
Address ____________________________________________________________________________
Phone ______________________________________________________________________________

(Please send a copy of this protocol with gross descriptions and preliminary diagnoses to SSP pathologist and SSP veterinary advisor. Send final report with histopathologic findings, laboratory results, and any pertinent digital or color slides to (and copy to SSP veterinary advisor):
Scott P. Terrell, DVM, Diplomate ACVP
Michele Miller, DVM, MS, PhD
SSP Pathology Advisor, Elephants
SSP Veterinary Advisor
Disney’s Animal Kingdom, 1200 N Savannah Circle, Bay Lake, FL 32830
michelemiller128@gmail.com
W (407) 938-2746; H (407) 251-0545; Cell (321) 229-9363
mail: Scott.P.Terrell@disney.com

Elephant Endotheliotropic Herpesvirus Research and Tissue Request Protocol, June 2015
CONSENT FORM FOR USE OF SAMPLES BY AZA ELEPHANT TAG/SSP

I give consent for the sample submitted to the AZA Elephant TAG/SSP serum/tissue bank to be used for research on any elephant related issues based on recommendations by the veterinary advisor and/or steering committee.

The results could be reviewed and used by the AZA Elephant TAG/SSP Veterinary Advisor in providing health-related recommendations and publications.

I understand that all results and recommendations regarding the individual elephant will be kept confidential.

_____ Yes, I agree to allow the AZA Elephant TAG/SSP to use our sample for designated research and testing results.

_____ No, I do not consent to the use of our sample and test results unless specified.

__________________________________________  ____________
Signature, title                                      Date

__________________________________________  __________________
Printed name                                         Phone number

__________________________________________  __________________
Institution                                          Email address

__________________________________________
Address

Comments: _____________________________________________________________________
FREQUENTLY ASKED QUESTIONS ABOUT EEEHV

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, eight of which are known to cause hemorrhagic disease. The viruses found in clinically ill 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 unknown reasons, primary or reactivated latent elephant herpesvirus infections can sometimes circulate 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 more 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. More than twenty cases of EEHV have been identified in elephant populations in India, Thailand, Myanmar, Sumatra, 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 is subclinically infected with one or more forms of elephant herpesvirus.

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 is transmitted. Viral shedding occurs when the virus comes out of latency and most human herpesviruses are transmitted predominantly in saliva. Until recently EEHV could only be detected when the virus was circulating in the blood by using a blood test, but studies now show that most healthy Asian elephants periodically shed low levels of EEHV1, 4, and 5 (which may or may not be infectious) in secretions from the trunk.

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. Therefore, the AZA Elephant TAG/SSP recommends that institutions continue to exchange elephants and elephant semen for breeding and artificial insemination as specified in the breeding recommendations.

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 herpesvirus 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 zoos from breeding elephants will severely impede the progress that is being made in studying EEHV and finding a cure. Discontinuation of captive breeding is not the way to solve the disease. When an outbreak of equine herpesvirus infection 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 due to canine distemper virus infection, captive propagation continued, and the 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 recovered 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, 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 for wild populations and those in human care.