GUIDELINES FOR MANAGEMENT

2ND EDITION

Elephant Endotheliotropic Herpesvirus (EEHV) in Asia

Recommendations from the 1st Asian EEHV Strategy Meeting

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On behalf of the Asian EEHV Working Group

Photo credit on the cover
Clockwise from left: Christopher Stremme, Khajohnpat Boonprasert, Chatchote Thitaram

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Asian EEHV Working Group

GROUP STATEMENT

28th January 2016

A recently recognised herpesvirus, EEHV (elephant endotheliotropic herpesvirus), can cause severe haemorrhagic disease in elephants, and is associated with a high fatality rate in young Asian elephants (1-8 years of age). Death frequently occurs within 1-2 days of the first visible signs, and early diagnosis and treatment is critical to survival.

The prevalence of EEHV in captive Asian elephants in North America and Europe has been well characterised, with an estimated mortality rate of 70% in captive born elephants that become ill from the virus. Little is known about the prevalence and impact of EEHV on captive and wild elephant populations in Asian elephant range countries.

From Nov 5th to 7th, 2015, Wildlife Reserves Singapore hosted the **1st Asian EEHV Strategy Meeting**. For three days, 38 (wildlife) veterinarians, researchers, conservationists, and elephant specialists shared information, identified regional needs, and prioritised future EEHV-related projects. Eight Asian elephant range countries were represented (Thailand, Myanmar, Indonesia, Cambodia, Sri Lanka, India, Vietnam, and Malaysia) along with delegates from Singapore, the United States, Canada, and the Netherlands.

As a result of this 1st Asian EEHV Strategy Meeting, an **Asian EEHV Working Group** was formed which together recognised:

- The epidemiology of EEHV in elephants in Asia and its impact on populations is currently unknown. Within the last 10 years, 59 fatal cases of EEHV disease in Asian elephants have been identified within the eight range countries represented at our meeting. Twelve of these deaths were wild elephants.

- The identification of EEHV-associated deaths in wild elephants in Asia is significant and it is the opinion of the Working Group that EEHV is a conservation concern requiring close monitoring and further study.

- Early diagnosis of EEHV-associated disease in young elephant calves allows early treatment and a better chance of a successful outcome. Therefore an important consideration is that the examination, sample collection, and treatment of young calves depend on the ability to handle and manage the calf from a very young age (less than 1 year old).

- Laboratories are critical to the routine monitoring, detection, and post mortem evaluation of elephants affected by EEHV. Currently, of 13 Asian elephant range countries, only three (Thailand, Indonesia, and India), have laboratories capable of confirming EEHV.

Based on the above concerns, the Asian EEHV Working Group seeks the support of regional governments and international stakeholders in the following areas of immediate focus:

- To build capacity and increase awareness and education of EEHV amongst elephant care staff in Asia including keepers (mahouts), veterinarians, and government officials.

- To develop region-specific medical protocols, "standard operating procedures" that outline routine monitoring, rapid and accurate detection, and appropriate treatment of EEHV-associated disease.

- To closely collaborate within the region and internationally to identify and implement research projects to continue advancing the understanding of EEHV.

Frequently Asked Questions

Elephant Endotheliotropic Herpesvirus (EEHV)

By The Asian EEHV Working Group

1 What is EEHV and EEHV-HD?

EEHV is an abbreviation for Elephant Endotheliotropic Herpes Virus, which is a virus that can cause fatal Elephant Endotheliotropic Herpes Virus hemorrhagic disease (EEHV-HD) in elephants. Endotheliotropic describes the tissue that the virus preferentially affects, i.e. endothelial tissue found on the inside of blood vessels. EEHV is carried by most juvenile and adult elephants, does not always cause overt disease, and is species-specific to elephants. There are many different strains of EEHV. Most of the deaths in Asian elephants have been caused by EEHV1A. Other fatal strains in Asian elephants are EEHV1B, EEHV3, EEHV4 and EEHV5.

2 How is EEHV transmitted?

Herpesviruses are spread by mucosal secretions. Mucosal secretions include saliva, breast milk, and nasal and vaginal secretions. Available evidence suggests that EEHV can be found in elephant mucosal secretions and may be spread via similar mechanisms, such as trunk-to-trunk contacts.

3 Can people or other animals get EEHV-HD?

No, the disease can only affect elephants and is not infectious to humans or other animals.

4 Should an elephant with EEHV be isolated?

We do not believe that elephants with EEHV need to be isolated from other elephants. This is because of the fact that most elephants carry EEHV without getting sick. In addition, the majority of cases of EEHV-HD have been sporadic. However, direct transmission from another acute case cannot be ruled out completely. Finally, elephants are social animals and separating them from their herd is apt to increase their stress.

5 What is the incubation period of EEHV-HD?

Available evidence suggests that the incubation period for EEHV is probably between 7-14 days. This is similar to herpesvirus infections in other animals.

6 Why is EEHV important?

EEHV is important because it has caused a very large number of deaths in young Asian elephants. Asian elephants are highly endangered and have a low reproductive rate. The further loss of young elephants from the population, animals that are potential future breeders, has the potential to be absolutely devastating to the future of this magnificent species.

7 How can I better understand EEHV and EEHV-HD?

There is, unfortunately a great deal of misinformation about the disease. However, we recommend the website www.EEHVinfo.org as an excellent source of accurate information about the disease. The website is maintained by the researchers, veterinarians, and elephant managers who are studying the disease, treating the disease and caring for elephants with EEHV-HD. The information there is scientific and evidence-based. There are also multiple scientific publications and textbooks that cover EEHV and EEHV-HD. *Fowler's Zoo and Wildlife Medicine 7 Current Therapy* (Saunders Press 2012) is devoted to the subject.

8 What happens to an elephant when it gets EEHV-HD?

EEHV causes damage to the lining of small blood vessels, primarily capillaries. When this happens, blood starts to leak out of the vessels. The result is progressive blood and fluid loss. As the damage to the blood vessels worsens, the heart starts to pump less efficiently, and ultimately the elephant dies of shock. This is similar to what happens when Ebola virus, a haemorrhagic virus, causes disease in people.

Facial oedema. Photo: Khajohnpat Boonprasert

Hyperemic tongue with ptechiae. Photo: Khajohnpat Boonprasert

Surprisingly, most elephants carry EEHV latently and show no signs of disease. A few elephants develop benign skin lesions. We do not know why some elephants develop fatal haemorrhagic disease with this virus.

9 What ages of elephants are affected by EEHV-HD?

EEHV-HD can affect elephants of any age, but the elephants that have the highest risk of dying of fatal haemorrhagic disease are young elephants between 1 and 8 years of age.

10 What are the signs of EEHV-HD?

Early signs of EEHV-HD are very non-specific. Some elephants will be sleepy (lethargic), others will not sleep at all. Mild gastrointestinal signs (colic) may be seen, including constipation or mild diarrhoea and a decreased appetite. Lameness (e.g. a stiff leg) has also been reported. As the disease progresses, signs associated with shock are seen. These include an increased heart rate and an increased breathing rate. As blood leaks from the heart, it becomes less efficient, and blood and oxygen do not circulate efficiently around the body. Late-stage signs include cyanosis (a blue colour) of the tongue and a swollen head, which represents oedema (fluid) leaking into the tissues. Elephants experiencing brain bleeds may show neurologic signs or severe sleepiness. Mouth lesions have been reported in several cases, a symptom that generally occurs later in the disease.

11 Can EEHV-HD be treated and what is the success rate?

Eleven survivors have been reported from the United States, two from Thailand and one from Cambodia. All elephants received extensive treatment. The primary treatment is aggressive fluid therapy. Antivirals such as famciclovir, ganciclovir, and acyclovir are also typically administered. Other supportive treatments include anti-inflammatories, antioxidants, diuretics, and plasma transfusions. The success rate remains low and the disease has a 70% mortality rate, which is exceptionally high and on par with Ebola virus. However, it is clear that the survival rate increases with early aggressive therapy.

The survival rate is low for several reasons. First, the virus is extremely virulent and disease progresses rapidly. Laboratory diagnosis of the disease can take more than 24 hours, yet the disease can kill in less than 24 hours from first observed signs of illness. Thus, treatment must begin before EEHV is confirmed. In addition, treatment requires aggressive, around-the-clock care, necessitating trained animals, experienced veterinarians, and access to testing and treatment supplies. See also Chapter 2 for treatment details.

12 How much does it cost to treat EEHV-HD?

EEHV-HD is an expensive disease to treat. The antivirals, which must be used for at least one week and often longer, can cost several thousands of dollars (US). EEHV-HD is also expensive in terms of personnel time because sick elephants require around-the-clock care. Costs of testing can also be high. However, the cost of not treating is the likely loss of an elephant's life. In the United States, all survivors of EEHV-associated clinical disease received treatment. While not all treated elephants survived, NO elephant that did not receive treatment survived EEHV HD.

13 How can we prevent EEHV-HD and is there a vaccine?

At this point, we do not have a vaccine or other ways to prevent the disease. We recognise however, that elephants identified early with the disease and treated in the early stages of disease have the best chances of survival.

Thus, training of staff, both mahouts and veterinarians, to recognise early signs of disease is important. Training calves ahead of time to tolerate sample taking (blood draws) and treatment is essential if they become sick. Having appropriate testing equipment and medications for treatment readily available is also an important component. Finally, monitoring a herd of elephants with routine blood draws and viral testing can alert caretakers to an impending problem.

14 What should we do if we have a suspected case of EEHV-HD?

If a calf or young elephant between the age of 1 and 8 years presents with vague signs of disease as described in #10 above, the first step in treatment should be administration of rectal fluids at a dose of 10-20 ml/kg. This can halt some of the early signs of shock and should be repeated several times a day. This is also an appropriate approach for the treatment of other diseases that may present similarly, since typically diagnosis will take a while. Starting antivirals should also be done as soon as possible even before diagnosis is confirmed. Collecting blood to test for EEHV as well as other possible diseases should also be started immediately. Because EEHV can mimic several bacterial diseases in their early stages, many sick elephants are typically started on antibiotics as well. There are excellent planning and treatment documents on the EEHVinfo.org website.

15 What other diseases cause signs similar to EEHV?

The early stages of EEHV can look extremely similar to various infectious bacterial diseases such as Salmonella, *E. coli*, Clostridium toxiaemia and Pasturella, as well as viral diseases such as encephalomyocarditis virus (EMCV). In all cases, fluid administration is an appropriate first step. Blood should also be collected and serum banked. For EEHV, polymerase chain reaction (PCR) testing of whole blood is necessary for confirmation of disease.

16 Which countries are affected by EEHV-HD?

EEHV-HD is a worldwide disease, and confirmed lethal cases have been reported in elephants in multiple Asian range countries including Myanmar, Laos, Malaysia, India, Thailand, Indonesia (Sumatra), Borneo, Nepal and Cambodia. Wild elephant deaths due to EEHV-HD have been confirmed in India. Several other Asian countries have had suspected cases. EEHV-HD has also occurred in multiple zoos around the world.

17 Who is performing EEHV research?

Multiple laboratories world-wide are studying the disease. In the United States, these include Baylor College of Medicine, Johns Hopkins University, Cornell University, and the Smithsonian's National Zoo. In Europe, these include Animal and Plant Health Agency in Weybridge (UK), Erasmus University Rotterdam (NL), Artemis One Health in Utrecht (NL), Free University Berlin (DE), Veterinary University Zürich (CH), and Institute for Zoo and Wildlife Research IZW (DE). In Asia, researchers laboratories include Faculty of Veterinary Medicine, Chiang Mai University and Kasetsart University (Thailand), National Trust for Nature Conservation (Nepal), University of Peradeniya (Sri Lanka) and Kerala Veterinary and Animal Sciences University (India).

18 Can all elephants get EEHV-HD?

EEHV-HD can affect all elephants, both Asian and African elephants. Furthermore, this is a disease of both wild and captive elephants. However, the group that is most at risk is young Asian elephant calves and juveniles, either wild or captive.

19 How long has EEHV existed?

EEHV most likely co-evolved along with the evolution of elephants. Thus, it has been around for millions of years.

20 What are risk factors for EEHV-HD in elephants?

Age appears to be a risk factor as young elephants are more often affected. Changes in immune status may be part of the picture, as the timing of the disease may, in some cases, be associated with loss of maternal antibodies or concurrent disease. Whether stress is part of the disease and what constitutes stress is still not clear. We are still working to identify other risk factors.

21 Should EEHV impact the translocation of elephants?

The movement of young elephants in high-risk age groups to a new facility or of other elephants into a facility that already hosts young elephants, has, in some cases occurred shortly before an EEHV-HD case. Thus, there may be a risk, but the extent of that risk and what other variables are involved are still being investigated.

22 How often should a healthy elephant be tested for EEHV?

Under ideal circumstances, juvenile elephants within vulnerable age groups (1-8 years of age) should be monitored every week (checking for the presence of EEHV in the blood). This is based on the incubation time of the disease (7-14 days).

However, it's recognised that the capacity or resources to achieve this goal may not be available. In these circumstances, other behavioral or simple clinical information can be used to identify possible emerging disease. Confirmation of EEHV involvement, even if sporadic or delayed, is encouraged.

23 Are there regulatory/legal issues involved in EEHV?

At this point, there are no regulatory or legal issues. Because the disease does not affect people or other animals, and because it is not usually directly transmitted from elephant to elephant, regulation has not been needed.

24 What do we still need to learn about the disease?

Unfortunately, a great deal still remains unknown. These include why some elephants die of haemorrhagic disease and others are unaffected by it, what antivirals would be best for treatment, and the pathophysiology of the virus (i.e., the physiological effects of the virus within the body of the elephant.) Because we have still not been able to grow the virus in culture, the virus has been difficult to study.

Fortunately, there is some good news. The virus has recently been completely sequenced which will enable virologists to learn a great deal about this very unusual virus. We also now know that early detection, diagnosis, and treatment can save lives. Educating those who care for elephants about this deadly disease is a priority and working together so that we can learn from each other's experiences is also essential.

25 How is the presence of EEHV confirmed?

Currently conventional polymerase chain-reaction (cPCR) and quantitative PCR (qPCR) are used to diagnose EEHV in Elephants. These assays look for the presence of viral DNA in the sample. Clinical pathology, including a complete blood count may show decreases in total while blood cell numbers, particularly monocytes, and platelets. A blood smear may show reactive white blood cells and the presence of band heterophils, a type of premature white blood cell associated with systemic inflammation. These blood cell changes may precede the appearance of clinical signs. The presence of clinical signs can provide suspicion of disease as well.

Post mortem necropsy findings include extensive haemorrhage within multiple body cavities, pericardial effusion, and oedema of multiple organs, including the brain. Histolopathology will show vasculitis and thrombosis, often most severe in heart, kidneys and liver. Basophilic intranuclear inclusion bodies are also characteristic of EEHV but can sometimes be difficult to find.

26 Can African Elephants transmit EEHV to Asian Elephants?

EEHV-HD can affect all elephants, both Asian and African, but naturally African and Asian elephants harbour different types of EEHV. EEHV viruses endemic to Asian elephants are EEHV1, EEHV4 and EEHV5. EEHV viruses endemic to African elephants are EEHV2, EEHV3, EEHV6 and EEHV7. Nowadays it is assumed that there is no cross infection between the species.

Medical Management of EEHV-HD

For Elephants Clinically Ill from Elephant Endotheliotropic Herpes Virus–Haemorrhagic Disease (EEHV-HD)

EEHV-HD Emergency Care

FLOW CHART

Sick elephant between 1-8 years of age

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Start rectal administration of fluids (Page 8)

↓

Collect baseline information (Page 8)
Blood sample: Lab testing (Page 21)
History (Page 23)
Physical exam (Page 24)

↓

Supportive fluid therapy*
Continue Rectal fluids TID-QID
IV fluids
IV plasma
Plasma and fluid administration (Page 8-9)
Plasma collection, separation, and storage information (Page 10)
Plasma cross matching information (Page 11)
Platelet rich plasma information (Page 10)
*may require sedation (Page 15)

↓

Start additional treatments*
Antivirals (Page 14)
Antibiotics (Page 14)
Adjunctive (opioids, NSAIDS, steroids) (Page 14)
*may require sedation (Page 15)

Time is essential when treating elephants with EEHV-HD. Extremely sick calves and juveniles may not look particularly ill, and may eat, drink, and participate in training, until literally moments before they die. Waiting until the animal looks very sick is associated with a poor prognosis and death. Even if a young elephant looks only mildly ill or uncomfortable, veterinarians and caretakers are strongly urged to **start rectal administration of fluids**. This technique can be life-saving because what appears to kill young elephants suffering from EEHV-HD is vascular shock. Rectal fluids can alleviate the early physiological effects of shock and prevent the spiralling of events that leads to death.

Collect baseline information

BLOOD COLLECTION

- **Essential**: EDTA (purple topped tube) whole blood and smear; EEHV qPCR (or cPCR if not available) and haematology (including platelets).
- Serum (red topped tube) or plasma (green topped tube): biochemistry.
- Citrated plasma: coagulation panel.
- Serum or plasma (EEHV-gB_ELISA antibodies)
- Samples should also be stored for future research (please store any leftover blood collected).

- If possible contact the nearest diagnostic lab that runs PCR and qPCR for emergency diagnosis and arrange sample transport. See the chapter 4 for addresses.

- Anamnesis: activity pattern, appetite, sleeping pattern.

- Physical examination: body posture, evidence of oedema around eyes, head, neck and ventral abdomen, temperature, blood pressure, changes in colour or ulceration of mucous membranes. Auscultation of the heart and lungs can be performed on calves weighing less than 3,000 lb (1,200 kg). Tachycardia, murmurs and arrhythmias should be noted.

- Blood samples should be tested frequently, even DAILY, using qPCR in order to adjust the treatment regime according to the viral load. If qPCR is not available, evaluation of the appearance, number and distribution of white blood cells can be an indication of how the elephant is responding internally.

Fluid therapy

RECTAL

Rectal administration of lukewarm, clean water is the first choice of fluid therapy in sick calves and is superior to intravenous administration. It should be given through a garden hose or rubber tubing after careful removal of faecal balls from the distal part of the rectum (use sufficient lubricant in order to avoid irritation of the rectum mucosa which causes peristaltic activity). When the hose is placed over the horizontal ridge in the rectum (approximately 1 elbow length from the anus), the tube can be advanced for another 100 cm (if possible). A gastric pump can be used; if not available use a large funnel.

Rectal fluids should be administered a minimum of 3-4 times per day, up to every 2 hours. A bolus treatment of 10 to 20 ml/kg dose is often used. When finished, the tail should be held down for at least one minute. Excess fluids will simply be expelled.

IV CATHETER PLACEMENT

Placement of an intravenous catheter (16-20G IV catheter, with a minimum length of 6 cm to prevent perivascular leaking) in a large, peripheral vein is recommended for:

- Plasma transfusion (supplementation of platelets) after cross matching recipient blood with donor plasma at 0.5-2 ml/kg BW. The donor should be an adult elephant, preferably PCR-screened on EEHV-viraemia at the time of blood collection.

- Administration of other IV-only medications. Please note that the ear veins are very susceptible to vasculitis, associated with perivascular administration of drugs. Sloughing of the ear pinna distal to the affected vein is likely in these cases. Extra care should be taken with drugs that are particularly caustic.

- IV fluid therapy, which will require follow up with rectal fluids.

Rectal fluid therapy. Photo: Christopher Stremme

IV FLUIDS

In addition to rectal fluids, a bolus of 'isotonic' IV fluids (2.5 to 4 ml/kg in a calf) can be given if the elephant is dehydrated or in shock as a resuscitative measure; this bolus could be repeated up to three times with re-evaluation of the patient and vital signs after each bolus. **Asian elephants have very low serum osmolarity and are hyponatraemic and hypochloraemic compared to other species. Therefore fluids considered isotonic in other species (0.9% saline, ringers etc.) will be hypertonic in an elephant, and draw fluid into the vascular space**. IV fluids should always be supplemented by large amounts of rectal fluids (tap water).

IV PLASMA

Plasma collection, storage and administration

Fresh plasma is currently considered one of the best supportive therapies to provide, as platelets, clotting factors and potentially protective antibodies may be provided. Note that the freezing process activates the platelets, which renders them useless at the time of transfusion. Therefore - where possible - freshly collected plasma is preferred. The following should be considered for plasma transfusions:

- If frozen plasma is available, this can be given in an early stage of the disease to save time (despite the activated and spent platelets).

- Blood collection from an adult elephant (plasma donor) should be initiated to provide fresh plasma as soon as possible.

- A sterile, closed collection system is needed for plasma collection. Open collection systems, such as those that use a syringe, cannot be left to sit for any period of time as they are subject to bacterial invasion.

- Cross-matching the donor animals with the recipients, especially if one donor will be used on multiple occasions. (*See page 11*)

- PCR screening the donor plasma for current EEHV DNA.

- If stored, storage at -80°C is essential (6-8 months maximum).

- Plasma separation does not require a centrifuge. Leaving to stand overnight followed by manual separation (*see below*) is feasible.

- For administration of plasma, a patent IV cannula and a filtered infusion giving set are required.

- Dose rate 0.5-2 ml/kg/day – the first 100 ml of each donor should be given slowly to monitor for anaphylaxis.

Note: Fresh plasma is not a good source of platelet unless specially prepared through spinning techniques into platelet rich plasma (PRP) – as described below.

Colloids, such as fresh or frozen plasma or hetastarch, are more effective than crystalloid fluids for immediate volume expansion in viraemic or seriously ill animals. The larger molecules in these fluids do not leak out of capillaries as easily, and increase plasma volume. In this respect, a (preferably fresh) plasma transfusion has high priority as it provides platelets and coagulation factors.
As the preparation of fresh plasma is time consuming, banked plasma can be administered as an emergency treatment.

To supplement platelets, frozen plasma is NOT suitable, because it contains activated platelets, which will be useless in case of Disseminated Intravascular Coagulopathy (DIC) as is likely the case in EEHV-HD. The best plasma to administer is the so called Platelet Rich Plasma (*See below*).

In addition, plasma from a donor with a high antibody titre may help to bind virus particles in the patient (although the role of antibodies is not yet well understood in EEHV-HD). Plasma should only be administered intravenously after cross-matching donor plasma and recipient whole blood samples (a minor cross-match) to assure compatibility. Additionally, it would be ideal if the donor animal's blood be PCR tested to ensure the donor does not have a high EEHV viraemia. This information would also be useful as retrospective information.

As there will probably be no time for PCR-screening, this can be performed later on using the stored sample (stored plasma should be PCR screened at the time of collection). The first 100 ml should be given slowly, and heart rate, respiratory rate, and temperature should be monitored. Possible transfusion reactions include fever, rash, or anaphylaxis. Mild signs can be treated by decreasing the rate of transfusion. More severe reactions should be addressed by stopping the transfusion.

If no reaction is seen, the transfusion dose can be increased to 0.5-2 ml/kg BW. Clinical improvement may be seen at a plasma dose of 0.5 ml/kg.

For **IV cannulation**, see Appendix 3 on page 26.

For **plasma separation**, see Appendix 4 on page 27.

Summary
Use banked (frozen) plasma for emergency treatment (coagulation factors, antibodies, colloids) and start preparing fresh plasma (platelets, coagulation factors, antibodies, colloids). Please note that a major cross-match needs to be carried out if whole blood is transfused.

Note: Plasma must be frozen within 6 hours to retain clotting factors.

How to collect Platelet Rich Plasma without specific blood bags:

A. Collect blood in a container with acid citrate dextrose (ACD) as an anticoagulant at the ratio of 6 to 1 and mix gently. In the absence of specific blood bags, empty NaCl-infusion bags or plastic infusion bottles can be used (maintain sterility!) The sample can be kept at room temperature (20-25°C).

B. Instead of ACD, heparin can be added to the donor blood (6,250 IU heparin/liter whole blood)

1. Centrifuge at 200G for 10 minutes at room temperature.
2. Remove plasma and change to a new tube.
3. Centrifuge at 1,650G for 10 minutes.
4. Platelet rich plasma (at bottom of tube) can be kept at 4°C and be used within 5 days.
5. If heparin was used as anticoagulant, this can be reversed by protamine HCl (10 mg protamine HCl/1,000 IU heparin given IV).

Plasma cross matching

Minor crossmatch
Used to assess the compatibility of a donor's serum/plasma with the red cells of a recipient. Used in elephants when recipient is getting plasma from another elephant.

Major crossmatch
Used to assess the compatibility of a donor's red blood cells with recipient's plasma. Typically not used with elephants unless the recipient is getting whole blood or packed red blood cells.

Materials needed

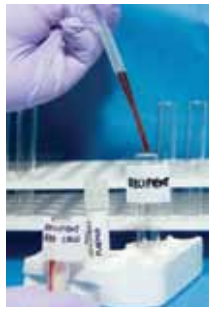
1. EDTA (preferred) or serum tube (without the separator gel) from donor and recipient animals (all animals involved).
2. Centrifuge.
3. Small tubes (glass preferred) for separating the plasma and for testing (estimate minimum 3 tubes/animal).
4. Physiologic saline (0.9% saline without preservatives).
5. Droppers or pipettes.
6. Incubator 35-37˚C
7. Markers for labeling tubes.
8. Paper for recording results.

Incubator

Step one

Prepare a 3-5% red cell suspension.

1. Collect blood from both donor and recipient in EDTA.
2. Centrifuge the tube and separate the plasma from the red cells. Save both.
3. Place 1 drop of recipient red cells into a small (2-5 ml) clean test tube.
4. Add approx. 1-2 ml of normal saline to the tube with the red cells. (Or 1 drop RBC to 40 drops saline)



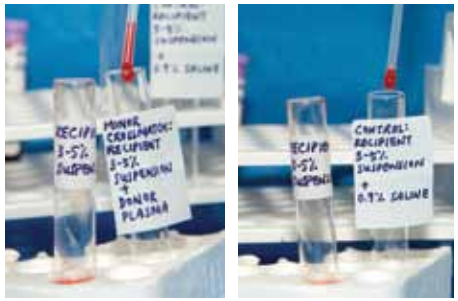
5. Centrifuge at 2500 RPM for 20 seconds.
6. Remove the supernatant, leaving the red cell button on the bottom.

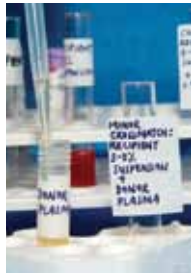
7. Repeat steps 4-6 three times (for a total of 4 washes).
8. Add 1 drop of newly washed recipient red cells to a new test tube.
9. Add approximately 20-40 drops of saline and mix to suspend the red cells. This should be an approximate 3-5% cell suspension to work with.

Step two

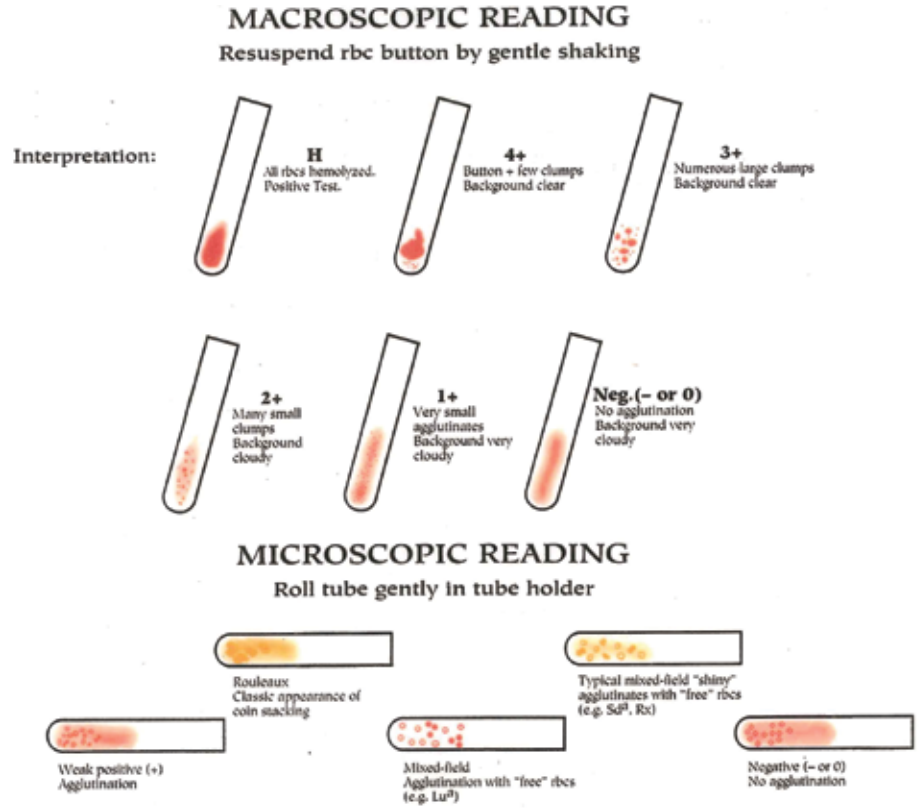
Minor crossmatch.

1. Add 1 drop of the recipient's 3-5% red cell suspension to a labeled test tube. Then add 1 drop of the recipient's 3-5% red cell suspension to another labeled test tube to be used as a control.

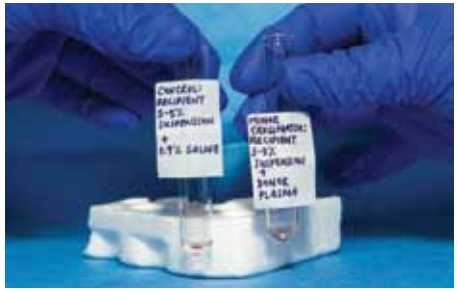




2. Add 2 drops of donor plasma or serum to the test tube.
3. Add 2 drops of saline to the control tube.
4. Incubate these tubes at 37˚C for 15 minutes.
5. Centrifuge the tubes for 20 seconds at 2500 RPM.



6. Observe the supernatant for signs of hemolysis. If present in the crossmatch tube and not the control tube, the match is not compatible. If present in both, start again with a new cell suspension.



7. If no hemolysis, then gently rock the test tube back and forth to re-suspend the cell button. Observe the cell button while rocking the tube and grade for the presence of agglutination. Grade on a 0-4 scale where 0 is no agglutination and 4 is heavy clumping. Record your results.

Step three

Major crossmatch

1. Add 1 drop of the donor's 3-5% red cell suspension to a labeled test tube.
   Add 1 drop of the donor's 3-5% red cell suspension to another labeled test tube to be used as a control.
2. Add 2 drops of recipient's plasma or serum to the test tube.
3. Add 2 drops of saline to the control tube.
4. Incubate these tubes at 35-37˚C for 15 minutes.
5. Centrifuge the tubes for 20 seconds at 2500 RPM.
6. Observe the supernatant for signs of hemolysis. If present in the crossmatch tube and not the control tube, the match is not compatible. If present in both, start again with a new cell suspension.
7. If no hemolysis, then gently rock the test tube back and forth to re-suspend the cell button. Observe the cell button while rocking the tube and grade for the presence of agglutination. Grade on a 0-4 scale where 0 is no agglutination and 4 is heavy clumping. Record your results.

Oxygen therapy

Supplemental oxygen therapy should be administered, when possible, to all patients with clinical signs undergoing treatment for EEHV-HD. Oxygen can be administered at 2-4 l/minute via a flexible tube passed into one nostril of the trunk. If the elephant will not tolerate oxygen therapy while awake, it may be possible to slip the tube into the trunk while the elephant is sleeping.

Equipment and supplies

The following equipment and supplies will need to be on hand for support during therapy.
Drugs and equipment needed:

- Banked plasma (frozen at -80°C)
- Antiviral (Famciclovir, Ganciclovir, Acyclovir)
- Sedatives (Detomidine, Butorphanol, Xylazine)
- Reversals (Atipamezole, Naltrexone)
- Antibiotics (Ceftiofur, Penicillin, Amoxicillin, Enrofloxacin, Cephalexin, etc)
- Glucocorticosteroids
- NSAIDs (Flunixin meglumine, Meloxicam, Ibuprofen, Phenylbutazone, etc)
- Plasma transfusion set
- "Plasma extractor" (*See Page 15*)
- I.V. fluids
- Syringes
- Needles
- 16-20 GA catheters, min 6 cm length
- Rectal fluid kit (tube and gastric pump or large funnel)
- I.V. administration sets with injection ports
- Standard extension set
- Tape for holding catheter in place and skin glue
- Stethoscope
- Thermometer
- Mortar and pestle
- Exam gloves
- OB sleeves and lube
- Gauze
- Flashlights/ head lamps
- Towels
- Inner tubes (various sizes)/gym mats — to be used for cushioning and support in the event of a full immobilisation procedure
- Surgical prep: Chlorhexidine scrub or Povidone iodine and alcohol
- Oxygen bottles and regulator

Antiviral administration

Antiviral drugs are thought to have an effect during the early stages of viral replication. It is therefore recommended that antiviral therapy starts as early as possible. The efficacy of the following drugs has not been proven, but all survivor cases have been treated with one or other of the following drugs:

- **Famciclovir**: 15 mg/kg orally or rectally TID (grind with mortar and pestle, mix with water to make into a watery paste for direct application into the cleaned rectum).
  - Medications should not be administered rectally within one hour of rectal fluid administration.
- **Ganciclovir**: in advanced stages of the disease, when a reduced absorption from the intestinal tract can be expected, IV administration may be considered more prudent and slow IV administration of ganciclovir at a dose of 5 mg/kg BID (dissolved in 1 litre of fluid given over 1 hour) should be considered.
- **Acyclovir**: therapeutic doses have yet to be established, but 15mg/kg BID was used in a survivor case: orally, rectally (grind with mortar and pestle, mix with water to make into a paste and further dilute with water) or intravenously.

Antibiotic administration

Antibiotics should be considered for treatment of underlying conditions and/or secondary infections associated with leukopenia and immunosuppression:

- Ceftiofur: 1.1mg/kg IV BID
- Enrofloxacin: 2.5mg/kg PO or rectally SID
- Marbofloxacin: 2mg/kg IV, IM, SQ SID has been used
- Amoxicillin: 11mg/kg IM SID
- Penicillin G: 20,000-50,000 IU/kg IM or IV TID-BID (BID administration has been used in EEHV survivor cases in Asia)
- Pendistrep LA: 20,00-50,000 IU/kg IM q24h, 36h, 48h or 72h (q72h administration has been used successfully in EEHV-HD cases in Asia)
- Any suitable antibiotic with presumed action against invasive gut flora

Adjunctive treatments

Opiods

Opioids are a useful adjunct to providing pain relief and, in some cases, mild sedation to assist in the management of animals being treated. There is the possibility of behavioural changes in the elephant when using opioids, and trained behaviours may well be lost or less responsive. A dose of 0.008-0.014 mg/kg Butorphanol IM (repeat every 3-4h) is recommended for analgesia.

NSAIDS

Although EEHV-HD is thought to be a vasculopathy as opposed to a vasculitis, anti-inflammatories may be indicated as part of the analgesic regime as well as to reduce inflammation. Non-steroidal anti-inflammatories (NSAIDs) may play a useful role in early management of the disease. However, it should be noted that in human medicine, NSAIDs are contraindicated in cases where peripheral oedema or haemorrhagic diathesis are present, due to the decreased glomerular filtration rate and the effects on coagulation seen when using NSAIDs. The analgesic and anti-inflammatory effects of these drugs should be weighed against these possible side effects. Flunixin meglumine or other NSAIDS should be administered to well hydrated patients, who are preferably receiving concurrent fluid therapy. Administration of omeprazole (0.7-1.4 mg/kg PO SID based on the equine dose) for gastrointestinal protection during NSAID treatment should be considered.

- Flunixin meglumine 0.25-0.5 mg/kg IV/IM SID
- Meloxicam 0.2mg/kg IM SID has been used
- Ibuprofen 6mg/kg PO BID
- Phenylbutazone 3mg/kg q48 hours (published dose), 1-2.5mg/kg PO, IV or IM SID (anecdotal dose) or suxibuzone (loading dose 6 mg/kg/day followed by 3 mg/kg/day).

Note: If drug allows IV administration it should be considered the route of choice as large amounts of NSAID's given IM are prone to cause abscesses. However, IV injections must be done with caution and ideally after catheter placement.

Steroids

- **A single high dose glucocorticosteroid therapy** has been used in Thailand in 2 clinical cases caused by EEHV1a, 1 survived EEHV-HD but died 34 days later from a *Clostrdium* infection.
- In 2017 glucocorticosteroids were also used in Kolmarden Zoo in a severely ill EEHV-HD calf (cyanotic tongue) which survived.
- Treatment of an EEHV-HD case with glucocorticoids has not been fully investigated and is considered "experimental", more so than other treatments.
  - Triamcinolone 0.067 mg/kg IV (dosage given in both cases mentioned above).
  - (Or: FulmenthasoneL 0.005 mg/kg IV or deep IM)
  - (Or: Dexamethasone 0.05-0.1 mg/kg IV or IM)
- Veterinarians that use this treatment are encouraged to report their experience to their representative on the EEHV in Asia Working Group, or to sonja.luz@wrs.com.sg

Sedation

Standing sedation

- Standing sedation can be performed using Xylazine or Detomidine (preferred) in combination with Butorphanol.
  - Xylazine: 0.04-0.08mg/kg IM (can be reversed with Yohimbine or Atipamezole)
  - If insufficient sedation is obtained by Xylazine alone, an additional (low) dose of Ketamine (0.03 – 0.06 mg/kg) can be given IM or IV.

  OR
  - Detomidine 0.01-0.022 mg/kg IM (can be reversed by Atipamezole at 3 times the dose of Detomidine)

  AND
  - Butorphanol 0.045-0.075 mg/kg given at same time as Detomidine. Butorphanol can be reversed with naltrexone at 2.5-5 times the dose of Butorphanol in emergency situations, but reversal is not essential and should preferably not be carried out if the calf is considered to be in pain.

- Provide supplemental oxygen via nasal cannula whenever possible.

Note: Butorphanol could be given at the higher end of the range, by itself (without Detomidine) for adequate sedation in some elephants.

Light sedation of adult elephants

- It may be necessary to sedate the dam or other adult herd mates so they are not stressed during manipulations of a calf
- Butorphanol 0.006 mg/kg IM and Detomidine 0.0026 mg/kg IM (In adult female Asian elephants, 20mg Butorphanol and 10mg Detomidine have been effective)
- Sedation can be reversed as described above but is not necessary
- Alternatively, Xylazine (0.04–0.08 mg/kg) or other sedative agents (e.g. Azaperone at 0.024–0.038 mg/kg) can be used if Detomidine is unavailable.

EEHV Sample Monitoring And Collection Protocol

Recommended sample collection
For elephants that are: A) healthy, B) suspected to be infected, or C) post-mortem.

	HEALTHY	SICK	POST-MORTEM
1. Pictures	X	X	X
2. Blood smear	X	X	
3. Blood collection: i.Biochemistry and ELISA	X	X	X*
ii. Whole blood for PCR	X	X	X*
4. Trunk wash (or saliva)	X	X	
5. Lesion swab		X	X*
6. Tissue samples: i. Histopathology			X
ii. PCR			X
iii. All organs, including bone marrow			X

** If recently deceased.*
Description of sampling methods and supplies necessary for each method is listed below.

1. Pictures

Pictures of elephant before, during, after infection and/or post-mortem are recommended.

2. Blood smear

Blood smear for CBC and blood morphology

Supplies
- Clean microscope slides
- Wright-Giemsa stain
- 100% methanol

Directions
– Take one drop of blood and make a blood smear on clean microscope slide. Allow to dry. To prevent damage, fix the slide by dipping it in 100% methanol for one minute and allow to dry. Prepare slide using Wright-Giemsa stain for microscopic analysis.

3. Blood collection

i. Serum for biochemistry and antibody ELISA testing (i.e. red-top tube)

Supplies
- Red-top blood collection tubes
- 18-20 gauge butterfly scalpel set
- Centrifuge
- Disposable Pasteur pipettes
- Storage tubes (2 ml)
- -20°C freezer (or cooler with ice until access to -20°C freezer)

Directions
Collect blood in red-top tube. Keep upright for 5-10 minutes and allow to clot at room temperature. Centrifuge for 1500 g x 10 min. With pipette, gently aspirate out serum. Place serum (›2ml) into multiple storage tubes. Store samples at -20°C. Under field conditions, place under ice and transport to -20°C as soon as possible.

ii. Methods to preserve blood until receipt in laboratory for PCR

A. EDTA whole blood

1. If the whole blood can be transported to the laboratory within a day or two, no preservation is necessary (although keeping on ice or frozen is preferred).
2. If transport to the laboratory will not be within 48 hours, whole blood or ground up tissues can be placed in the wells of a GenPlate (#GVN3P-20, Gentegra.com) or FTA/FTA Elute Card (GE Healthcare Life Sciences, or Sigma-Aldrich) and dried at room temperature. This allows storage and shipment at room temperature or higher. DNA can be recovered from the GenPlate and FTA/FTA Elute Card for testing.

B. Buffy coat

Supplies
- Purple-top blood collection tubes
- 18-20 gauge butterfly scalpel set
- Centrifuge
- 20-gauge syringe
- 1-cc blue-tip or Pasteur tip micropipette
- Anti-DNase solution or anti-RNase solution:
  - DNAgard® Blood (Biomatrica, San Diego, CA; Sigma Aldrich 62501)
  - RNA *later* (Fischer Scientific AM7022; Sigma Aldrich R0901)
- Storage tubes (~2 ml)
- Cooler with ice
- -80°C freezer

Directions
Collect blood in purple-top blood collection tube. Gently invert ~10 times. Allow to sit for 1 hour at 4°C if possible; room temperature okay. Centrifuge at 1500 g x 10 min. Use 20-gauge syringe to remove plasma and discard. If possible, with 1-cc blue-tip or Pasteur-tip micropipette, carefully remove the clear buffy coat without disturbing the layer. Place buffy coat into equal amount of anti-DNase or anti-RNase solution. Place into multiple storage tubes (~2 ml). Keep at 4°C for shipment (can be kept up to 1 week). For long-term storage, keep at -80°C. If under field conditions, place under ice and transport to -80°C as soon as possible.

4. Trunk wash or saliva

Trunk wash (or saliva) for surveillance of healthy or clinically ill patients. Note: Trunk wash or saliva testing cannot be used for diagnosing a case of EEHV viremia; only blood can be used for diagnosis.

Supplies
- 60 ml sterile saline solution
- Clean ziplock bag
- 50 ml conical vials
- Centrifuge
- Disposable pipette
- Anti-DNase solution or anti-RNase solution:
  - DNAgard® Blood (Biomatrica, San Diego, CA; Sigma Aldrich 62501)
  - RNA *later* (Fischer Scientific AM7022; Sigma Aldrich R0901)
- Cooler with ice

Directions
Recover minimum of 30 ml of trunk wash fluid. Use 60 ml sterile saline solution infused into trunk, have elephant raise trunk, then collect saline into clean zip-lock bag. Transfer trunk wash into clean 50 ml conical vials. Centrifuge conical tubes at 900 g x 5 min. Carefully remove supernatant without disturbing pellet. Keep pellet. Place equal volume of anti-DNase or anti-RNase solution over pellet. Mix tube. Keep over ice for shipment. Freeze pellets at -80°C if banking for later processing.

5. Lesion swabs

If clinically ill patient has visible lesions, take swabs of lesions if possible. Note: Lesion swabs cannot be used for diagnosis of EEHV-HD; only whole blood (or tissues post-mortem) can be used for EEHV-HD diagnosis.

Supplies
- Swabs in tubes with anti-DNase solution or anti-RNase solution. Any of the following can be used to preserve the swabs until receipt by laboratory:
  - DNAgard® Blood (Biomatrica, San Diego, CA; Sigma Aldrich 62501)
  - RNA *later* (Fischer Scientific AM7022; Sigma Aldrich R0901; Qiagen)
  - RNAprotect Cell Reagent (#76526, Qiagen)
- Cooler with ice

Directions
Swab local lesions and store in anti-DNase solution, anti-RNase solution, or PBS. Preserve in -80°C until analysis. Under field conditions, place under ice and transport to -80°C as soon as possible.

6. Tissue samples (Post-mortem)

Sample all organs that exhibit haemorrhagic lesions.

i. Histopathology

Supplies
- Scalpel
- 10% buffer formalin
- Container

Directions
Sample all organs that exhibit haemorrhagic lesions. Tissue size: 1 cm^3. Store in 10% buffer formalin. Store 1 part tissue : 10 parts 10% buffer formalin. Okay to put all tissue samples in one container. Store at room temperature. Submit samples within 1 month of collection.

ii. PCR analyses (cPCR and qPCR)

Supplies
- Scalpel
- 50 ml conical tube
- Anti-DNase solution or anti-RNase solution:
  - DNAgard® Tissue (Biomatrica, San Diego, CA; Sigma Aldrich 62501)
  - RNA *later* (Fischer Scientific AM7022; Sigma Aldrich R0901)
- 96-99% molecular grade alcohol/regular alcohol
- Cooler/cooler with ice/-80°C (if not available, -20˚C) freezer

Directions
Sample all organs that exhibit haemorrhagic lesions. Tissue size: 1 cm^3. Place tissue in 50-ml conical tube. Storage and shipping preference: in order of high to lowest preference.

1) Place tissue in 5cc conical tube with equal volume of **RNA *later***. Transport over ice. Place -80°C (if not available, -20˚C) until analysis.
OR
Place tissue in 5cc conical tube for 1 gm tissue 1 ml of **DNAgard® Tissue** solution. Transport over ice and freeze it till the extraction

2) Place tissue in conical tube with equal volume of **96-99% alcohol** (prefer molecular

SAMPLE COLLECTION SUPPLIES

Red-top blood tube

Purple-top blood tube

Butterfly scalpel set (18-20 gauge)

Syringe (20 gauge)

Microscope slides

Disposable pipettes

Anti-DNase solution

Wright-Giemsa stain

Anti-RNase solution

Sterile saline solution

10% buffer formalin

Ziplock bag

96-99% alcohol

Alcohol

Swabs

Storage tubes (15 ml)

Conical vials (50 ml)

Scalpel

Storage tubes (2 ml)

Storage tubes (5 ml)

FTA Cards

GenPlates

Tissue container

Cooler with ice

-20°C freezer

-80°C freezer

Grade ethanol or HPLC grade ethanol). Transport over ice. Place -80°C (if not available, -20˚C) until analysis.

3) If 96-99% alcohol is not available, place tissue in regular ethanol and ship under ambient temperature.

4) If alcohol is not available, ship tissue in conical vial over ice.

iii. All organs including bone marrow

Essential organs for diagnosis

- Heart
- Liver
- Spleen
- Kidney
- All tissues with extensive haemorrhaging
- Blood
- Bone marrow (ribs)

Organs for research purpose

- Adrenal
- Penis
- Thymus
- Large intestine
- Pituitary
- Tongue
- Prostate
- Trachea
- Bulbo-urethral gland
- Lung
- Salivary gland
- Trunk cross section
- Brain
- Parathyroid
- Temporal gland
- Salivary gland
- Cecum
- Mammary gland
- Skin
- Seminal vesicles
- Diaphragm
- Muscle
- Small intestine
- Ureter
- Esophagus
- Nerve (sciatic)
- Spinal cord
- Urinary bladder
- Eye
- Ovary/testis
- Vaginal/urogen. canal
- Hepatic bile duct
- Epididymus
- Tonsillar lymphoid tissue
- Uterus/cervix
- Pancreas
- Stomach
- Thyroid gland
- Hemal node
- Lymph nodes (tracheobronchial, submandibular, tonsillar, mesenteric)

Supplies

- Scalpel
- Anti-DNase solution or anti-RNase solution:
  - DNAgard® Tissue (Biomatrica, San Diego, CA; Sigma Aldrich 62501)
  - RNA *later* (Fischer Scientific AM7022; Sigma Aldrich R0901)
- 5 ml storage tube
- Cooler with ice

Directions

If carcass is highly putrefied (>4 days old), take long bone and obtain the bone marrow. Place bone marrow (1-2 g) into equal amounts of anti-RNase solution in 5 ml tube. Keep at 4°C for shipment. Or place tissue in 5cc conical tube for 1 gm tissue 1 ml of DNAgard® Tissue. Transport over ice and freeze until extraction. For long-term storage, keep at -80°C. If under field conditions, place under ice and transport to -80°C as soon as possible.

Note

Tissues and blood can also be stored on GenTegra products and FTA cards for years at room temperature and can be shipped at room temperature. GenPlates are used for storing whole blood and tissue slurries; GenTegra-DNA (RNA) tubes are good for storing purified DNA (RNA). They do not currently have a storage system for plasma or serum. FTA cards are used for storing whole blood, serum, plasma, cultured cells, buccal cells, plasmids, tissue swabs and tissue smears. Products can be bought at www.gentegra.com for GenePlates and www.sigmaaldrich.com or www.gelifesciences.com for FTA cards. These products are well-tested and have been used for up to 20 years by the military, forensics and hospitals. Protocols can be found on the GenTegra website or email latimere@si.edu, or GELifeSciences and Sigma-Aldrich websites for information.

EEHV Diagnostic Testing

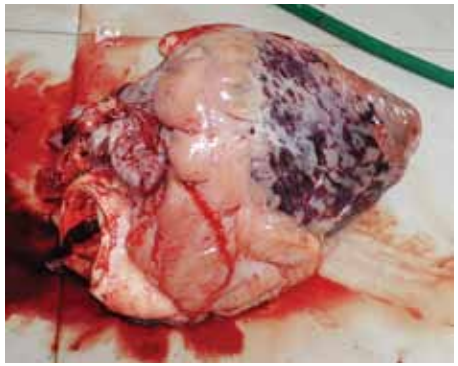
In Southeast Asia

Prompt EEHV-HD diagnosis is essential for optimal care of elephants. Molecular methods are the current test of choice. The gold standard is quantitative Polymerase Chain Reaction (qPCR); conventional PCR (cPCR) will suffice if qPCR testing is not available. EEHV qPCR is a rapid specific test that provides viral loads in blood, an important value for determining whether to treat with antivirals. cPCR can take somewhat longer and is only semi-quantitative, but has the advantages of less expensive reagents and equipment, requires less technical training and is a method that allows DNA sequencing of the PCR product, which is useful epidemiologically.

Asian elephants should be tested for EEHV1 (1A/1B), EEHV4, and EEHV5. qPCR assays for EEHV1, EEHV1A, EEHV1B, EEHV4 and EEHV5 are available (as well as assays for the EEHVs found in African elephants—EEHV2, EEHV3, and EEHV6). One of the qPCR tests for EEHV4 also detects EEHV3 and is sometimes referred to EEHV3/4, while another one detects EEHV4 only. If cPCR testing is being done, pan pol primers (reference below) and EEHV1, 3-4, and 5-specific primers should be used. Please check with the researchers listed under Resources below for the current preferred EEHV-specific primers.

Sampling

For an active case, EDTA whole blood is the desired sample; heparin blood can also be used. In a pinch, a clot from a serum separator tube can be tested. Ideally, the blood will be stored refrigerated or frozen until testing; although not ideal, untreated blood and tissue have been tested after several days at room temperature and were positive for EEHV. Post mortem samples to collect include blood, heart, liver, spleen, kidney and any tissues with extensive haemorrhaging.



Haemorrhagic heart lesions. Photo: Chatchote Thitaram

If refrigeration is not available, tissue samples can be stored in RNAprotect (#76526, Qiagen) or RNA Later (#76104, Qiagen). These products allow short-term storage at room temperature for transport to the laboratory. Blood/homogenised tissue can be stored in GenPlates (#GVN12P-20, GenTegra); purified DNA can be stored in GenTegra tubes (#GTD2100-S, GenTegra). Both GenTegra products allow room temperature or higher shipment and storage for years.

Check with the testing laboratory for their desired samples and sample handling.

Current labs

At this time, the following laboratories in Southeast Asia are able to test for EEHV. Check with the laboratory contact to set up testing. We are working to increase the testing capacity in SE Asia and hope to have EEHV qPCR testing available soon in SE Asia.

India
Kerala Veterinary and Animal Sciences University - Dr. Arun Zachariah
Email: zacharun@gmail.com

Indonesia
Medika Satwa Lab - Dr. Adin Priadi
Email: adinpriadi@yahoo.com

Singapore
DSO National Laboratories - Dr. Boon-Huan Tan
Email: tboonhua@dso.org.sg

Thailand
Chiang Mai University
Dr. Chatchote Thitaram
Email: cthitaram@gmail.com

Kasetsart University
Dr Supaphen Sripiboon
Email: ssripiboon@gmail.com

Mahidol University
Dr Witthawat Wiriyarat
Email: witthawat.wir@mahidol.ac.th

The Veterinary Research and Development Centre (North-eastern region)
Bopit Puyati
Email: bpuyati@gmail.com

References for qPCR and cPCR

cPCR

1. GARNER, M. M., HELMICK, K., OCHSENREITER, J., RICHMAN, L. K., LATIMER, E., WISE, A. G., MAES, R. K., KIUPEL, M., NORDHAUSEN, R. W., ZONG, J. C. & HAYWARD, G. S. (2009) Clinico-pathological features of fatal disease attributed to new variants of endotheliotropic herpesvirus in two Asian elephants (Elephas maximus). Veterinary Pathology 46, 97-104

2. LATIMER, E., ZONG, J.C., HEAGGANS, S.Y., RICHMAN, L.K., & HAYWARD, G.S. (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). Veterinary Microbiology 147 (1-2), 28-41

qPCR

1. STANTON, J. J., ZONG, J. C., LATIMER, E., TAN, J., HERRON, A., HAYWARD, G. S. & LING, P. D. (2010) Detection of pathogenic elephant endotheliotropic herpesvirus in routine trunk washes from healthy adult Asian elephants (Elephas maximus) by use of real-time quantitative polymerase chain reaction assay. American Journal of Veterinary Research 71, 925-933

2. STANTON, J.J., NOFS, S.A., PENG, R., HAYWARD, G.S., & LING, P.D. (2012) Development and validation of quantitative real-time polymerase chain reaction assays to detect elephant endotheliotropic herpesviruses-2, 3, 4, 5, and 6. Journal of Virological Methods 186 (1-2), 73-77

Serology

Serology cannot be used for EEHV diagnostics, but may be useful for determining serostatus of the herd. Currently, two groups are working on serological assays for EEHV:

1. Dr Byron Martina's group is working on a gB-based EEHV1 ELISA (VAN DEN DOEL PB, PRIETO VR, VAN ROSSUM-FIKKERT SE, SCHAFTENAAR W, LATIMER E, HOWARD L, CHAPMAN S, MASTERS N, OSTERHAUS ADME, LING PD, DASTJERDI A AND MARTINA B. A novel antigen capture ELISA for the specific detection of IgG antibodies to elephant endotheliotropic herpes virus. BMC Veterinary Research 2015, 11:203 doi:10.1186/s12917-015-0522-6)

2. Dr Gary Hayward's group is working on a chip assay to differentiate between the subtypes of EEHV.

Trunk wash and swab testing

Trunk washes and swabs collected over a 1-2 month period may be useful for elucidating what EEHV types are in a herd, with the caveat that only EEHVs that are shed during the collection period will be detected. Latent EEHVs will not be detected by this testing. Check with your preferred testing laboratory to see if they offer trunk wash and/or swab testing.

Helpful resources

1. Arun Zachariah: zacharun@gmail.com
2. Supaphen Sripiboon: ssripiboon@gmail.com
3. Erin Latimer: latimere@si.edu
4. Willem Schaftenaar: w.schaftenaar@rotterdamzoo.nl
5. Lauren Howard: lhoward@sandiegozoo.org
6. Gary Hayward: gary.s.hayward@gmail.com
7. Paul Ling: pling@bcm.edu
8. Ellen Wiedner: Ebwvmd@yahoo.com
9. Eehvinfo.org

EEHV Evaluation Form [OPD card]

OPD. No. _____
Date _____

Elephant's name _____ Microchip No. _____
Sex ☐Male ☐Female Age _____ (month/year)
Birth Date _____ ☐ Wild born ☐ Captive born ☐ Hand reared ☐Parent reared
Type of work ☐ Zoo ☐ Tourism ☐ Logging ☐ Patrol ☐ Other _____
Mahout's name _____ Owner's name _____
Address _____
_____ Tel. _____
Weight _____kg. ☐ True ☐ Calculated from bod measurements ☐ Estimated
Nutrition status ☐ Obese ☐ Good ☐ Fair ☐ Poor

History

Is this elephant still parent-fed? ☐ Yes ☐ No ☐ Unknown Weaning age _____ year
Recent transport ☐ Yes ☐ No ☐ Unknown
When _____ From _____ To _____

Unusual event

- Extreme environmental changes ☐ Yes, when_____ ☐ No ☐ Unknown
- Human-animal interaction ☐ Yes, when_____ ☐ No ☐ Unknown
- Management changes ☐ Yes, when_____ ☐ No ☐ Unknown
- Mahout changes ☐ Yes, when_____ ☐ No ☐ Unknown
- Training procedure changes ☐ Yes, when_____ ☐ No ☐ Unknown
- Herd status changes ☐ Yes, when_____ ☐ No ☐ Unknown
- Others _____

Exposure history Has this elephant been exposed to the following?

- EEHV confirmed cases ☐ Yes, when_____ ☐ No ☐ Unknown
- Other ill animals ☐ Yes, when_____ ☐ No ☐ Unknown
- Wild elephant ☐ Yes, when_____ ☐ No ☐ Unknown

Medical record

- Vaccination history _____
- Deworming history _____
- Previous illness, testing and treatment history _____

EEHV Evaluation Form [OPD card] 2/3

Clinical observation

Behavior changes

- Eating ☐ Normal ☐ Abnormal ☐ Not observed
- Drinking ☐ Normal ☐ Abnormal ☐ Not observed
- Defecation ☐ Normal ☐ Abnormal (constipation/diarrhea) ☐ Not observed
- Urination ☐ Normal ☐ Abnormal ☐ Not observed
- Sleeping ☐ Normal ☐ Abnormal ☐ Not observed
- Locomotion ☐ Normal ☐ Abnormal ☐ Not observedd
- Activity/play behaviour ☐ Normal ☐ Abnormal ☐ Not observed

EEHV related signs

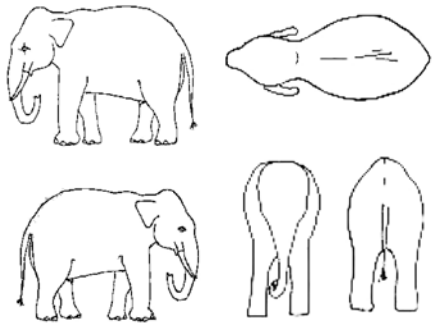
- Blood-shot eyes ☐ Normal ☐ Abnormal ☐ Not observed
- Oral mucosa - Lesion: ☐ Present ☐ Not present ☐ Not observed
  - Colour: _____
- Temporal gland swelling ☐ Present ☐ Not present ☐ Not observed
- Head, face or neck swelling ☐ Present ☐ Not present ☐ Not observed
- Mobility/lameness ☐ Present ☐ Not present ☐ Not observed
- Visible skin lesion ☐ Present ☐ Not present ☐ Not observed
- Tongue cyanosis ☐ Present ☐ Not present ☐ Not observed

Physical examination

HR _____ best/min Pulse _____ time/min RR _____ best/min

Temp. _____ °C / °F MM _____ CRT _____ second

Lesions



Other examination _____

EEHV Evaluation Form [OPD card] 3/3

Sample Collection

☐ Whole Blood ☐ Serum ☐ Feces ☐ Trunk wash ☐ Tissue ☐ Swab from _____

☐ Other _____

Collected for _____ Date _____

Recommended sample collection for EEHV diagnosis

Aims	Test method	Whole Blood	Serum	Swab	Trunk Wash	Tissue
Presence of virus**	PCR	X		X	X	X
Viral load	qPCR	X				
Haematology		X				
Chemistry			X			
Serology			X			

*** In active case of EEHV, blood samples (or tissue samples from dead elephants) are recommended. Swabs and trunk wash are not likely to be positive in an active case, but can be used for monitoring shedders in a herd.*

Camp Form

Current visit date _____ Previous visit date _____

Camp's name _____ Address _____

_____

Contact number _____ Email _____

Type of management ☐ Zoo ☐ Tourism ☐ Logging ☐ Patrol ☐ Other _____

Average work hours per day _____ hours

Number of elephant: Total ___ Babies ___ (newborn to 1 years old)

Young ___ (1-10 years old) Adult ___ (›10 years old)

Changes in herd status from last visit; (please specify number of animal, location and date)

Birth _____ Death _____ Arrival _____ Departure _____

Feeding system (please specify type and amount of food) _____

_____

_____

_____

Unusual events record (i.e. flooding, drought, disease outbreak) _____

_____

_____

_____

Frequency of your vet visit _____ Previous vet visit date _____

Any concerns from your previous vet visit _____

_____

APPENDIX 2

GUIDELINE

Placement of an intravenous cannula into an ear vein in a juvenile Asian elephant

Source: ZSL Whipsnade Zoo

Aseptic preparation of the ear pinna after numbing the area with sedative cream one hour previous.

Insertion of the cannula. If needed, cut down skin to create easy vein access for catheter

Fixing the cannula to the skin with skin glue.

Waiting for the glue to dry.

Attaching the giving set and creating a loop to prevent removal of cannula on movement of the head.

Fixing the giving set to the head.

Boluses of medication can be given swiftly through giving set ports, e.g. fluids and antibiotics.

Antivirals, fluids and nutraceuticals can be given slowly.

APPENDIX 3

GUIDELINE

How To Make A 'Plasma Extractor'

If you do not have one of these manufactured Plasma Extractors, you can make one!

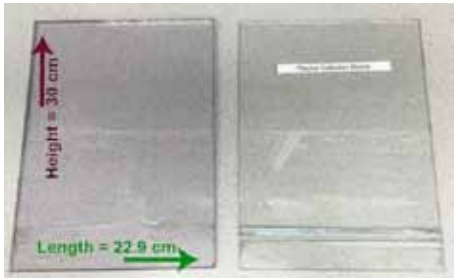
Materials

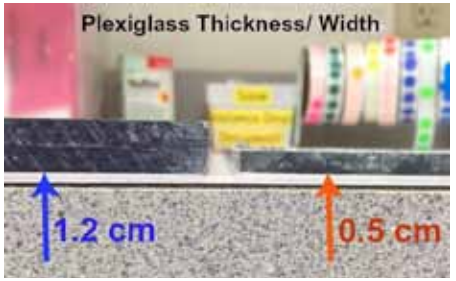
- Two pieces of Plexiglas
- Duct tape

Step 1

Prepare two pieces of Plexiglas to match the following measurements. Note difference in thickness to provide sturdiness.

- 1st piece: Length= 22.9 cm, Height= 30 cm, Width= 1.2 cm
- 2nd piece: Length= 22.9 cm, Height= 30 cm, Width= 0.5 cm





Step 2

Align the pieces of Plexiglas together evenly and hold them together. Then wrap duct tape around the bottom ends of the pieces to keep the Plexiglas together.

Make sure that you can pry the untaped edges apart. The Plexiglas must be able to part wide enough for a full bag of whole blood to fit in between the pieces.

In-house Plasma Separation Procedure For Elephants

Design elaborated by Houston Zoo, Inc.

Materials

- Sterile blood collection bag containing anticoagulant citrate phosphate dextrose adenine solution (CPDA-1) USP for collection of 450 ml of whole blood. Establish weight of the empty plasma bag prior to collection (*See Procedure 13*).
- Refrigerator with temperature 0-4 °C
- Scale (g)
- Plasma Extractor (*See previous page on how to make one*)
- 1-2 Kelly or Crile haemostats
- 1 smooth-jaw haemostat
- Plasma Extractor – handmade vs. commercial
- Hand-held blood bag tube stripper/cutter/sealer tool
- 4 plastic clamps
- Metal clips. Establish weight of a single clip. (*See Procedure 13*)

Procedure

1. Receive bag of whole blood with citrate phosphate dextrose adenine solution (CPDA-1) USP coagulant.

2. Hang the bag in refrigerator for 6-24 hours to allow for gravitational separation of plasma from red cells. Temperature should be between 0-4 °C. (Figure A)

3. Carefully remove the blood bag from the refrigerator. Avoid re-suspending the separated red blood cells into the plasma (minimise abrupt motions when handling the collection bag).

4. Begin plasma separation process by inserting the blood bag into:
a.) the "Plasma extractor" or
b.) 2 pieces of Plexiglas duct-taped together. Lay the empty plasma bag beside the extraction apparatus. (Figure B)

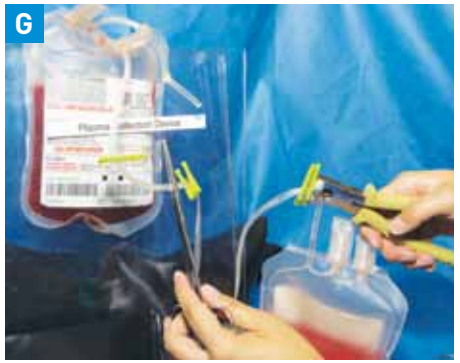
5. Break the plastic barrier piece connecting the blood bag to the empty plasma bag. (Figure C)

6. With one hand, slowly apply gradual pressure to the Plexiglas pieces and with the other hand, use haemostats to hold the connection tubing. The plasma from the blood bag should be flowing into the plasma bag. Be cautious of disrupting the sediment. (Figure D)

7. When most of the plasma has separated into the plasma bag, quickly clamp off the connecting line with haemostats. Add secondary plastic clamps for extra security. (Figure E)

8. Using the handheld stripping tool, begin easing the remaining plasma in to the collection bag. (Figure F)

9. Using another set of haemostats, clamp the line closer to the plasma bag, leaving approximately 30 cm of tubing. Add secondary plastic clamps if necessary. (Figure G)



10. Cut the connecting line so that the plasma bag separates from the blood bag.

11. To properly seal the plasma bag for storage, tie 1-3 knots at the open end of the tubing. (Figure H)

12. Make a loop with the tubing and apply 2-3 evenly spaced metal clips. (Figure I) Slide the first metal clip as close to the bag as possible. Clamp the clips down with the multi-tool. (Figure J)

13. Weigh the full plasma bag. To determine actual plasma volume, subtract established materials weights (empty plasma bag and metal clips) from the weight of the full plasma bag.

14. Label the plasma bag with animal ID number, collection date and plasma volume.

15. Store the plasma in a freezer (preferably -80°C). However, use fresh plasma for treating EEHV-HD as freezing will activate the thrombocytes, making them useless for EEHV-HD treatment.

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NOTES

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