

# Elephant Endotheliotrophic Herpes Virus (EEHV) Protocol

# **Elephant Endotheliotropic Herpes Virus**

**Jonathan Cracknell BVMS CertVA MRCVS**

<b>INTRODUCTION.....</b>	<b>2</b>
<b>AETIOLOGY .....</b>	<b>2</b>
<b>HISTORY OF HERPES VIRUSES IN ELEPHANTS .....</b>	<b>4</b>
<b>EEHV EPIDEMIOLOGY .....</b>	<b>11</b>
<b>EEHV PATHOLOGY .....</b>	<b>12</b>
<u>CLINICAL PATHOLOGY .....</u>	<u>12</u>
<u>GROSS PATHOLOGY.....</u>	<u>13</u>
<u>HISTOPATHOLOGY .....</u>	<u>16</u>
<u>ELECTRON MICROGRAPHY .....</u>	<u>17</u>
<b>EEHV CLINICAL SIGNS.....</b>	<b>18</b>
<b>THERAPEUTIC PROTOCOLS .....</b>	<b>20</b>
<u>ANTI-HERPES DRUGS .....</u>	<u>20</u>
<u>ANTIBIOTICS.....</u>	<u>22</u>
<u>ANALGESIA.....</u>	<u>23</u>
<u>FLUID THERAPY.....</u>	<u>24</u>
<u>OXYGEN THERAPY.....</u>	<u>28</u>
<u>DIURETICS.....</u>	<u>29</u>
<u>PERICARDIAL TAP.....</u>	<u>30</u>
<u>VASOCONSTRICTORS .....</u>	<u>30</u>
<b>DIAGNOSTIC TESTING.....</b>	<b>31</b>
<u>POLYMERASE CHAIN REACTION (PCR) .....</u>	<u>31</u>
<u>ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA).....</u>	<u>32</u>
<b>HUSBANDRY AND MANAGEMENT GUIDELINES .....</b>	<b>34</b>
<u>DAILY MANAGEMENT OF THE HERD BY THE KEEPERS .....</u>	<u>34</u>
<u>MANAGEMENT BY THE VETERINARY DEPARTMENT .....</u>	<u>34</u>
<u>MANAGEMENT BY THE CURATOR OF MAMMALS .....</u>	<u>35</u>
<u>TRANSLOCATIONS OF ELEPHANTS INTO A COLLECTION .....</u>	<u>35</u>
<u>TRANSLOCATION OF ELEPHANTS TO OTHER COLLECTIONS .....</u>	<u>36</u>
<u>RISK PERIODS .....</u>	<u>36</u>
<u>FREE CONTACT VERSES PROTECTED CONTACT.....</u>	<u>36</u>
<b>REFERENCES .....</b>	<b>37</b>
<b>APPENDIX A:ALTERNATIVE NAMES FOR EEHV .....</b>	<b>40</b>
<b>APPENDIX B: FAMCICLOVIR DOSAGES AND COST .....</b>	<b>41</b>
<b>APPENDIX C: ACYCLOVIR DOSAGES AND COST .....</b>	<b>42</b>
<b>APPENDIX D: NORMAL ELEPHANT PHYSIOLOGICAL PARAMETERS .....</b>	<b>43</b>
<b>APPENDIX E: SAMPLE REQUIREMENTS.....</b>	<b>44</b>
<b>APPENDIX F: TESTING FACILITIES.....</b>	<b>45</b>
<b>APPENDIX G: PROPOSAL FOR USE OFANTIVIRAL AGENTS .....</b>	<b>46</b>

## Introduction

Elephant Endotheliotropic Herpes Virus (EEHV), also known as Proboscis  $\beta$ -3-herpes virus, was first described in 1990 by Ossent et al and has now become one of the most important emerging infectious diseases of elephants. The virus has resulted in massive mortalities and after reproductive management is the most important limitation for successful management of captive populations. It has been identified in many European and American captive collections, but the World Wide significance has yet to be determined. This report attempts to identify the current knowledge regarding Elephant Endotheliotropic Herpes Virus, with an emphasis on collection management and possible treatment strategies for individual cases.

## Aetiology

Elephant Endotheliotropic Herpes Virus is classed as a member of the  $\beta$ -herpesviridae. This group includes the cytomegaloviruses but is evolutionary distinct from the more commonly know mammalian alpha and gamma herpes viruses.

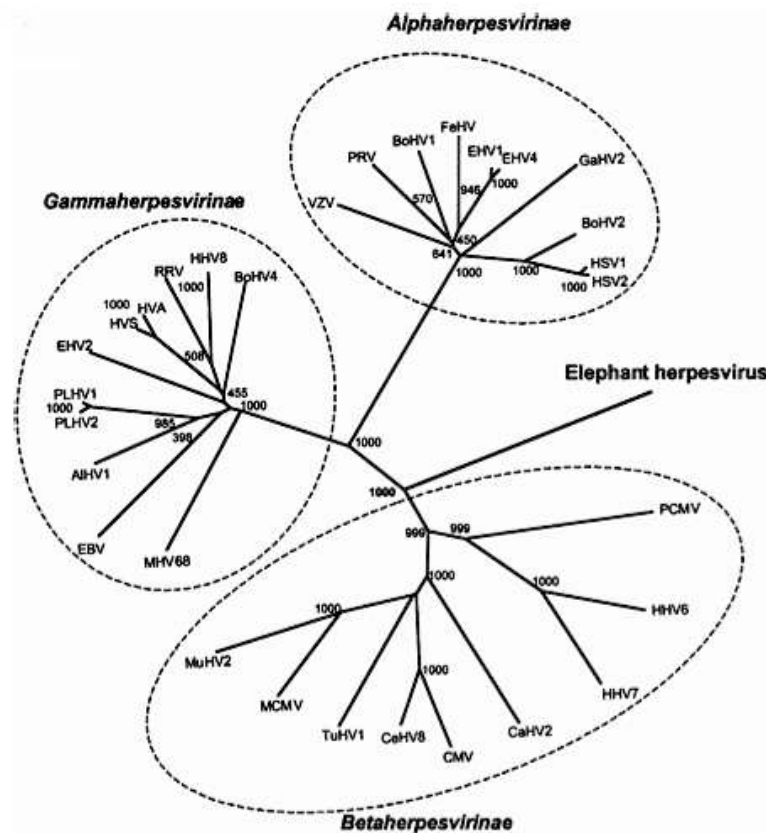


Fig 1. Phylogenetic analysis of gB and DPOL of EEHV-1 (From Ehlers, et al, 2001).

Divergence of alpha herpesviruses from beta and gamma herpesviruses occurred approximately 374-413 million years ago, whilst the divergence of beta from gamma herpesviruses was approximately 331-351 million years ago, with the earliest divergence within rhadinoviruses (Gamma 2, EHV2 & HHV8) occurring 70-73 million years ago (McGeoch et al, 2005). What this means clinically is that the elephant herpesviruses are an ancient virus that have been present in a natural reservoir for a long period despite this disease having only been recognized for the first time in the last 10 years or so.

Herpes literally means creeping, which refers to the latent infections that are usual in these viruses. Their genome is a single linear molecule of dsDNA and the virus replicates in the nucleus and matures by budding through the nuclear membrane where they acquire an envelope. In the case of EEHV the intracytoplasmic virus particles are 120nm in size, with intranuclear capsids 70-92nm in size (see Figure 2). Pathology follows the replication of the virus in target organs resulting in lysis of the cells and subsequent dysfunction of those organs. A feature of all herpesviruses is lifelong persistence of the virus in the body, usually in a latent form (Fenner et al, 1993).

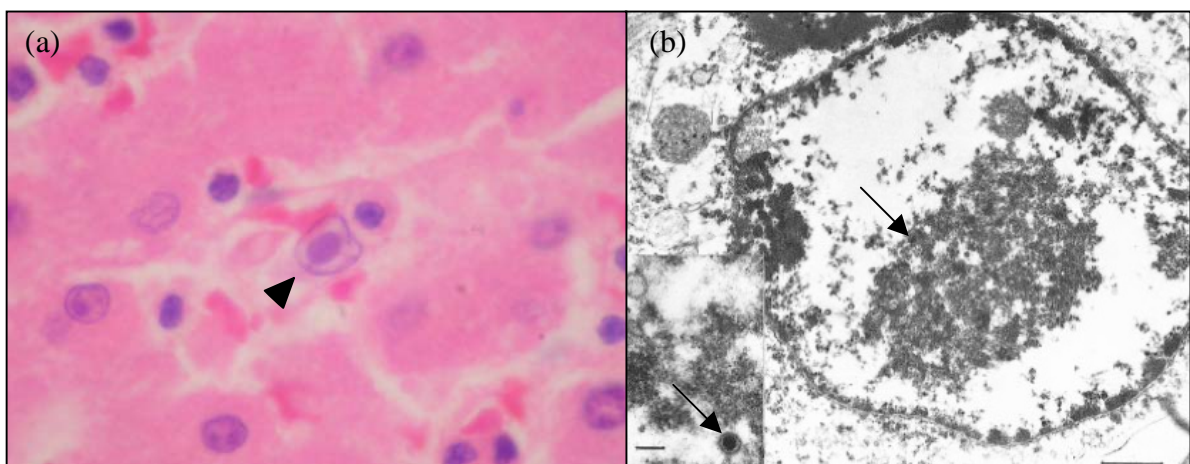


Fig 2. (a) Intranuclear inclusion body (arrowhead) of hepatic sinusoidal endothelial cell infected with EEHV. (Courtesy of Mark Stidworthy, IZVG). H & E stain. (b) Electron micrograph of an intranuclear inclusion body in the liver with the individual virions being clearly visible (arrow). Bar 1 $\mu$ m. (From Ehlers et al, 2001).

## **History of herpes viruses in elephants**

Previous to the 1980s little tissues or blood samples remain to allow retrospective analysis. Descriptions prior to 1980 exist but the majority of the elephant herpes virus work is recent, and is mainly due to the advent of appropriate diagnostic tests that only came in to use at the end of the last century.

Herpes viruses were first identified as incidental inclusions in pulmonary nodules in asymptomatic African elephants (*Loxodonta africana*) in the early 1970s (Basson et al, 1971, McCully et al, 1971). Isolation was not confirmed but was based on ultrastructural findings. These were later identified as being caused by EEHV2.

Leach (1983) describes a non-irritant blister-causing vaginal virus of African elephants in a mixed species herd that later caused grossly similar lesions in Asian elephants (*Elephas maximus*). This may have been herpes related but was not confirmed.

In 1986 Jacobson described skin lesions that were contained intranuclear inclusions that were morphologically consistent with herpes viruses. A group of 33 African elephants aged 2-8 years were transported from Zimbabwe to Texas and then to Florida. Seven of the animals had nodular skin lesions on the trunk, head, limbs and lateral body when acquired. Eight months later a further 63 elephants were transported directly to Florida from Zimbabwe. None of these animals had lesions when purchased over the next 18 months 20 elephants from the second group developed similar lesions. The lesions were 0.7-1.4cm in diameter and formed inverted cutaneous papillomas (see figure 3). All contained intranuclear inclusion bodies (Jacobson et al, 1986).

Material was retained from the lesions and they were found to retrospectively be positive for EEHV on PCR (Richman et al, 1999).

1988 Pilaski described herpes virus related nodular skin lesions in Asian elephants.

The first reported death attributed to a herpes virus occurred in an Asian elephant and was reported by Ossent et al (1990) with the loss of Lohimi, a three year old female Swiss circus elephant, in 1988. In the morning she had appeared normal but in the evening she was assessed due to abnormal trunk carriage. She was dull, had a swollen, cyanotic tongue, and swelling of the head and neck. Her temperature was



Fig 3. Inverted cutaneous papillomas that were later confirmed to be caused by EEHV.

normal. She died 2 hours following examination. Extensive haemorrhages were found throughout, especially within the heart, stomach, trunk musculature, and the intestines. There was severe widespread oedema, and the pericardial sac contained 2 litres of colourless fluid. Histologically intranuclear inclusions were found within the sinusoidal cells of the liver as well as endothelial cells from multiple tissues.

Comparisons were drawn to similarities of the Varicella-zoster virus in neonatal or deficient cell-mediated immunity humans, which has a similar pathological picture despite this being an alpha herpes virus (taxonomic classification was not available at this point). Virus isolation was attempted but failed.

That same year, following the loss of the 3 year old Asian elephant, Ossent collaborated with Metzler et al (1990) to assess the prevalence of infection with herpes virus in the contact animals and elephants from other herds. At this time PCR nor ELISA were available towards EEHV and it was decided to use tests for bovine herpes virus 1 (BHV1), 2 (BHV2), and 4 (BHV4) as a close comparison.

BHV1 and BHV2 are alpha herpes viruses, whilst BHV4 is a gamma herpes virus. Sera were tested for neutralising antibody to BHV1, BHV2 and BHV4 (figure 4). All the animals tested were Asian elephants and included animals from the circus where Lohimi died, Zurich Zoo and animals freshly arrived from Burma. All of the animals were clinically normal except for Lohimi. The

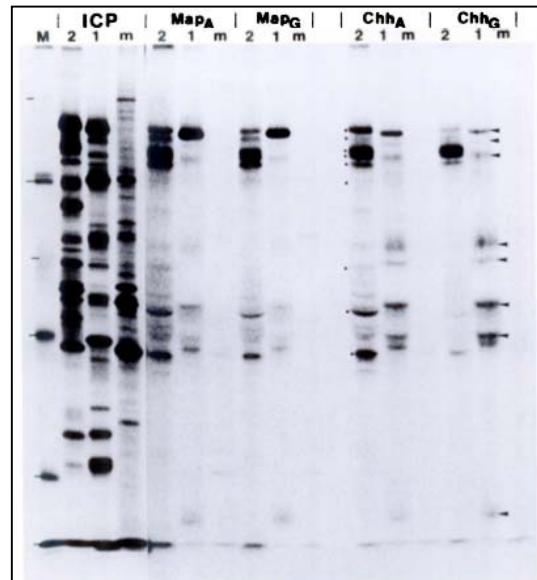


Fig 4. SDS-PAGE results showing immunoprecipitation products using serum from the elephants in Metzler et al paper. BHV1 (lane 1) and BHV2 (lane 2) are represented.

reactions were weak but were considered seropositive. BHV2 was the strongest overall but Lohimi reacted strongest to BHV4. As BHV has not been isolated outside of Artiodactylids the positive results were deemed significant but it was assumed that the positive response was to a similar, heterologous to BHV with cross-reactive antibodies. At this time it was thought that a virus similar to BHV2 (an alpha herpes virus) was the most likely candidate but artefact such as herpes simplex infection from humans was not ruled out as a contaminant. This paper represented the first attempt to look at the serology of herpes virus infections in elephants as well as creating the potential for screening programmes. However it was assumed that as there were no clinical signs in any of the elephants that elephant herpes virus was unlikely to be a primary pathogen and that the death of Lohimi was an unusual occurrence.

In 1995 at the National Zoo, Washington D.C., Kumari, a 16 month old female calf died and became the index case for EEHV. This was first reported in 1996 (Richman et al). In the first report the clinical signs were described as intermittent anorexia, lethargy, decreased stool production, mild colic, and lingual cyanosis terminally. Mild leukopaenia and mild renal dysfunction were found on haematology and biochemical tests respectively. Post mortem findings included oral ulcers, mesenteric and serosal haemorrhages and oedema, extensive cardiac haemorrhages, and a swollen pale red liver. Basophilic intranuclear inclusion bodies were found in the capillary endothelial

cells and electron microscopy confirmed the presence of particles morphologically similar to herpes virus. Three elephants in this herd had serum antibodies reactive to BHV. In addition a retrospective review of the studbook mortality records identified a further six additional cases; five of the animals ranged from 18 months to 7 years, and one was 26 years old. This case was instrumental in identifying the need for further characterisation of the virus and determination of the epidemiological aspects of the disease, which allowed the development of EEHV specific PCR and ELISA techniques that would not be discussed for another three years later.

Following on from Metzger et al's work Narayana et al (1997) investigated the prevalence of antibodies to BHV1 and BHV2 in 109 Asian elephants in India in the wild and in zoological collections. They did not look at BHV4. Four animals had titres against BHV1 and twenty five had titres to BHV2 which was similar to the results seen with Metzger's work except that he saw 100% seropositivity and similar conclusions were drawn about the elephant herpesvirus being similar to BHV2.

By 1999 it was well recognised that herpes virus infections were having a detrimental affect on both African and Asian elephants in North American and European zoological collections. In August 1998 Kiba, an eleven year old male housed at the Zoological Garden at Berlin but previously from Houston Zoo, Texas died following a EEHV infection. Virology techniques confirmed that the DNA sequence obtained showed an identity 97% with the terminase sequence of the EEHV described in the USA. This case was used for genetic and ultrastructural characterisation of EEHV that was utilised to set up the German PCR assay for EEHV (Ehlers et al, 2001). Implications for management and breeding of elephants were discussed. Texas had had three confirmed cases of EEHV between 1988 and 1991 and this case showed the possibility of institutional transfer being a cause for the dissemination of EEHV.

Failing to culture the virus clinicians turned to molecular techniques and with the

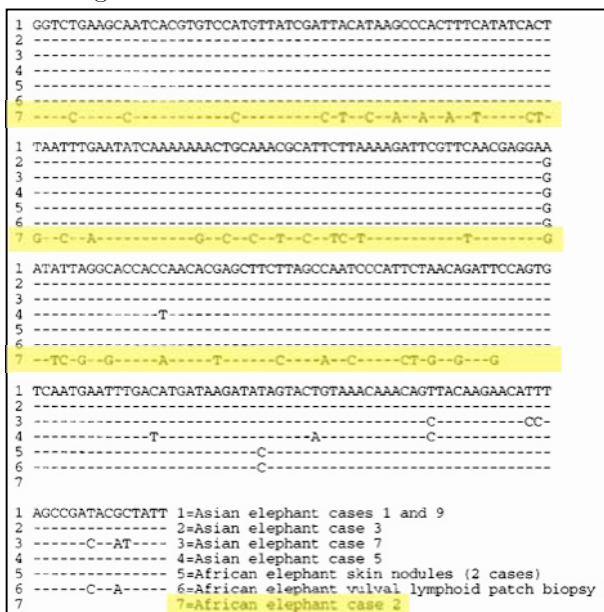


Fig 5. Comparison of DNA sequences from terminase PCR products from the heart tissue of 4 Asian elephants and 1 African that died of EEHV. The dashes indicate identical nucleotides to the top line reference case: NOTE the differences of case 7 showing the terminal EEHV African elephant case but the similarity of the non lethal African lesions to the fatal Asian lesions (lines 1-6)

development of diagnostic tests Richman et al first described the PCR in 1999. At this point ten animals had been confirmed to have had EEHV in USA; 7 Asian elephants died, 1 survived and 2 African animals died. Here the first description of the capillary endothelium as a predilection site for EEHV was described and that the cause of death was due to acute myocardial failure from capillary injury and leakage due to endothelial cell damage caused by the presence of herpes virus. The paper described the identification of two separate EEHV viruses for the first time: one that was fatal to African elephants and one that was nonlethal to African elephants but when found in Asian elephants were lethal. The authors

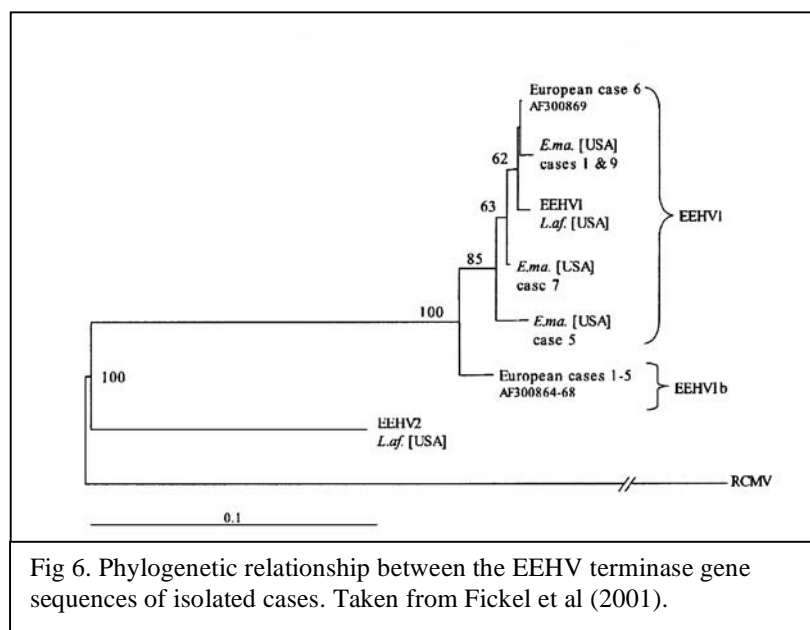
also described that the virus had pathology and histomorphology similar to the beta herpes viruses or a new previously unrecognised group of herpes viruses. The authors made an important observation: in collections where there had been deaths from EEHV they had mixed African and Asian elephants together and this could be a possible route of infection. This was further strengthened by the identification that the EEHV virus responsible for the Asian elephant deaths had the same terminase gene as that found in the EEHV responsible for the African inverted cutaneous papillomas and in vulvar lymphoid patches both in the USA and Kruger National Park. This virus was distinct from that causing the death of the African animals. It was also hypothesised that a nonlethal EEHV existed in Asian elephants that was fatal to Africans.

Now a test was available to identify infected animals or confirm the cause of death but there still lacked an ability to be able to screen infected or latent animals: only currently sick or viraemic animals could be screened.

Following on from the work over the last five years Richman et al (2000) reviewed and described the clinical and pathological findings of EEHV for several animals all confirmed by PCR to have died from EEHV. The descriptions can be seen in the pathology section.

In addition Richman et al (2000b) produced a detailed review of the current knowledge on EEHV with recommendations for future research and management.

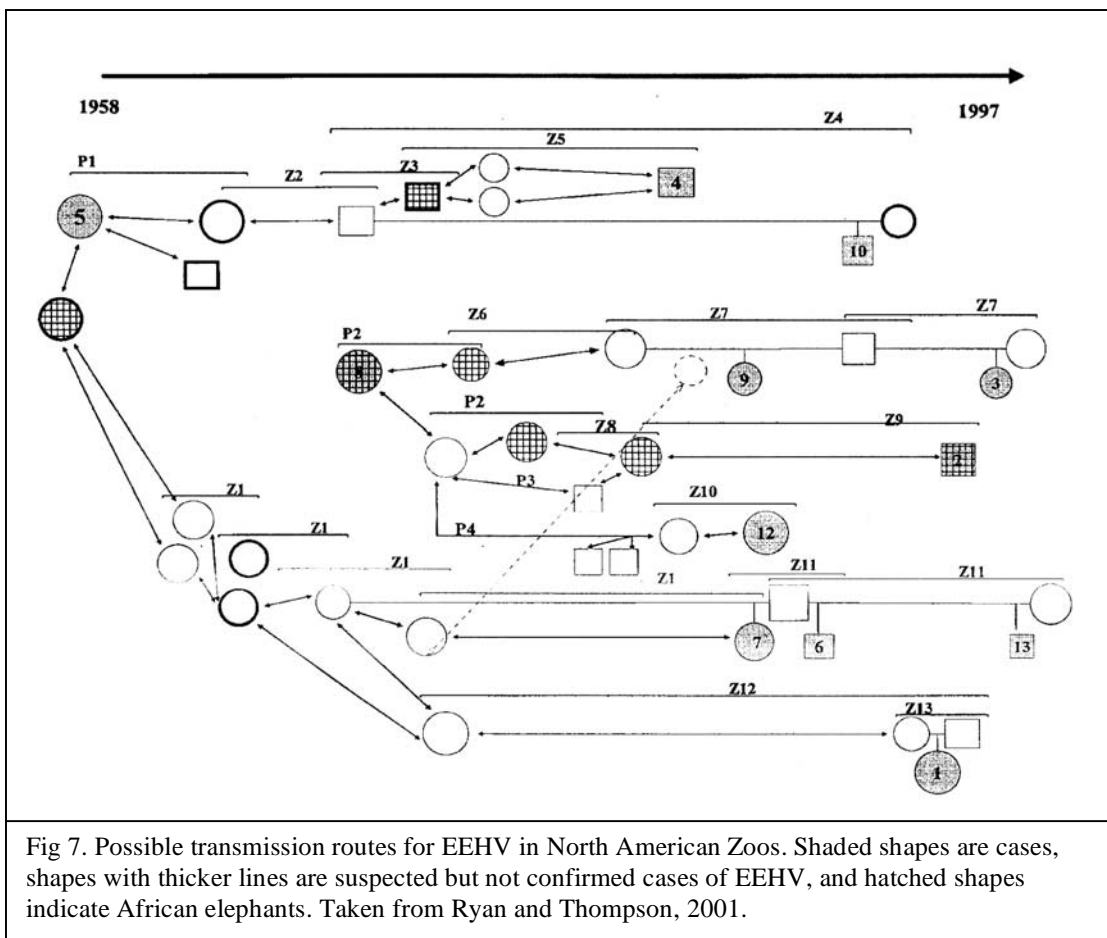
2000 also saw the beginning of the development and publication of therapeutic regimes. Schmitt first reported the use of famciclovir in 1998 in the journal of the Elephant Managers Association. However widespread publication did not occur until 2000 (Schmitt et al) where the therapeutic regimes for the surviving animals were published. These included the clinical use of the PCR as a monitoring tool for response to therapy. Further therapeutic descriptions followed with the treatment of an Asian elephant bull (Schaftenaar et al, 2001), and the appropriate use of famciclovir in suspected cases of EEHV (Schaftenaar and Mensink, 2005). The pharmacokinetics for famciclovir being described in 2003 (Isaza et al).





Fickel et al (2001) differentiated the two types of EEHV into three types: EEHV1, EEHV1b, and EEHV2. Respectively EEHV1 was found in both Africans and Asians, EEHV1b Asians only, and EEHV2 Africans only (Figure 6). One of the animals in the EEHV1b cluster was wild caught in Malaysia, which possibly indicates that Asian elephants have an endogenous EEHV that exists independent of African elephant reservoirs.

Ryan and Thompson (2001) produced a model to predict the disease risk of Inter-institutional transfer of specimens in cooperative breeding programs using EEHV and the elephant species survival plan. They reviewed the AZAA SSPs and studbook mortality records and correlated all of the deaths and movements during 1983 until 1996 to the then 12 cases of EEHV that had occurred in North America. Although based on a theoretical model their findings showed that concerns of the risk of disease transmission associated with animal moves is justified, especially when considering EEHV. Moves for Asian elephants during the study period ranged from 18-30 transfers/year and for Africans 10-66/year in North America. When correlated to the 12 confirmed cases they identified between 2-14 direct contacts/year and 2-270 indirect contacts between the cases and other elephants. Out of these indirect contacts 30% were stillborn calves, and 47 contacts were with born elephants (15 of which were common to two or more of the cases). A possible transmission route is indicated in figure 7. They highlighted that despite low frequency of movement of large animals it can still result in a large number of potential disease exposures. With EEHV this is further complicated by the lack of knowledge regarding the circumstances where disease becomes infectious or even fatal, risk factors, or routes of transmission.



Hildebrandt et al (2001) looked at the prevalence of EEHV in Asia. They considered the spread of EEHV may have occurred following the movement of African elephants to Thailand where a large population of Asian elephants resides. Eighty three elephants were assessed using PCR on tissues, however they all tested negative for EEHV. However this does not rule out the possibility that EEHV has been introduced into the Thai wild or captive elephant populations.

Montali et al (2001) identified that despite being thought of as a captive disease of elephants following the mixing of African and Asian elephants fatal disease of Asian elephants had occurred in North America, Europe and Asia without direct exposure to African elephants.

Fickel et al (2003) knowing that the PCR was limited to detecting active infection and unsuitable for screening for carriers or latent infections looked at glycoprotein B (gB) for serological assay development. Glycoprotein B is an envelope protein known to induce virus neutralising antibody in other herpes virus infections. The protein is relatively homologous across the other herpes viruses and determines the rate of entry into a cell, membrane anchorage, syncytial plaque formation, and antigenicity. They compared the gB gene from 5 Asian elephants with EEHV (from a possible 9 cases). They found 88% similarity between EEHV1 and EEHV1b that they suggested was evidence that it was unlikely that EEHV originated in African elephants and was passed to Asian as this would require massive, fast evolution of the EEHV gB in the Asian animals. The authors went on to say that gB was likely to be an important antigenic target for the immune system and that high selection pressure had resulted in the sequence variation. This is a useful starting point for the development of a serological assay. In addition the variation in gB indicated that the model as designed by Ryan and Thompson (2001) was probably too simple as a male animal that died from EEHV sired a calf that later succumbed to a different EEHV based on EEHV gB variations suggesting that multiple EEHV can exist within the same herd.

This was used to develop the EEHV serological antibody titre testing of elephants in North America, which became available at the end of 2002 and was recommended for testing when transporting elephants between institutions.

September 2005 saw the first EEHV workshop with International delegates accumulating the known knowledge and identifying the areas that required work, highlighting areas of high importance including what aspects needed to be known and what resources were available for the study of EEHV. Ten years after Richman et al (1996) identified the need for elucidation of the epidemiology there was still very little known.

This was followed up a year later by the second EEHV workshop in Copenhagen. Little development had been made from the last meeting except for improvements in the ELISA technology at Erasmus Medical Centre, Rotterdam, and the development of possible screening techniques using ultrasound guided biopsies of retropharyngeal lymph nodes. Therapeutic techniques were reviewed and guidelines were drawn up as well as the proposed development of education material for elephant keepers.

Hildebrandt et al (2005) published the technique for ultrasound-guided biopsy of the retropharyngeal lymph nodes as a possible way to obtain suitable ante mortem tissues for EEHV PCR. Thirty nine elephants were biopsied and blood samples were taken. All tested negative by PCR. This may be of use in detection of carriers of EEHV but at present has not resulted in a positive case.

Ehlers et al (2006) published a paper detailing their continued work on Kiba's EEHV genetic structure. They aimed to extend the genomic characterisation of EEHV 1 and in doing so identified a thymidine kinase (TK) and protein kinase (PK) gene amongst other open reading frames (ORFs). This not only confirmed that EEHV is a distant relative of the beta herpes viruses but also identified the suitability of the use of famciclovir and other therapeutics such as ganciclovir, acyclovir, penciclovir and the orally available valganciclovir. The authors identified that cell cultures, once available, could be used to perform in vitro phosphorylation of the pro-drug therapeutics therefore testing their suitability in treatment protocols.

At the second EEHV workshop there were concerns that despite the TK gene existing there is the possibility that it has a different functionality to that in humans and that may not activate the pro-drugs such as famciclovir. An interesting point was that the TK gene was similar between the animals that survived and those that did not. However there is no evidence that it is not active either. Further testing continues.

Wellehan et al (2006) identified two novel herpes viruses that result in epiphora, blepharitis, conjunctivitis and other ocular pathology in eight Asian elephants. Nucleotide sequencing indicates that these are probably gamma herpes viruses.

Reid et al (2006) confirmed the first fatal case of EEHV in Asia (Cambodia). The virus was PCR processed and was assigned to the EEHV1 cluster. Phylogenetically this was related to the USA and European strains despite no transport or contact with elephants to these countries. The paper highlights that still little is known and that further work is required in the areas of basic virology and epidemiology.

## **EEHV epidemiology**

Very little actually known. What is known is based on clinical cases and assumptions.

- EEHV1 found in both Asian and African elephants (vestibular and skin lesions)
- EEHV1b found only in Asian elephants
- EEHV2 found only in African elephants in pulmonary nodules and has resulted in the death of two African elephants in North America
- Acute (days to hours) and peracute (sudden death) presentations
- Animals are viraemic before they are clinical
- Unpredictable/unknown disease transmission
- Possibility of oral or genital touching as route of transmission
- Transplacental infection possible as PCR positive on still births or in foetuses in utero at euthanasia
- Fomites unlikely to have a roll in transmission as fragile virus
- Stress is often associated with the disease
- It is thought that an EEHV viraemia can occur following a stressful event that may well be clinical or subclinical in nature e.g. bull at Rotterdam was viraemic following the loss of his trunk tip and another animal was positive on PCR following a traumatic mesenteric hernia
- Transportation probably not stressful for animals that are used to it
- Early treatment with famciclovir is important for recovery
- Majority of fatalities have been young animals
- No sex predilection
- There have been a number of still born animals tested PCR positive for EEHV
- EEHV present in free ranging as well as captive elephants
- World Wide problem
- Cannot culture on usual cell culture media, suspect need elephant endothelial cell line which is presently unavailable.



Fig 8. Oral and genital touching are thought to be likely routes in the transfer of EEHV between animals

## **EEHV pathology**

### **Clinical pathology**

**Haematology:** Leukopaenia, thrombocytopaenia and a low erythrocyte count are a common finding. Anaemia persisted for one week in one animal. One animal had a leukocytosis with a lymphopaenia and monocytosis. However haematology values can be normal. It must be noted that elephants have a relatively low erythrocyte count normally and that young Asian and African elephants can have high WBCC as a normal feature. Normal values are given as a guideline:

<b>Parameter</b>	<b>Range</b>	<b>Unit</b>	<b>Range</b>	<b>Unit</b>
<b>PCV</b>	30-40	%	-	-
<b>Hb</b>	11-15	g/dl	-	-
<b>RBC</b>	2.5-5.0	$\times 10^{12}/l$	-	-
<b>MCV</b>	80-160	fl	-	-
<b>MCH</b>	35-50	Pg	-	-
<b>MCHC</b>	25-40	g/dl	-	-
<b>Platelets</b>	200-600	$\times 10^9/l$	-	-
<b>Reticulocytes</b>	0	%	-	-
<b>RDW</b>	28-32	%	-	-
<b>ESR</b>	65-150	mm/hr	-	-
<b>WBCC</b>	5-24	$\times 10^9/l$	-	-
<b>Heterophils</b>	10-18	$\times 10^9/l$	25-30	%
<b>Lymphocytes</b>	5-8	$\times 10^9/l$	30-40	%
<b>Monocytes</b>	2-4	$\times 10^9/l$	25-30	%
<b>Eosinophils</b>	0.1-1	$\times 10^9/l$	<5	%
<b>Basophils</b>	0.0-0.03	$\times 10^9/l$	<1	%

**Biochemistry:** Mild renal dysfunction was noted in the North American index case and a case at Rotterdam zoo but little else has been noted presumably due to the peracute to acute onset of the disease. WWAP hypoalbuminaemia was noted and was assumed to result from either protein losing vasculopathy or as a negative acute phase protein, mild increase in urea and inorganic phosphorus. AST was mildly elevated and was assumed to be as a result of trauma (ataxia) and prolonged recumbency.

**Blood gas analysis:** Blood gases have received little attention. WWAP case arterial samples were attempted but venous samples could only be obtained. A respiratory acidosis was noted with reduced  $pO_2$  (67mmHg) despite maintenance on intranasal oxygen. Normal blood gas values:

<b>Parameter</b>	<b>Arterial</b>	<b>Venous</b>	<b>Unit</b>
<b>pH</b>	7.38-7.44	7.36-7.4	
<b><math>pCO_2</math></b>	38.2-43.4	40.4-45.6	mmHg
<b><math>pO_2</math></b>	95-103	-	mmHg
<b><math>HCO_3^-</math></b>	23.6-27.0	23.7-27.1	mEq/l

## **Gross pathology**

Haemorrhagic diathesis (abnormal risk or susceptibility to bleeding) is the hallmark of EEHV infection. Once an elephant becomes viraemic, productive infection of capillary endothelial cells by the herpesvirus is cytotoxic, leading to capillary leakage and widespread haemorrhage. Severe diffuse myocardial haemorrhage is likely associated with circulatory shock and death.

The gross pathology, extent and severity, vary depending on the time course of the disease and therapeutic measures. The common gross findings as outlined by Richman et al (2000) include;

- Oedema of the trunk, head, neck, limbs and dependant abdomen
- Pericardial effusion
- Extensive petechial to ecchymotic haemorrhages involving the epi- and endocardial heart surfaces and throughout the myocardium
- Diffusely scattered petechiae within all of the viscera and parietal peritoneal serous membranes
- Cyanosis of the tongue
- Hepatomegaly (not always)
- Variable oral, laryngeal, and large intestinal ulcers (hard palate ulcers not be seen in UK cases)

Additional pathological findings reported include;

- Intracranial haemorrhages
- Blood in the cranial cavities
- Perivascular haemorrhage in the subcutaneous tissues when skin carcass
- Haemorrhages throughout the stomach, small intestine and large intestine,
- No intravascular thrombi
- Skin masses in Asian elephants similar to those of African elephants (non-lethal)

African elephant (*Loxodonta maximus*) skin and genital lesions (non-lethal form);

- Multifocal, proliferative, nodular cutaneous lesions located on the trunk (but also the palpebrae, head, limbs, and lateral sides of the body. Two types seen (can be on the same animal);
  - Small nodules 0.7-1.4cm diameter, well-circumscribed, central area of grey keratinaceous and cellular material that extend below the skin surface to form a flask-like base. Termed inverted cutaneous papillomas.
  - Large growths 1.2-10cm diameter, raised, fibromatous in appearance. These are thought to be inflammatory fibrous polyps in response to the EEHV.
- Lymphocytic vulvitis: ulceration of the mucous membranes of the vulva.
- Pulmonary nodules



**Fig 9. Gross post mortem findings with EEHV.**

(a) Swollen head, neck, and trunk is a common finding at post mortem, (b) ulceration of the hard palate is commonly described at post mortem examination, however the UK cases confirmed by PCR to have died from EEHV did not show this lesion, (c) the frequently reported hard palate ulceration is a common finding in most cases (taken from Fickel), (d) cyanosis of the tongue with petechial to ecchymotic haemorrhages is common to all of the EEHV cases reported so far, and (e) swelling of the head can be severe (ventral aspect)



**Fig 10. Gross post mortem findings with EEHV (continued)**

(a) pericardial effusion is common and large volumes can be collected (g), (b) on opening of the pericardium the heart is often haemorrhagic (the pale areas are due to fat), (c) petechial to ecchymotic haemorrhages are found throughout the epi-, endo-, and myocardium, (d) mesenteric haemorrhages are also a common findings, as are intracranial bleeds (e: arrows), (f) here the subcutaneous vessels have perivascular haemorrhages.



## **Histopathology**

The histological findings are an important part of confirming the diagnosis of EEHV as some of the gross aspects can be seen with other diseases. The findings as described by Richman et al (2000) include;

- Extensive microhaemorrhages throughout the heart and tongue associated with oedema and mild infiltrates of lymphocytes, monocytes, and neutrophils between myofibres.
- Multi-focal hepatic sinusoidal expansion with mild sub-acute inflammation, and mild hepatocellular vacuolar degenerative changes.
- The capillary endothelial cells in the myocardium, tongue, muscle, and within the hepatic sinusoids of the liver contained amphophilic to basophilic intra-nuclear viral inclusion bodies that were in close association with the microhaemorrhages
- Ulcers of the oral and laryngeal mucous membranes are often acute with necrotic surface cells still intact in some areas
- Placenta: inclusion bodies seen within the trophoblastic layer

Recent reviews of the histopathology has revealed that the vascular lesions are not associated with inflammation and therefore the term vasculopathy rather than vasculitis applies. Only capillary endothelium in targeted organs seems to be affected by the virus with no evidence of changes in venous or arterial endothelium. The actual molecular mechanism of endothelial damage is unknown but includes direct viral injury, apoptosis, immune mediated destruction, and DIC but light micrographic findings are suggestive of direct injury by the virus.



Courtesy of Mr Mark Stidworthy, IZVG

African elephant (*Loxodonta maximus*) skin and genital lesions (non-lethal form);

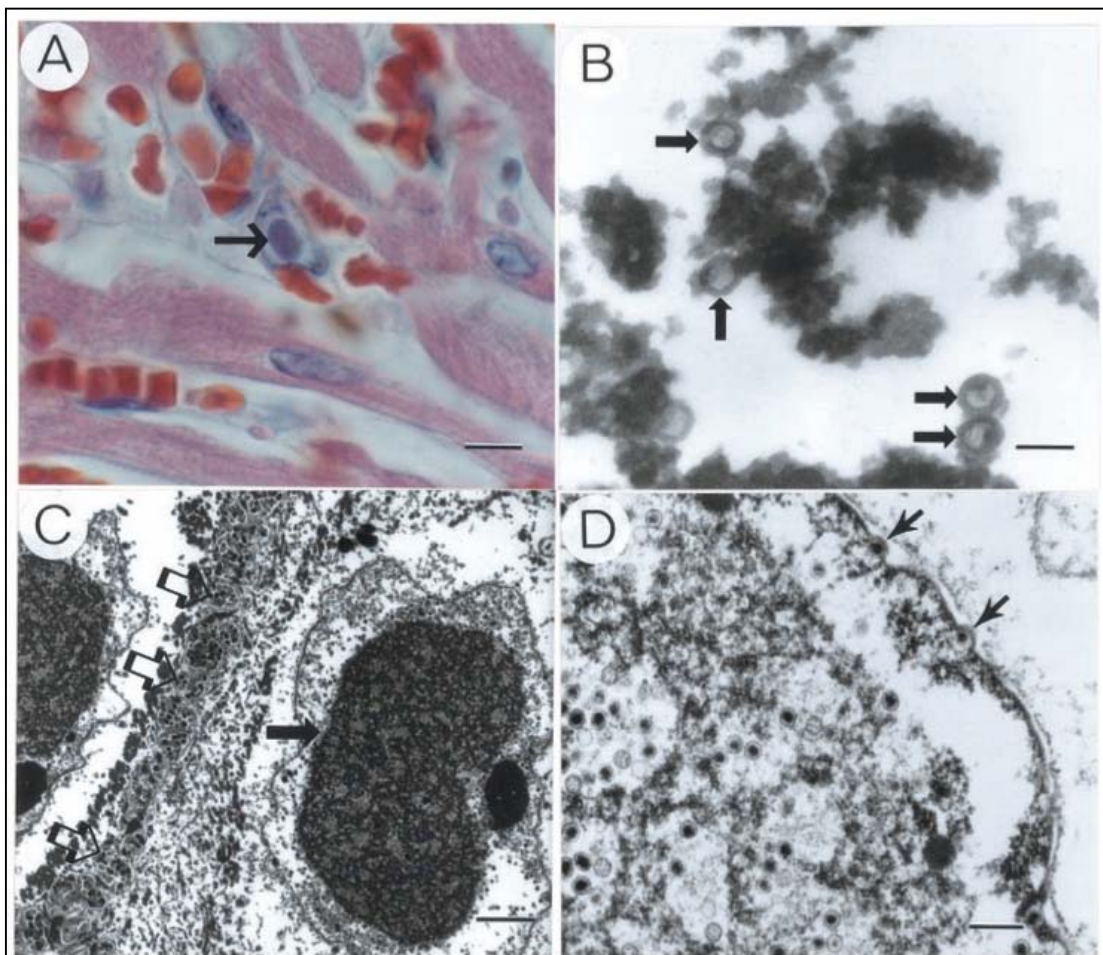
- African elephant cutaneous inverted papillomas:
  - Hyperplastic epithelial cells with acanthosis and amphophilic intranuclear inclusion bodies in the cells of the stratum spinosum

- African hyperaemic nodules from the distal urogenital canal:
  - Reactive lymphoid follicles and have been attributed to non specific antigenic stimulation as no inclusion have ever been found, but they do test positive for EEHV PCR
- African pulmonary nodules:
  - Multiple large lymphoid follicles that surround epithelial cells that contain intranuclear inclusion bodies. The epithelial cells often form syncytia.

### **Electron micrography**

Taken from Jacobson et al (1986);

- Virus particles found in large aggregates within the inclusion bodies
- Virus particles also found in the intercellular spaces
- 80-92nm nucleocapsids morphologically consistent with herpes viruses
- Electron dense and electron lucent cores
- Once left nucleus become enveloped, round to ellipsoid in shape, 136-182nm in diameter
- Particle envelopment often seen at the nuclear membranes



**Fig 11. Electron micrographs of EEHV.**

(a) Tissue from the USA index case demonstrating diffuse myocardial haemorrhage and a basophilic intranuclear viral inclusion body within a capillary endothelial cell (arrow), (b) Transmission electron micrograph (TEM) of the same tissue showing details of the intranuclear inclusion. Herpes virus capsids are evident (arrow), (c) African elephant skin papilloma TEM showing the inclusion body (solid arrow) and intercellular enveloped herpesviruses (open arrows), and (d) shows a higher magnification of (c) showing details of the intranuclear nucleocapsids. Arrows show the viruses undergoing envelopment at the nucleus membrane.

Taken from Richman et al (1999)

## **EEHV clinical signs**

Two clinical presentations are recognised: Peracute and acute. Peracute refers to a duration of a few hours only and includes animals that are classed as sudden deaths, whilst acute refers to having severe signs and a short course of hours to days.

The following clinical signs are associated with EEHV infections;

- (Sudden death)
- Lethargy
- Dullness
- Anorexia
- Mild colic
- Oedema of the head, neck, trunk and thoracic limbs (and ventral abdomen)
- Cyanotic, swollen tongue: starts at tip and moves caudally typically
- Oral ulceration (not seen in UK cases)

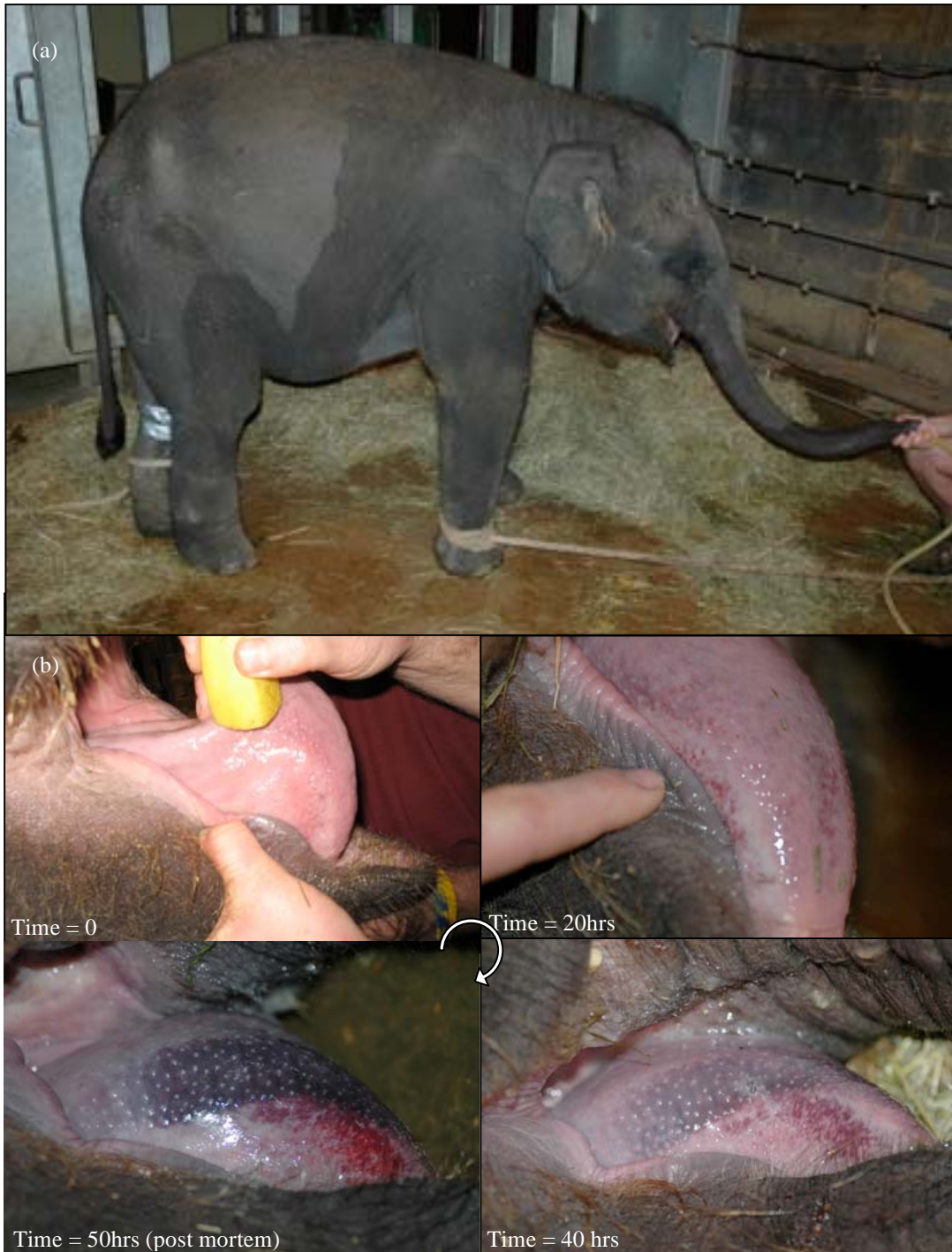
Other clinical signs sometimes seen as the disease progresses include;

- Dribbling due to the swollen tongue
- Reduced trunk movement
- Ataxia due to intracranial bleeds or cerebral hypoxia
- Recumbency
- Decrease filling of venous vascular system as animal becomes shocked
- Difficulty in auscultation
- Weak thready pulses
- Unresponsive to commands

Differential diagnoses include;

- Encephalomyocarditis
- Clostridial enterotoxaemia
- Anthrax (sudden death)
- Salmonellosis
- Hypovitaminosis E

Coagulation profiles should be considered as part of the assessment and your local lab should be contacted for their preferred method of sample collection and processing. In addition ultrasound guided biopsy of retropharyngeal lymph nodes should be attempted of all in contacts as described by Hildebrandt et al (2005) for EEHV PCR.



**Fig 12. Clinical signs commonly associated with EEHV infection**  
 (a) shows the swelling of the head and ventral oedema, the trunk, neck and thoracic limbs can also be affected, (b) shows the development and progression of the cyanosis and haemorrhages that occur within the tongue leading to dysphagia and dribbling of saliva and oral fluids. Hard palate ulcers are also reported and are seen in the pathology section.

## **Therapeutic protocols**

There are no set guidelines for treatment regimes as of yet.

The last EEHV workshop in Copenhagen (2006) outlined some recommendations based on a review of the surviving (and dead but treated cases). The overall consensus was that therapy needs to be started immediately and often without a confirmed diagnosis of EEHV. If you wait then therapy is unlikely to be efficacious and treatment regimes should be put in place before the time when you may need them.

Treatment should be aggressive from the beginning.

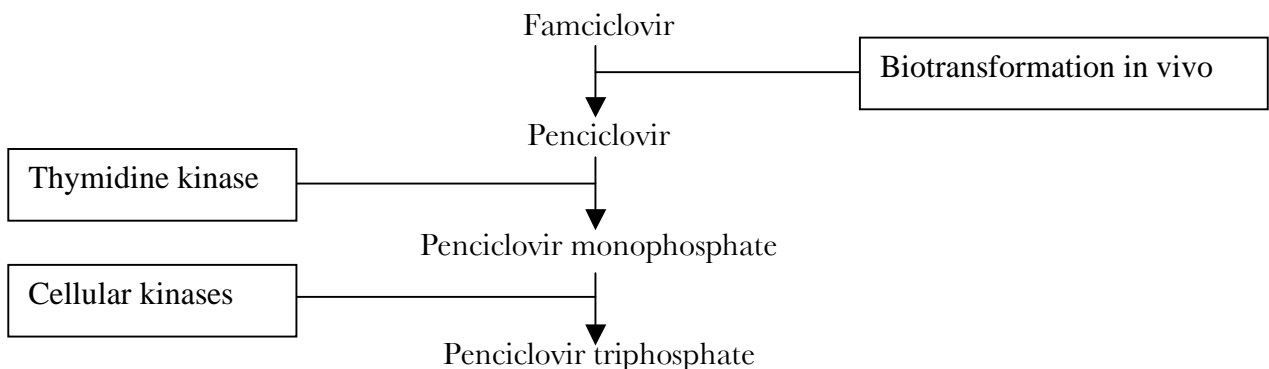
## **Anti-herpes drugs**

### **Famciclovir**

Manufacturer:	Novartis Pharmaceuticals
Trade name:	Famvir
Forms:	125mg, 250mg, 500mg, and 750mg tablets Cream (Denavir)
Dose:	12mg/kg qid 1 <sup>st</sup> day then bid 3 weeks Alternative: 15mg/kg bid/tid then 8-15mg/kg bid/tid
Route:	Oral or rectal (mix with ultrasound gel not KY jelly for rectal administration)

Famciclovir is an antiherpes virus drug used in the treatment of herpes simplex and Varicella zoster virus in man. It is the one of the core drugs used to treat EEHV in elephants and was first described by Schmitt and Hardy (1999) and Schmitt et al (2000).

Famciclovir is a pro-drug that undergoes rapid biotransformation to the active antiviral compound penciclovir that is a second-generation guanosine nucleoside with antiviral properties:



Penciclovir triphosphate then inhibits DNA polymerase which is an enzyme used by herpes viruses for herpes virus DNA synthesis, if inhibited the virus can no longer replicate.

This is why aggressive early therapy is indicated: famciclovir does not support any pathology that has already occurred; it only reduces or prevents further pathology from occurring. If given too late when pathology is too severe then famciclovir can have no effect. A case in USA was given famciclovir 5 days after clinical signs occurred and the animal survived, conversely an animal in the UK was given famciclovir 32 hours after clinical signs developed and went on to die. The difference is likely to be due to individual variation of the severity of pathology at the time of therapy and the time course of the disease.

In man penciclovir triphosphate has an intracellular half life of 7-20 hours depending on the herpes virus present. Isaza et al (2003) published pharmacokinetic data for famciclovir in Asian elephants. They found that the oral and rectal routes were both suitable and that at a dose of 8-15mg/kg qid will result in penciclovir concentrations that are considered therapeutic in humans.

Schaftenaar and Mensink (2005) describe the use of famciclovir in their collection and advocate the use of famciclovir early on in cases even when a firm diagnosis of EEHV is not available. They also describe the rectal administration of the drug.

In man penciclovir is relatively safe with little or no side effects, however there is no data available for long term use of famciclovir in elephants.

In North America at the time of writing there have been four survivors of EEHV and all were treated with famciclovir. However nine other animals treated with famciclovir died despite therapy. The question is whether these four animals would have survived without therapy or because they were treated?

As can be noted above famciclovir requires the presence of thymidine kinase and cellular kinases before it becomes active. Recent work by Ehlers et al (2006) has indicated that the genes are there but there are concerns that the proteins have a different functionality and that famciclovir has little or no effect. Further work is required to assess the efficacy of this potentially useful but expensive drug.

Note: alternative anti herpes viral drugs are available and can be potentially used. There are no published doses for these yet due to the large amount of data on the use of famciclovir in elephants but they include;

- Acyclovir
- Ganciclovir (not recommended due to side effects)
- Penciclovir
- Valganciclovir (in man better bioavailability)

## Conservative symptomatic therapies

### Antibiotics

Although antibiotics have no effect in treating EEHV the animal's immune system will be severely compromised and the clinical situation could be complicated by secondary opportunistic infections and therefore antibiotics should be instigated immediately.

Antibiotics available and their dose (based on elephant data unless indicated) include;

<b>Drug</b>	<b>Dose</b>	<b>Frequency</b>	<b>Route</b>
<b>Amoxicillin</b>	11mg/kg	SID	IM
<b>Ampicillin</b>	8mg/kg	QID/BID	PO
<b>Ceftiofur</b>	2mg/kg	SID/BID	IM
<b>Doxycycline (Equine dose)</b>	10mg/kg	BID	PO
<b>Enrofloxacin (Equine dose)</b>	2.5-5mg/kg	BID	PO/SC
<b>Marbofloxacin (Equine dose)</b>	2mg/kg	SID	IV/SC/ PO
<b>Procaine penicillin and benzathine penicillin</b>	1600-2275iu/kg	EOD	IM

## **Analgesia**

Although EEHV is thought to be a vasculopathy as opposed to a vasculitis anti-inflammatories are indicated as part of the analgesic regime as well as reducing secondary inflammation resulting from peripheral oedema and haemorrhage.

NSAIDs are part of the recommendations outlined by the EEHV workshop and they play a useful part 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 is 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 side effects.

Opioids are also a useful adjunct to providing relief and in some cases mild sedation to assist in the management of animals being treated. Be aware that there is the possibility with behavioural changes in the elephant when using opioids and that animals should be treated with extra care, as trained behaviours may well be lost or less responsive.

Most important is not to forget tender loving care, providing a supportive environment and to provide a comfortable stall with keepers that the elephant knows well.

Analgesics available and their dose (based on elephant data unless indicated) include;

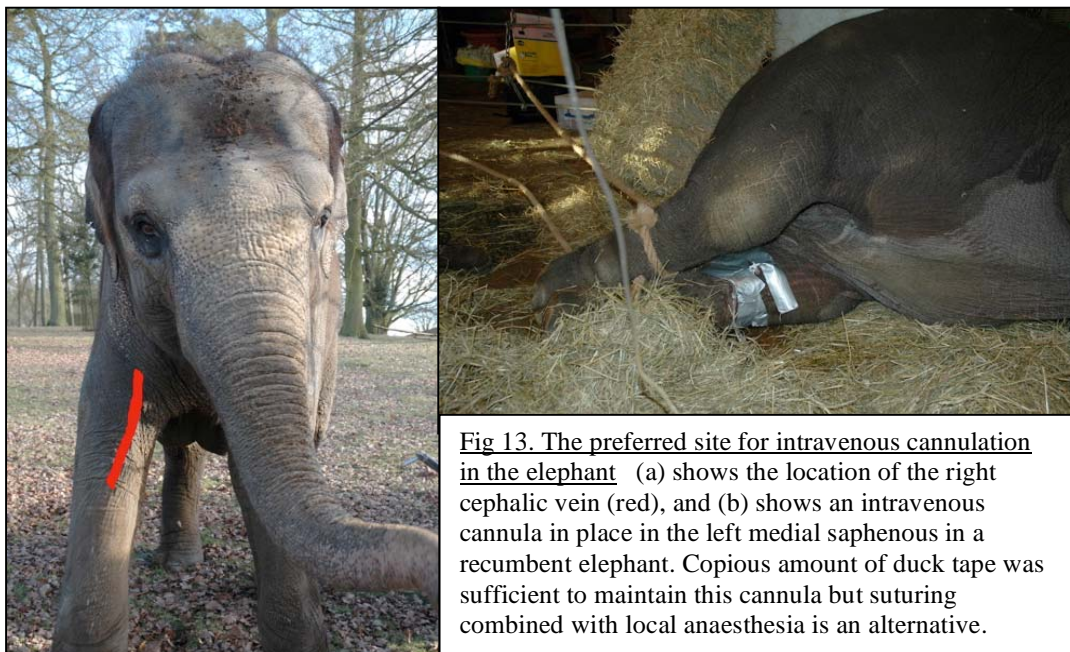
<b>Drug</b>	<b>Dose</b>	<b>Frequency</b>	<b>Route</b>
<b>NSAIDS</b>			
<b>Carprofen (Equine)</b>	0.7mg/kg	SID	IV/PO
<b>Meloxicam (Equine)</b>	0.6mg/kg	SID	IV then PO
<b>Flunixin meglumine</b>	0.8mg/kg	BID	IM
<b>Flunixin meglumine (Equine)</b>	1.1mg/kg	SID	PO/IM
<b>Phenylbutazone (Equine)</b>	4.4mg/kg	BID	IV
<b>Phenylbutazone (Equine)</b>	2.2-4.4mg/kg	BID	PO
<b>Aspirin</b>	25mg/kg then 10mg/kg	BID SID	PO
<b>Opioids</b>			
<b>Morphine (analgesia)</b>	0.03-0.06mg/kg	As req	IM
<b>Morphine (analgesia + sedate)</b>	0.06-0.2mg/kg	As req	IM
<b>Methadone (analgesia)</b>	0.03-0.06mg/kg	As req	IM
<b>Butorphanol</b>	0.015mg/kg	As req	IV/IM



## **Fluid therapy**

The role of fluid therapy in EEHV cases has not been fully appreciated. Clinically animals become dehydrated from a reluctance to drink, in addition fluid is lost from the vascular compartment as animals haemorrhage into peripheral tissues.

Fluid therapy not only provides maintenance requirements but is also essential for supporting the cardiovascular system. Early intravenous access should be attempted and maintained in any EEHV case, when the animal becomes shocked it is more difficult to obtain IV access. Maintenance can be difficult in bright animals but as soon as they become depressed this is relatively easy. The preferred sites for intravenous access are the medial saphenous (often deeper than it appears) or the cephalic vein. The cannula can be sutured in place or stabilised with liberal use of duck tape (see figure 13). Multiple cannulas maybe required. The auricular vein is not recommended for long term or any intravenous drugs due to the risk of perivascular injection and ear sloughing.



Formulating a fluid therapy plan is extremely challenging in elephants with EEHV for several reasons;

- Large volumes of fluid required
- Maintenance of the intravenous access can be difficult
- On-going pathology results in compromised cardiac function and pumping ability whilst capillary endothelial cell damage results in increased leakage of fluid from the vasculature thereby exacerbating peripheral oedema
- Diuresis is often performed to decrease peripheral oedema and it seems contraindicatory to increase fluid loads using intravenous fluid therapy
- Monitoring fluid therapy is challenging in elephants
- What are the suitable choices of fluids?

### Large volumes are required

Maintenance fluid therapy requirements have not been determined for elephants but are assumed to be similar to other mammals (Mikota, 2006);

- Maintenance (adult) = 2ml/kg/hour = 2litres/1000kg/ hour
- Maintenance (calf) = 4ml/kg/hour = 4litres/1000kg/hour
- Surgical rate = 10ml/kg/hour = 10litres/1000kg/hour
- Shock rate = 50-90ml/kg/hour = 50-90litres/1000kg/hour
- Volume replacement fluid (litres) =  $\frac{\text{Body weight (kg)} \times \text{percentage dehydration}}{100}$

The biggest limitation (apart from availability) to the rate of fluid therapy is the gauge and the length of the intravenous cannula. The following is a rough guide but indicates the need for large short catheters when cannulating elephants (Raffe and Wingfield, 2002, p453);

- 14G Short maximum flow rate = 200ml/min
- 16G Short maximum flow rate = 150ml/min
- 16G Long maximum flow rate = 100ml/min
- 16G Extra long max flow rate = 50ml/min

When cannulating elephants long catheters (approx 4inches) are generally used and therefore the smallest gauge should be used (larger diameter). A minimum of two or more catheters should be placed: this allows suitable volume infusions and guaranteed intravenous access without at least one cannula at all times if one were to become dislodged: maintenance of continual intravenous access is essential. The use of fluid pumps can increase the volume given but is limited by the pressure increases in the system shutting down the pump.

### Maintenance of intravenous access can be difficult

Elephants that are bright will attempt to remove or play with intravenous lines. If still eating and drinking then a cannula should be placed but not attached to any fluid lines. This ensures access is available if needed and makes management of the catheter easier.

Duct tape has been found to be more useful and easier to apply then suturing catheters by this author.

When fluid therapy is instigated care must be taken to maintain the drip line. A member of staff should baby sit the fluid bag at all times to prevent the animal from pulling or kicking the line out. If more lines are placed then each should have a member of staff dedicated to that bag and recording the volumes administered.

It is better to invest time maintaining these lines as re-cannulating is stressful and can be technically difficult in shocked animals.

### Compromised cardiac function and capillary leakage

On going pathology results in several challenges in designing a fluid plan;

- cardiac function becomes compromised as pathology continues resulting in the heart being less able to cope with increased fluid loads or preload from the use of vasoconstrictors, in addition pericardial effusion results in tamponade reducing further the actual ventricular filling and cardiac output
- increasing leakage of capillary endothelium means that crystalloids and even colloids are less likely to remain in the vascular compartment
- together these two pathologies alone will result in exacerbation of peripheral oedema and potentially pulmonary oedema
- however if no fluid support is given then the fluid loss to peripheral tissues will result in a shock syndrome which ultimately will result in myocardial hypoxia which hastens death in severe cases

Obviously in terminal stages of EEHV fluid therapy is unlikely to be beneficial but in early stages cardiovascular support is essential to prevent multiple organ dysfunction exacerbating an already compromised animal.

In addition to the pathological considerations the clinician must remember that diuretics are often used and if excessive amounts of crystalloid are used then careful attention must be paid to the elephants electrolytes. Any abnormalities should be monitored and corrected accordingly;

- Hyponatraemia correct at a rate of 1mEq/l/hour
- Hypokalaemia correct rate at 0.5mEq/l/hour

If a metabolic acidosis is present in conjunction with hyponatraemia or hyperchloraemia then the use of sodium bicarbonate should be considered;

- Bicarbonate deficit (ml of 8.4% molar) =  $0.3 \times \text{base excess} \times \text{body weight (kg)}$

However it should be noted that in Equids sodium bicarbonate will raise the blood pH but will actually decrease CSF and intracellular pH, which may be detrimental to the acidotic patient. It may further increase the plasma lactate and sodium concentrations and may decrease the ionised calcium concentration. In effect it corrects pH without addressing the underlying pathophysiology. Bicarbonate is contraindicated in respiratory acidosis and in cases with normal or increased plasma carbon dioxide tensions.

### Monitoring efficacy of fluid therapy

Due to the concerns of fluid overload in these patients it is recommended that additional support be brought in for the intensive care of these animals when required. Monitoring the ICU patient, which is ultimately what may be required with EEHV cases, is a specialist discipline and is limited by experience and available diagnostic equipment and modalities.

The recommendations for fluid therapy monitoring by the general practitioner include;

- Monitor oral intake of fluid volumes
- Monitor intravenous administration of fluid volumes
- Attempt to monitor urine output (catheterise recumbent males if possible)
- Clinical signs of hydration (difficult in oedematous patients)
- Auscultation of lung fields for rales and evidence of pulmonary oedema
- Monitoring of peripheral pulse character
- Monitor total protein and PCV

The previous techniques are extremely limited and are not accurate and more efficacious monitoring tools should be used, none of which have been validated or even attempted in elephants as far as this author is aware;

- **Central venous pressure:** Difficult in elephants but should be considered as is the gold standard for monitoring the efficacy of fluid therapy load in a patient as well as assessing pericardial effusion effects. Would require central venous cannulation.
- **Arterial blood pressure monitoring:** simple systems can be designed for invasive blood pressure monitoring and clinicians are advised to monitor blood pressure. Drip tubing, pressure veils, and electronic transducers are available and should be used for monitoring cardiovascular health. Auricular arteries are easily accessible. Essential if using vasoconstrictors.
- **Blood Gas analysis:** monitoring of the alveolar-arterial oxygen tension gradient (A-a gradient) is useful as the gradient increase in the face of pulmonary oedema

### Choice of fluids

Several routes and fluids are available, all with limitations;

- **Oral:** The most physiologically normal and easily regulated method for administering fluids. The animal regulates own fluid requirements as long as they are able. In EEHV the swollen tongue can lead to difficulties in drinking.
- **Rectal:** Reasonable volumes of water can be given per rectum; however can have implications on temperature management if used copiously.
- **Intravenous - Crystalloids:** Easily accessible and useful for maintenance requirements. It must be remembered that only approximately 10% of the fluid administered remains in the vascular compartment and in severe shock cases these are not a useful first line fluid choice. In long term use electrolyte abnormalities should be monitored for.
- **Intravenous - Colloids:** Larger molecular weight fluids that are retained within the vasculature in larger volumes. More useful in volume resuscitation but little is known about their use in elephants with EEHV and which types are better indicated depending on the level of endothelial damage. This author

recommends starting with a medium weight colloid initially (e.g. starch) and moving on to larger molecular weights as indicated.

- **Intravenous - Oxyhaemoglobin:** Useful in very sick animals but maybe prohibitively expensive and if finances are an issue then concentrate on purchasing famciclovir.
- **Intravenous - Blood/Plasma:** Very useful in supporting the cardiovascular system but little known about transfusion medicine in elephants and accordingly considerations for the risk of the effects of increased viscosity by increasing PCV in cardiovascularly compromised patients, and the unknown epidemiology or carrier status of herd members (i.e. will the transfusion contain an EEHV inoculant or beneficial antibodies?) on the patient must be considered. Care must be taken and the suitable blood taking and giving sets should be available before this is attempted. It is possible that serum banking of sero-positive animals could be an option for the near future.

### **Oxygen therapy**

Oxygen therapy is useful in providing support for the compromised cardiovascular system. Nasal oxygen can be easily supplied to recumbent patients or in standing patients with a keeper maintaining a nasal (trunk) oxygen tube (see figure 14).

A flow rate of 5 litres a minute is suitable for most patients and allows relative conservation of the oxygen supply for the duration of therapy. If at all possible oxygen should be supplied in this form continually until the elephant is deemed not to need supplementation or has died. At this flow rate this will not provide 100% inspired oxygen but will however increase oxygen tensions above room air and therefore provide overall better tissue oxygenation than when not used.



**Fig 14: Intranasal oxygen administration**  
Intranasal oxygen support is an important part of management of the cardiovascular compromised patient.

## **Diuretics**

### **Furosemide**

Manufacturer:	Intervet
Trade name:	Dimazon 5% solution
Form:	50mg/ml (tablets also available)
Dose:	0.6mg/kg (Schmitt et al, 2000) 0.8mg/kg (EEHV workshop recommendations) 1-5mg/kg (equine dose, 5mg/kg max)
Route:	IV/IM QID

Management of the peripheral oedema has been achieved successfully with the use of furosemide (Schmitt et al, 2000) at a dose of 0.6mg/kg.

Furosemide is an anthracillic acid derivative classed as a loop diuretic. For the drug to have an effect it must reach the vasculature adjacent to the proximal renal tubules, there must be no tubular obstruction, and it is highly bound to albumin and if this plasma protein leaks into the tubule or tissues the drug will be bound in an inactive form. All of these factors result in a variable response to the drug.

Furosemide acts to block the sodium-potassium- chloride cotransporter that results in the loss of fluid through the nephron. Despite being relatively safe side effects in Equids include hypokalaemia, arterial vasoconstriction via rennin release, convulsions, ataxia, metabolic alkalosis, bronchodilation, and increased prostaglandin effects. The main drug actions occur through decreasing blood volume with little effect from the increased prostaglandin release. However it must be noted that the concurrent use of NSAIDs block extra renal effects and can decrease diuresis effect by 30-40% in horses, although this has not been reported in elephants it should be considered (see NSAIDs above).

In horses there is evidence to suggest that intramuscular administration is more efficacious as the intravenous route produces a rapid saturation of receptors that soon dwindles as the drug is excreted. With intramuscular injection the same plasma levels are not reached but the longer duration produces a greater diuresis in comparison. In horses furosemide lasts approximately 4-6 hours.

### **Other diuretics**

The use of other diuretics has not been recorded in elephants but there use should be considered e.g. mannitol.

## **Pericardial tap**

Regular monitoring of the heart using trans-thoracic ultrasonography should be performed at least daily with increasing frequency depending on the presence of pericardial effusions if present.

If cardiac tamponade occurs secondary to pericardial effusion occurs then pericardiocentesis should be considered. If severe and the animal is severely compromised then the only other option is euthanasia. Pericardiocentesis in elephants is a theoretical technique, adapted from domestic species, that to the author's knowledge has not been attempted and therefore should not be undertaken likely;

- Suitable site should be identified using ultrasonography where the pericardium is closest to the thoracic wall and a needle can be placed between the ribs.
- Suitable length and gauge needle should be attached to an extension set and a three way tap (long spinal needles are suitable).
- The surgical site should be prepared aseptically in the usual manner.
- Local anaesthesia should be infiltrated at the proposed site for needle insertion ensuring that anaesthetic is placed to the level of the pleura.
- Repeat surgical scrub.
- Using ultrasound the needle should be guided into the pericardial space and the fluid aspirated.
- All precautions should be taken to prevent air retrograding through the needle into the pericardial space and to prevent the needle penetrating the heart itself
- Once sufficient fluid has been aspirated the needle should be removed and the fluid sent for cytology, total protein, and culture.
- Pneumothorax or pyothorax are described as complications in other species but the lack of a pleural space means that this is unlikely, however penetration of blood vessels and capillary vasculopathies could potentially result in a fatal haemorrhage which should be explained to all concerned.

## **Vasoconstrictors**

Vasoconstrictors are a useful adjunct to the management of circulatory failure. However their use should be limited to animals where blood pressure can be measured or in cases where it is a last resort as inappropriate use can compromise an animal further.

The agents are administered as a continuous infusion, usually by administering them into a 500ml bag of saline with the dose titrated to effect from a standard starting point.

Vasoconstrictors with the dose for equids include;

- Noradrenalin 0.1-0.75µg/kg/min IV to effect
- Phenylephrine 0.1-0.2µg/kg/min IV to effect (not to exceed 0.01mg/kg)
- Metaraminol To effect in humans

## **Diagnostic testing**

Clinical signs combined with the history of the herd, gross post mortem and histology findings can be indicative of EEHV infection. However in certain cases, especially in the living animals, further diagnostic testing is required for confirmation of infection.

**It must be noted that therapy should be instigated before the results of further diagnostic tests have been received.**

The main tests available are the PCR and ELISA.

### **Polymerase chain reaction (PCR)**

Polymerase chain reaction (PCR) is a method for cloning DNA rapidly. Primers are created that are known to bind to specific parts of the DNA of the EEHV virus that are particular to that individual virus. Fragments of the terminase gene are used in the EEHV PCR. The sample, the primers, and a solution of the constituent parts of DNA are incubated together along with DNA polymerase an enzyme that is capable of forming new strands of DNA. Once incubated the sample DNA separates and a new strand will form using the DNA polymerase which creates 2 strands in place of the first, this process is repeated until a massive amplification of the DNA has occurred to detectable levels.

PCR is a very useful technique and is used to detect the presence of small amounts of virus but does not allow the identification of where the virus is found within tissues. Because EEHV virus has to be present for the PCR to work this means that the EEHV PCR is never positive in a healthy elephant.

The EEHV PCR is the principle diagnostic aid in confirming infection or the cause of death in suspected cases of EEHV.

Both blood and tissues can be presented for PCR. The preferred samples include;

- EDTA whole blood (lymphocytes)
- (Heparin blood possible)
- Heart
- Liver
- Tongue
- Retropharyngeal lymph node
- Bone marrow in juveniles
- (Lung)
- (Placenta)
- (Any bizarre lesions)

The main disadvantage of PCR is that it can only be used to identify animals with active infection. Elephants in the suspected latent phase where no active virus production is occurring will test negative with the PCR. This means that the PCR is



limited to confirming cases and not as a screening tool for potentially infected latent carrier animals.

At present the PCRs available are qualitative and not quantitative (but this is hoped to change later in 2007).

PCR is available at the following institutions;

- Rotterdam Zoo/ Erasmus University, Netherlands
- Institut für Zoo- und Wildtierforschung (IZW), Germany
- Institute of virology, Vetsuisse-Faculty, Switzerland
- Smithsonian National Zoo, USA
- (UK, along with labs in Bangkok and India, hopefully later this year)

### **Enzyme Linked Immunosorbent Assay (ELISA)**

Enzyme linked immunosorbent assay (ELISA) is a technique that looks at the serological response of an animal to a disease i.e. the antibody produced by an animal towards a specific disease. This technique looks at antibody-antigen responses in vitro. In the case of EEHV serum is mixed with artificial EEHV antigen (in this case glycoprotein B) and if anti-EEHV antibodies are present in the elephants blood then they will bind to the antigen embedded in the plate wall. Rabbit anti-elephant antibodies labelled with biotin are then added and if a positive antibody-antigen response has occurred then the rabbit antibodies will bind to the elephant antibodies and the response will be visually positive. The results are given as an optical density (OD) when light at a wavelength of 405nm is passed through the sample. A positive response indicates either previous exposure or potential carrier status.

Rotterdam, which are similar to the USA tests, provide the following results;

- <0.5 OD      negative
- 0.5-0.8 OD    borderline
- >0.8 OD      positive

Serology is more useful as it can be used in conjunction with PCR for diagnosis and evaluation of the immune response to active infection as well as monitoring the levels of exposure to EEHV, period of detectable antibodies, and the general epidemiology of the disease itself. However there are many problems, especially in the validation of the test that make use of the test limited. In fact serum from other species has resulted in high OD in some of the ELISAs. To this author's knowledge the ELISA is only available for Asian elephants at present. Current validation of the ELISA is occurring in the USA and work on further developing the ELISA is being undertaken in Rotterdam.

The ELISA requires serum.

With respect to the ELISA the following assumptions are made (taken from the AZAA ESSP recommendations);

- Elephants with high EEHV titres have been previously exposed/infected and may have protective immunity or resistance to developing potentially fatal clinical disease
- Elephants with high EEHV titres and a clinical history of disease are presumed to be carriers of EEHV and may periodically shed virus (with or without clinical signs)
- Elephants with no EEHV titres are probably immunologically naïve. Other risk factors including age (fatal cases have occurred mostly in younger animals under 10 years of age), health status, and previous exposure to other elephants, may alter the susceptibility of these individuals to EEHV infection and the development of clinical disease. However, these elephants should be considered susceptible to infection.
- Elephants with intermediate EEHV titres may have previous exposure and/or be potential carriers. These animals require serial sampling to determine their EEHV status.
- Currently, results of individually tested animals may provide preliminary guidelines for risk assessment on a case-by-case basis; however, sufficient data is not available to make broad-based management recommendations on risk of EEHV infection at this time. Immediate action should be taken to screen the current population and develop a long term monitoring programme to provide information for future management guidelines.
- There are several different strains of EEHV circulating around the world and it is not yet known if exposure to one strain will confer protection against other strains of EEHV

ELISA is available at the following institutions;

- Rotterdam Zoo/ Erasmus University, Netherlands (nearly operational)
- Institute of virology, Vetsuisse-Faculty, Switzerland (difficulties in construction)
- Smithsonian National Zoo, USA (clinically active)

However the American ELISA is the only present clinically active test with the European ELISA still being in the validation and construction process. At present the ELISA is still experimental and further testing is required before the ELISA will be available in the UK. Despite the availability of the American ELISA there are logistical difficulties regarding transportation and CITES permits for the elephant blood samples required which makes it very difficult to send from outside of America.

The ELISA is likely to become an important tool for the management and movement of elephants as well as for studying the epidemiology of EEHV.

<b>Results of EEHV ELISA</b>		<b>Animal/herd to be transported</b>	
		<b>Positive titre</b>	<b>Negative titre</b>
<b>Receiving herd</b>	<b>Positive titre</b>	Low risk	Risk to incoming animal (naïve animal going into herd that may have carriers)
	<b>Negative titre</b>	Risk to receiving herd of exposure to potential carrier (incoming animal)	Low risk

Taken from AZAA ESSP Recommendations for EEHV Testing and Transport of Elephants (2002)

## **Husbandry and management guidelines**

At present very little is known about the epidemiology of EEHV: we do not know the risk factors involved in clinical cases, we do not know the route of transmission, we are not able to detect latent carriers of the disease, and we do not have the knowledge to provide firm guidelines for movements. However common sense, knowledge from the behaviour of herpes virus infections in other species and the little knowledge that we do have regarding EEHV can be used to provide practical guidelines. These guidelines must be adapted as new information and improved tests become available, especially if assumptions are proven to be wrong.

### **Daily management of the herd by the keepers**

Daily checks should be carried out on every elephant;

- Oral examination for ulcers.
- Oral examination for tongue lesions.
- General assessment of the elephants.
- Comparisons can be made with the EEHV diagnosis poster (available mid 2007)
- Faecal ball temperature monitoring: however hyperthermia has not been seen in all cases of EEHV

Any concerns, however minor, should be reported to the veterinary department and the relevant animal collection manager immediately.

### **Management by the veterinary department**

The daily checks should be performed with a veterinarian on a weekly basis if at all possible to reduce the stress of unfamiliar staff on elephants at times when elephants are sick. An EEHV standing operating procedure document should be produced that collates all the known information and the doses of drugs which can act as a quick reference guide for suspected cases, as part of this suitable drugs or suppliers at short notice must be sourced.

If EEHV infection is suspected in a case then a minimum database should be established that includes;

- Complete general examination of the animal
- Blood sample to be taken (10ml minimum, see appendix B). To include;
  - EDTA for Complete blood count and PCV
  - EDTA for viral PCR (1-2ml minimum, 5ml preferred)
  - Heparin plasma for EEHV ELISA (1-2ml minimum, 5ml preferred)
  - Serum (for storage)
  - Other tests as indicated

- If no other disease process is diagnosed or suspected and EEHV is still a main differential diagnosis then start on famciclovir and supportive therapy immediately
- If death occurs then post mortem tissues should be submitted for EEHV PCR

Annual comprehensive health checks should be undertaken, and these should include EEHV ELISA of both positive and negative animals.

The veterinary department should regularly review EEHV literature and update the protocol as new information becomes available.

It should be noted that titre monitoring is a theoretical concept at present and the actual values at present are meaningless until further research has been performed. This is why the veterinarians should update the protocol as changes occur in the literature based on current trends and epidemiological data.

### **Management by the Curator of Mammals**

The Curators of Mammals should regularly review any protocols with the keepers and the veterinarians to ensure the efficacy of the procedures and that the protocols are implemented. They should ensure that all visitors to the Elephant house be informed of any protocols and that the protocol is adhered to, without exception. Curators should inform any other collections of the herd EEHV viral status whenever translocations to and from other collections occur. The Curators should ensure that both collections are aware of the relevance of the EEHV status in both the sending and receiving herd. This should be performed in conjunction with the keepers and veterinary department.

### **Translocations of Elephants into a collection**

- Prior to shipping the medical and pathological histories of the elephant herd from the shipping collection should be reviewed.
- Prior to shipping the animal should undergo a complete health check as per the usual requirements of the collection's veterinary department and DEFRA requirements if from overseas. With respect to EEHV the following should occur when available;
  - EEHV ELISA two weeks prior to shipment
  - EEHV ELISA one week prior to shipment
  - EEHV PCR one week prior to shipment
  - PCV/ CBC on day of shipment
  - Full clinical examination of animal on day of shipment
- If there are any concerns of active EEHV infection then the animal should not be shipped

- For elephants with a positive ELISA they should, within 24 hours of arrival and again at one week, have both the EEHV ELISA and PCR repeated to make sure stress of transport has not lead to recrudescence of clinical disease. If ELISA is not available then PCR should be repeated on the first, seventh, and fourteenth day of arrival into the collection
- Usual quarantine procedures should be in place and managed accordingly.

### **Translocation of elephants to other collections**

- The receiving collection should be informed of the herd's health and the current EEHV status within the herd.
- The receiving collection's herd EEHV status should be assessed. If this information has not been collected then the collection should be advised to assess the status prior to shipping. If unwilling then the risks should be outlined in writing and the EEP should be contacted for guidance. Ultimately however responsibility lies with the receiving institution.
- EEHV ELISA and PCR should be undertaken one week prior to shipping to the other collection in addition to other tests required by the carrier or receiving collection.

### **Risk periods**

It is not known what causes the recrudescence of disease or the factors that result in an animal becoming clinically sick. However in other herpes viruses periods of stress are thought to be important for the virus to become clinical or shed.

The main periods of stress, where there is a potential risk of EEHV infection include;

- Pregnancy
- Weaning
- Transport (although animals used to transportation this is unlikely to be a problem e.g. circuses)
- Social disharmony or introduction of new animals
- Periods of sickness with other diseases

During any of these periods, especially transportation consideration must be given to monitoring for clinical signs of EEHV. As outlined above it is prudent to monitor using ELISA or PCR techniques whenever cases are suspected.

### **Free contact verses protected contact**

When an animal becomes sick it can be very difficult to manage an animal in an intensive way using a protected contact system. Planning and training must be put in place so that access can be obtained for catheterisation, rectal temperatures, and other techniques required for the medical management of the elephant.

The only alternative is to put in place a free contact system when needed but the stress of this unusual system being implemented when an animal is already sick must be considered.

As part of an EEHV protocol the elephant keepers, veterinary staff and curatorial staff should discuss the method of management and the techniques/training that will be required to obtain the necessary access. This should be part of the medical management of any elephant for any possible disease process and should not be forgotten.

## **References**

- Basson, P.A., McCully, R.M., de Vos, V., Young, E., and Kruger, S.P. (1971). Some parasitic and other natural diseases of the African elephant in the Kruger National Park. *Onderstepoort Journal of Veterinary Research* 38:239-254
- Ehlers, B., Burkhardt, S., Goltz, M., Bergmann, V., Ochs, A., Weiler, H., and Hentschke, J. (2001). Genetic and ultrastructure characterisation of a European isolate of the fatal Endotheliotrophic elephant herpesvirus. *Journal of General Virology*, 82, 475-482
- Ehlers, B., Dural, G., Marschall, M., Schregel, V., Goltz, M., and Hentschke, J. (2006). Endotheliotrophic elephant herpesvirus, the first betaherpesvirus with a thymidine kinase gene. *Journal of General Virology*, 87: pp 2781-2789
- Fenner, F.J., Gibbs, E.P.J., Murphy, F.A., Rott, R., Studdert, M.J., and White, D.O. (1993). *Veterinary Virology*, Second Edition, Published Academic Press, London
- Fickel, J., Richman, L.K., Montali, R., Schaftenaar, W., Goritz, F., Hildebrandt, T.B., Pitra, C. (2001). A variant of the Endotheliotrophic herpesvirus in Asian elephants (*Elephas maximus*) in European zoos. *Veterinary Microbiology*, 82, pp103-109
- Fickel, J., Lieckfeldt, D., Richman, L.K., Streich, W.J., Hildebrandt, T.B., and Pitra, C. (2003). Comparison of glycoprotein B (gB) variants of the elephant Endotheliotrophic herpesvirus (EEHV) isolated from Asian elephants (*Elephas maximus*), *Veterinary Microbiology*, 91, pp11-21
- Hildebrandt, T.B., Fickel, J., Goritz, F., Rietschel, W., Lieckfeldt, D., Montali, R.J., Richman, L.K., and Ratanakorn, P. (2001). Survey on presence of the endotheliotrophic elephant herpesvirus (EEHV) in Thai camp elephants. *Proceedings of AAZV, AAWV, ARAV, NAZWV Joint Conference*. Pp183-184
- Hildebrandt, T.B., Hermes, R., Ratanakorn, P., Rietschel, W., Fickel, J., Frey, R., Wibbelt, G., Reid, C., And Goritz, F. (2005). Ultrasonographic assessment and ultrasound-guided biopsy of the retropharyngeal lymph nodes in Asian elephants (*Elephas maximus*). *Veterinary Record*, 157, 544-548
- Isaza, R., Hunter, R.P., Richman, L.K., Montali, R.J., Schmitt, D.L., Koch, D.E., and Lindsay, W.A. (2003). Famciclovir pharmacokinetics in young Asian elephants (*Elephas maximus*). *Proceedings of American Association of Zoo Veterinarians*, pp82-83
- Jacobson, E.R., Sundberg, J.P., Gaskin, J.M., Kollias, G.V., O'Banion, M.K. (1986). Cutaneous papillomas associated with a herpesvirus-like infection in a herd of captive African elephants, *Journal of the American Veterinary Medical Association*, 189: 1075-1078

- Leach, E. (1983). Vaginal virus in a mixed elephant herd. Proceedings of the Fourth Annual Elephant Workshop, Missouri, pp79-80
- McCully, R.M., Basson, P.A., Pienaar, J.G., Erasmus, B.J., and Young, E. (1971). Herpes nodules in the lung of the African elephant (*Loxodonta africana*). Onderstepoort Journal of Veterinary Research 38: 225-236
- McGeoch, D.J., and Gatherer, D. (2005). Integrating Reptilian Herpesviruses into the Family Herpesviridae. Journal of Virology, Jan, pp 725-731
- Metzler, A.E., Ossent, P., Guscetti, F., Rubel, A., and Lang, E.M. (1990). Serological evidence of herpesvirus infection in captive Asian elephants (*Elephas maximus*). Journal of Wildlife Diseases, 26(1), pp 41-49
- Montali, R.J., Richman, L.K., Mikota, S.K., Schmitt, D.L., Larsen, R.S., Hildebrandt, T.B., Isaza, R., Lindsay, W.A. (2001). Management aspects of herpesvirus infections and tuberculosis in elephants. In Recent Research on elephants and Rhinos, Schuling Verlag, Munster, p25
- Narayana Bhat, M., Manickam, R., and Kumanan, K. (1997). Serological evidence of Bovine Herpesviruses 1 and 2 in Asian elephants. Journal of Wildlife Diseases, 33(4), pp919-920
- Ossent, P., Guscetti, F., Metzler, A.E., Lang, E.M., Rubel, A., and Hauser, B. (1990). Acute and Fatal Herpesvirus Infection in a Young Asian Elephant (*Elephas maximus*). Veterinary Pathology, 27: 131-133
- Pilaski, J., Hentschke, J., Sinn, D., Francke, R., Rosenbruch, M., Olderding, P., and Molle, G. (1988). Zwei Viruserkrankungen unterschiedlicher Aetiologie bei Asiatischen Elefanten (*Elephas maximus*) in einem kleinen Wanderzirkus. Verhandlungsbericht des Internationalen Symposiums über Erkrankungen der Zootiere, 30: 263-269
- Reid, C.E., Hildebrandt, T.B., Marx, H., Hunt, M., Thy, N., Reynes, J.M., Schaftenaar, W., and Fickel, J. (2006) >Endotheliotrophic elephant herpesvirus (EEHV) infection, The first PCR confirmed fatal case in Asia. Vet Q, 28 (2): pp 61-64
- Richman, L.K., Montali, R.J., Cambre, R.C., and Lehnhardt, J. (1996). Endothelial Inclusion Body Disease: A newly recognised fatal herpes-like infection by Asian elephants. Proceedings of the American Association of Zoo Veterinarians, pp483-485
- Richman, L.K., Montali, R.J., Garber, R.L., Kennedy, M.A., Lehnhardt, J., Hildebrandt, T., Schmitt, D., Hardy, D., Alcendor, D.J., and Hayward, G.S. (1999). Novel Endotheliotrophic herpesvirus fatal for Asian and African elephants. Science, Vol 283, 19<sup>th</sup> February
- Richman, L.K., Montali, R.J., Cambre, R.C., Schmitt, D., Hardy, D., Hildebrandt, T., Bengis, R.G., Hamzeh, F.M., Shahkolahi, A., and Hayward, G.S. (2000). Clinical and pathological findings of a newly recognised disease of elephants caused by Endotheliotrophic herpesviruses. Journal of Wildlife Diseases, 36(1), pp1-12
- Richman, L.K., Montali, R.J., and Hayward, G.S. (2000b). Review of a newly recognised disease of elephants caused by Endotheliotrophic herpesviruses. Zoo Biology, 19: 383-392
- Ryan, S.J., and Thompson, S.D. (2001). Disease risk and Inter-institutional transfer of specimens in cooperative breeding programmes: Herpes and the Elephant Species Survival Plans. Zoo Biology, 20: pp 89-101
- Schaftenaar, W., and Mensink, J.M.C.H., Boer, A.M. D.E., Hildebrandt, T.B., and Fickel, J. (2001). Successful treatment of a subadult Asian elephant bull

(*Elephas maximus*) infected with the endotheliotropic elephant herpes virus. Proceedings of the 40<sup>th</sup> International Symposium on Disease of Zoo and Wild Animals, 4, 141-146

- Schaftenaar, W., and Mensink, J.M.C.H. (2005). The use fo famciclovir with and without proper indications. *Verh.ber.Erkrgr.Zootiere* 42, pp100-104
- Schmitt, D.L., Hardy, D.A. (1998). Use of famciclovir for the treatment of herpesviruses in an Asian elephant. *Jounral of Elephant Managers Association*, 9: pp103-104
- Schmitt, D.L., Hardy, D.A., Montali, R.J., Richman, L.K., Lindsay, W.A., Isaza, R., and West, G. (2000). Use of famciclovir for the treatment of Endotheliotropic herpesvirus infections in Asian elephants (*Elephas maximus*). *Journal of Zoo and Wildlife Medicine*, 31(4), 518-522
- Wellehan, J.F.X., Johnson, A.J., Childress, A., and Isaza, R. (2006). Identification of two novel herpesviruses associated with ocular inflammation in Asian elephants (*Elephas maximus*). *Proceedings of American Association of Zoo Veterinarians*
- Wingfield, W.E., and Raffe, M.R. (2002). *The Veterinary ICU Book*, Publisher Teton Newmedia, Wyoming.

### **Other sources of information**

- [www.elephantcare.org](http://www.elephantcare.org)
- Fowler, M.E., and Mikota, S.K. (2006). *Biology, Medicine, and Surgery of Elephants*, Pubilsher Blackwell Publishing, Iowa.
- Bertone, J.J., and Horspool, L.J. (2004). *Equine Clinical Pharmacology*, Publisher Saunders.



## **Appendix A**

### **Alternative names for EEHV in the literature**

- Elephant Endotheliotrophic Herpes Virus (EEHV)
- Elephantid Herpesvirus
- Proboscivirus
- Proboscis  $\beta$ -3-herpes virus
- Endothelial Inclusion Body Disease

## **Appendix B**

### **Famciclovir dosage and cost**

<b>Weight (kg)</b>	<b>Dosage (g)</b>	<b>First tid dose (g)</b>	<b>Daily bid dose (g)</b>	<b>Total dose 7 days (g)</b>	<b>Total dose 30 days (g)</b>	<b>Total Cost 7 days (£)</b>	<b>Total cost 30 days (£)</b>
100	1.2	3.6	2.4	18	73.2	489.96	1,992.50
200	2.4	7.2	4.8	36	146.4	979.92	3,985.00
300	3.6	10.8	7.2	54	219.6	1,469.88	5,977.50
400	4.8	14.4	9.6	72	292.8	1,959.84	7,970.00
500	6	18	12	90	366	2,449.80	9,962.50
600	7.2	21.6	14.4	108	439.2	2,939.76	11,955.00
700	8.4	25.2	16.8	126	512.4	3,429.72	13,947.50
800	9.6	28.8	19.2	144	585.6	3,919.68	15,940.00
900	10.8	32.4	21.6	162	658.8	4,409.64	17,932.50
1000	12	36	24	180	732	4,899.60	19,925.00
1100	13.2	39.6	26.4	198	805.2	5,389.56	21,917.50
1200	14.4	43.2	28.8	216	878.4	5,879.52	23,910.00
1300	15.6	46.8	31.2	234	951.4	6,369.48	25,902.50
1400	16.8	50.4	33.6	252	1024.8	6,859.44	27,895.00
1500	18	54	36	270	1098	7,349.40	29,887.50
1600	19.2	57.6	38.4	288	1171.2	7,839.36	31,880.00
1700	20.4	61.2	40.8	306	1244.4	8,329.32	33,872.50
1800	21.6	64.8	43.2	324	1317.6	8,819.28	35,865.00
1900	22.8	68.4	45.6	342	1390.8	9,309.24	37,857.50
2000	24	72	48	360	1464	9,799.20	39,850.00
2100	25.2	75.6	50.4	378	1537.2	10,289.16	41,842.50
2200	26.4	79.2	52.8	396	1610.4	10,779.12	43,835.00
2300	27.6	82.8	55.2	414	1683.6	11,269.08	45,827.50
2400	28.8	86.4	57.6	432	1756.8	11,759.04	47,820.00
2500	30	90	60	450	1830	12,249.00	49,812.50
2600	31.2	93.6	62.4	468	1903.2	12,738.96	51,805.00
2700	32.4	97.2	64.8	486	1976.4	13,228.92	53,797.50
2800	33.6	100.8	67.2	504	2049.6	13,718.88	55,790.00
2900	34.8	104.4	69.6	522	2122.8	14,208.84	57,782.50
3000	36	108	72	540	2196	14,698.80	59,775.00

Famciclovir prices based on current prices at time of writing (BNF 2007) at a dose rate of 12mg/kg

## Appendix C

### Acyclovir dosage and cost

Weight (kg)	Dosage (g)	Five x daily dose (g)	Total dose 7 days (g)	Total dose 30 days (g)	Total Cost 7 days (£)	Total cost 30 days (£)
100	1.9	9.5	66.5	285	21.95	94.05
200	3.8	19	133	570	43.89	188.10
300	5.7	28.5	199.5	855	65.84	282.15
400	7.6	38	266	1140	87.78	376.20
500	9.5	47.5	332.5	1425	109.73	470.25
600	11.4	57	399	1710	131.67	564.30
700	13.3	66.5	465.5	1995	153.62	658.35
800	15.2	76	532	2280	175.56	752.40
900	17.1	85.5	598.5	2565	197.51	846.45
1000	19	95	665	2850	219.45	940.50
1100	20.9	104.5	731.5	3135	241.40	1,034.55
1200	22.8	114	798	3420	263.34	1,128.60
1300	24.7	123.5	864.5	3705	285.29	1,222.65
1400	26.6	133	931	3990	307.23	1,316.70
1500	28.5	142.5	997.5	4275	329.18	1,410.75
1600	30.4	152	1064	4560	351.12	1,504.80
1700	32.3	161.5	1130.5	4845	373.07	1,598.85
1800	34.2	171	1197	5130	395.01	1,692.90
1900	36.1	180.5	1263.5	5415	416.96	1,786.95
2000	38	190	1330	5700	438.90	1,881.00
2100	39.9	199.5	1396.5	5985	460.85	1,975.05
2200	41.8	209	1463	6270	482.79	2,069.10
2300	43.7	218.5	1529.5	6555	504.74	2,163.15
2400	45.6	228	1596	6840	526.68	2,257.20
2500	47.5	237.5	1662.5	7125	548.63	2,351.25
2600	49.4	247	1729	7410	570.57	2,445.30
2700	51.3	256.5	1795.5	7695	592.52	2,633.40
2800	53.2	266	1862	7980	614.46	2,727.45
2900	55.1	275.5	1928.5	8265	636.41	2,821.50
3000	57	285	1995	8550	658.35	2,915.55

Acyclovir prices based on current prices at time of writing (BNF 2007) at a dose rate of 19mg/kg, this dose rate has not been recorded for use in elephants and is an extrapolated dose rate and dosing schedule from human famciclovir/acyclovir usage in the management of herpes. It is included here for the UK market where obtaining famciclovir can be difficult or cost prohibitive for some collections.

**The author does not take responsibility for the use of acyclovir and any side effects that may occur when used in elephants.**

## **Appendix D**

### **Normal elephant physiological parameters**

<b>Parameter</b>		<b>Normal values</b>
Weight (kg)	<i>E.m.maximus</i>	2000-5500
	<i>E.m.indicus</i>	2000-5500
	<i>E.m.sumatranus</i>	2000-4000
	<i>L.a.africana</i>	4000-7000
	<i>L.a.cyclotis</i>	2000-4500
Skin thickness (cm)		1.9-3.2
Rectal temperature	°C	36-37
	°F	97.5-99
Heart rate (bpm)	Standing	25-30
	Lateral recumbency	72-98
Respiratory rate (bpm)		4-12
Blood pressure (standing) (mmHg)	Systolic	178.6+/- 2.9
	Mean	144.6 +/- 2.9
	Diastolic	118.7 +/- 3.1

Taken from Mikota (2006) Elephant Vital Signs and Physiological Parameters, [www.elephantcare.org](http://www.elephantcare.org).

## **Appendix E**

### **Sample requirements**

#### **Ante mortem samples**

- EDTA whole blood for PCR
- (Heparin blood possible for PCR but not preferred)
- Serum for ELISA
- Biopsy masses and keep frozen, not formalin for PCR
- Biopsy masses in formalin for histology

#### **Post mortem samples**

All samples to be stored frozen and sent for PCR in a suitable container

- Heart
- Liver
- Tongue
- Retropharyngeal lymph node
- Bone marrow in juveniles
- (Lung)
- (Placenta)
- (Any bizarre lesions)
- (Brain)

#### **Other samples**

Discuss with your local testing facility as to other tissues that they may require as the epidemiology and research continues.

## **Appendix F**

### **EEHV testing facilities**

#### **Institutions for EEHV sample sending**

##### **Mario Wickert**

Institute of Virology  
Vetsuisse-Faculty, Veterinary Medicine  
Winterthurerstr. 266a  
CH - 8057 Zürich  
Switzerland

Tel. +41 44 635 87 10  
Fax. +41 44 635 89 11

- **Prefer frozen (-80° C) 5ml EDTA blood for PCR, minimum 1-2ml**
  - **Also would like serum for ELISA production**
  - **Will also take tissues**
- 

##### **Willem Schaftenaar DVM**

Head of the Veterinary Department  
Rotterdam Zoo  
Van Aerssenlaan 49  
3039 KE Rotterdam  
The Netherlands

Tel: 31-10-4431485  
Fax: 31-10-4431414

(Actually Erasmus University, Byron Martina)

- **Prefer frozen (-80° C) heparin plasma for ELISA, minimum 1-2ml**
  - **Tissues from post mortem for PCR:**
  - **Will serotype the virus**
  - **Also will perform PCR on EDTA blood**
- 

##### **Thomas Hildebrandt/Joerns Fickel**

Institut für Zoo- und Wildtierforschung (IZW)  
Im Forschungsverbund Berlin e.V  
Postfach 601103  
D-10252 Berlin  
Germany

Tel: 0049 (0) 30 51680  
Fax: 0049 (0) 30 5126104

- **As for Rotterdam.**

**Michelle Quinlivan**

Department of Virology  
Irish Equine Centre  
Johnstown  
Naas  
County Kildare  
Ireland

Ph +3534586626

- **PCR available**

**USA EEHV samples**

**Dr. Laura Richman**

John Hopkins Medical Institution  
1650 Orleans Street, CRB 316  
Baltimore, MD 21231

Tel: (410) 995-8682

Fax: (410) 955-8685

**Erin Latimer (EEHV tech)**

Dept. of Pathology  
Smithsonian National Zoo  
3001 Connecticut Ave., NW  
Washington, D.C. 20008

Tel: (202) 633-4252

Fax: (202) 673-4660

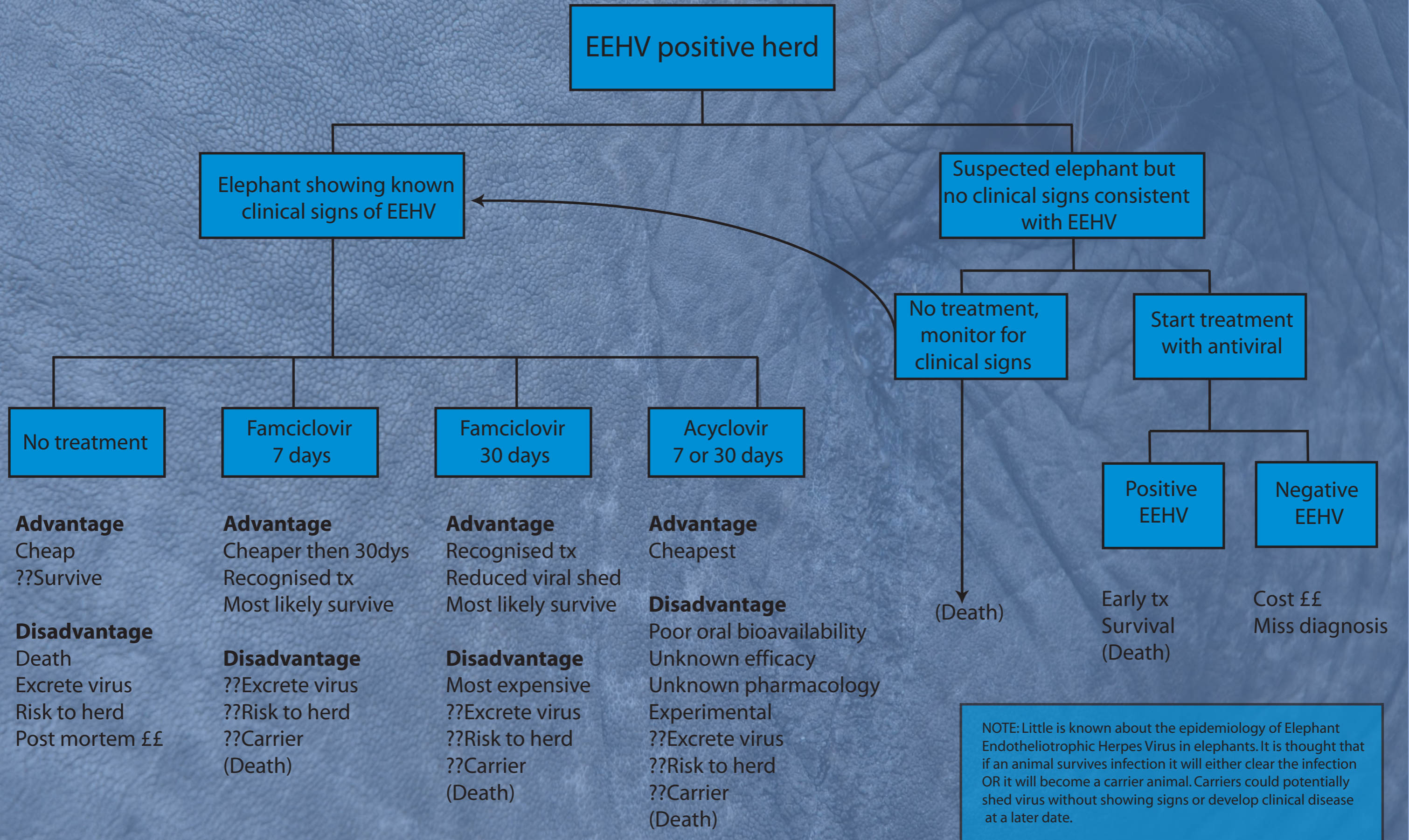
- **Both PCR and functional ELISA available**
- **Contact regarding CITES import and shipping requirements**

**Appendix G**

**Proposal for use and selection of antiviral agents**

**(Over page)**

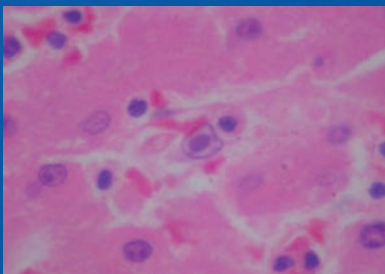
# Elephant Endotheliotrophic Herpes Virus Selection of Antiviral Drugs





# Elephant Endotheliotropic Herpes Virus Protocol

## Version 1.3



Elephant Endotheliotropic Herpes Virus (EEHV) is one of the most important emerging infectious diseases of elephants. It has implications for the future of captive as well as wild population management programmes. Ossent et al (1990) described the first death of an Asian elephant (*Elephas maximus*) attributed to EEHV, but the first detailed description of EEHV was in 1996 (Richman et al). Despite being recognised for over ten years little is known about the epidemiology of the disease.

Risk factors may include, but are not limited to; young age (under ten years), stress such as weaning or transportation, and contact with African elephants (*Loxodonta africana*). EEHV is caused by a virus of the subfamily  $\beta$ -herpesviridae. EEHV can be subdivided into three principle types which include; EEHV1 which causes acute disease in Asian elephants and can be carried asymptotically in African elephants; EEHV1b, which has so far been found in Asian elephants only; and EEHV2, which is found only in African elephants. Others may exist.

This protocol aims to review the growing body of literature regarding the management of EEHV and includes recommendations for the management and therapy of this devastating disease.