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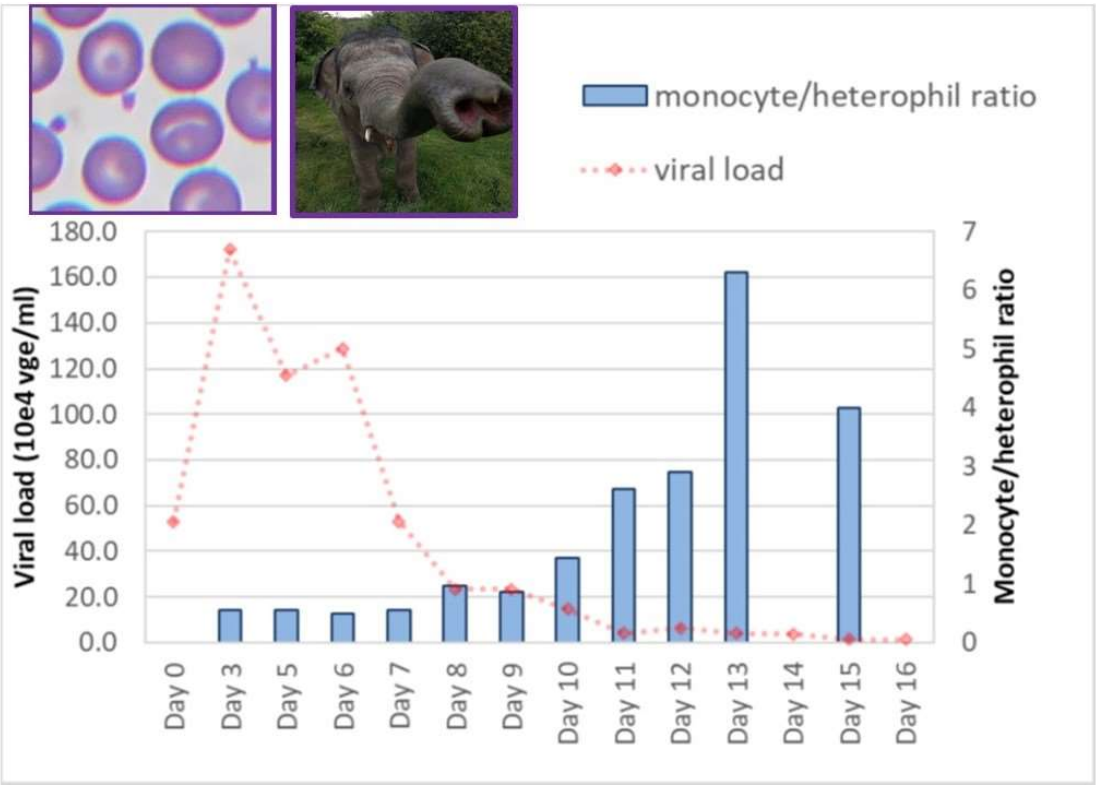
Elephant Endotheliotropic Herpes Virus (EEHV)



Latest research and current protocols 2019

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Contents

1. Introduction

Elephant endotheliotropic herpes viruses (EEHVs) can cause acute haemorrhagic disease (HD) in young Asian elephants (*Elephas maximus*). Clinical infections account for the majority of fatalities in calves in captivity, posing the greatest threat for sustaining a viable captive breeding population. The first reported death due to HD of unknown causes occurred in Switzerland in 1988 [33] and in 1999 it was reported that herpes viruses could be detected in tissues of necropsy cases.[39] Up to 2016, well over 80 confirmed cases have been reported in Asian elephants worldwide, with only 10 confirmed survivors.[25] In Europe alone, of the 109 calves born between 1985 and 2017, 25 have died of EEHV-HD.[37] Since then, changes in approach, which include early detection and aggressive intervention, have increased the chances of survival.[31,14] EEHV has also been confirmed and suspected in both free-living and captive Asian elephant calf deaths in Cambodia,[38] India,[54,53,26] Thailand,[47,46] Sri Lanka (Perera, pers comm 2018), Laos,[4] Myanmar,[32] Sumatra and Nepal.[25]

1.1 Etiology

Although previously grouped under the β-herpes viruses,[55] EEHVs differ significantly from the current α-, β- and γ-herpes viruses. It has therefore been proposed that the genus Proboscivirus, encompassing the elephant herpes viruses responsible for HD, is grouped in a new subfamily, the δ-herpes viruses.[24,34,40,58] Within the Probosciviruses, there are seven recognized species of EEHVs. EEHV1a, EEHV1b, EEHV4, EEHV5 all infect Asian elephants, and EEHV2, EEHV3, EEHV6 and EEHV7 infect African elephants. In the African elephant (*Loxodonta africana*), until recently there are reports of only two confirmed lethal cases and two confirmed survivors of HD caused by EEHV.[25] However, in 2019 two African elephants have died of EEHV.[19] EEHVs should be distinguished from elephant γ-herpes viruses, which can be isolated from nodules in the skin and mucosae of both Asian and African elephants.[22,27]

1.2 Epidemiology

Viruses in the family *Herpesviridae* have the ability to become latent and reactivate. EEHVs behave no different. Once an elephant becomes infected, it remains a life-long carrier. Reactivation of the virus can occur during periods of stress, excitement or illness due to other causes, and leads in adult elephants to excretion of virus in all bodily fluids, especially in trunk secretions [49,16] and in females also in vaginal-vestibular secretions.[42] Transmission routes for the virus have not yet been proven, but one can assume that contact with infected bodily fluids is the most likely route, and vertical transmission leading to subclinical infection has been seen (Dastjerdi, pers comm 2019). The incubation period anecdotally appears to be 7-14 days. Haemorrhagic disease caused by EEHVs has been seen in elephants ranging from 9 months to 40 years of age, but the general age range is 1-8 years, especially affecting the 1-4 year olds.[25]

Genetic analysis has shown that each captive elephant facility harbors its own EEHV strains [55,56,25] and co-infection of different EEHV species in one individual is possible.[43] Of all the EEHVs present, EEHV1a appears to be the most pathogenic and responsible for most cases of fatal HD in Asian elephants.[25] Although there have also

been many EEHV1b fatalities, elephants infected with this genotype appear to respond better to intervention. [31,15] Serological studies in Asian elephants in European and North America zoos and in captive Asian elephants in Thailand using a gB-ELISA have demonstrated that most elephants in all herds are infected with EEHV.[51] Most if not all adult elephants can therefore be considered to have survived a subclinical EEHV infection as juveniles.

2. Monitoring and clinical signs

2.1 Monitoring of clinically healthy juveniles

2.1.1 Haematology – in-house white blood cell differentials

If a juvenile Asian elephant is exposed to a high level of virus during a period of immunosuppression, especially if it concerns a primary exposure,[13] there is a likelihood of viral replication with subsequent clinical disease. Initial findings in the very early stages of the disease are a sudden decrease in lymphocytes and especially monocytes compared to a fairly stable heterophil level, which can be established via a white blood cell differential count on an air-dried stained blood smear (Appendix 1). This monocyte/heterophil ratio has proven to be a good prognostic indicator in EEHV infections.[7,52,31] The relative and absolute drop in mainly the monocytes causes an overall reduction of the absolute white blood cell count. This can be detected prior to the virus reaching detectable limits in the circulation. This can be accompanied by a gradual reduction in platelets. An estimated platelet count can be made manually by counting ten high-power (x100) fields and multiplying the total by 1.5 to obtain platelets*10^9/L.[41] Due to the variation between white blood cell counts in individual elephants, it will be essential to establish a normal range for each juvenile.[36] Minor changes in the initial stages of the disease can thus be detected in-house using basic veterinary equipment such as microscope slides, DiffQuick staining and a decent microscope.[28]

In elephants with an appropriate immune response to the virus, leucopenia will not be evident but initial viral loads will coincide with an elevated white blood cell count with high monocyte levels, an increased monocyte/heterophil ratio[30] and elevated platelet count.[2] This is also seen in subclinical episodes following survival of a clinical case.[52] Treatment in subclinical cases with such an adequate immune response is not required.

A detailed recent publication on leucocyte response in inflammatory processes has been written by Nicole Stacy, and is a good point to start when familiarizing oneself with elephant blood smear reading.[48]

2.1.2 qPCR on EDTA blood samples

Viral DNA can be detected in whole EDTA blood samples up to several weeks prior to

the manifestation of clinical signs. The quantitative PCR (qPCR) methods that have currently been developed for EEHVs can detect viral DNA from as little as 500 viral genome equivalents (vge) per ml blood.[16] Using Taqman PCR techniques, different EEHV species can be distinguished within a single blood sample.[17] Not all viral DNA positive elephants at this detection limit will go on to develop clinical disease, as in most cases the elephant's immune response will be able to overcome the initial infection. If, however, there is immunosuppression during these mild viraemic periods, immense viral replication can be triggered, with subsequent HD.[28]

Figure 1: Elephant haematology

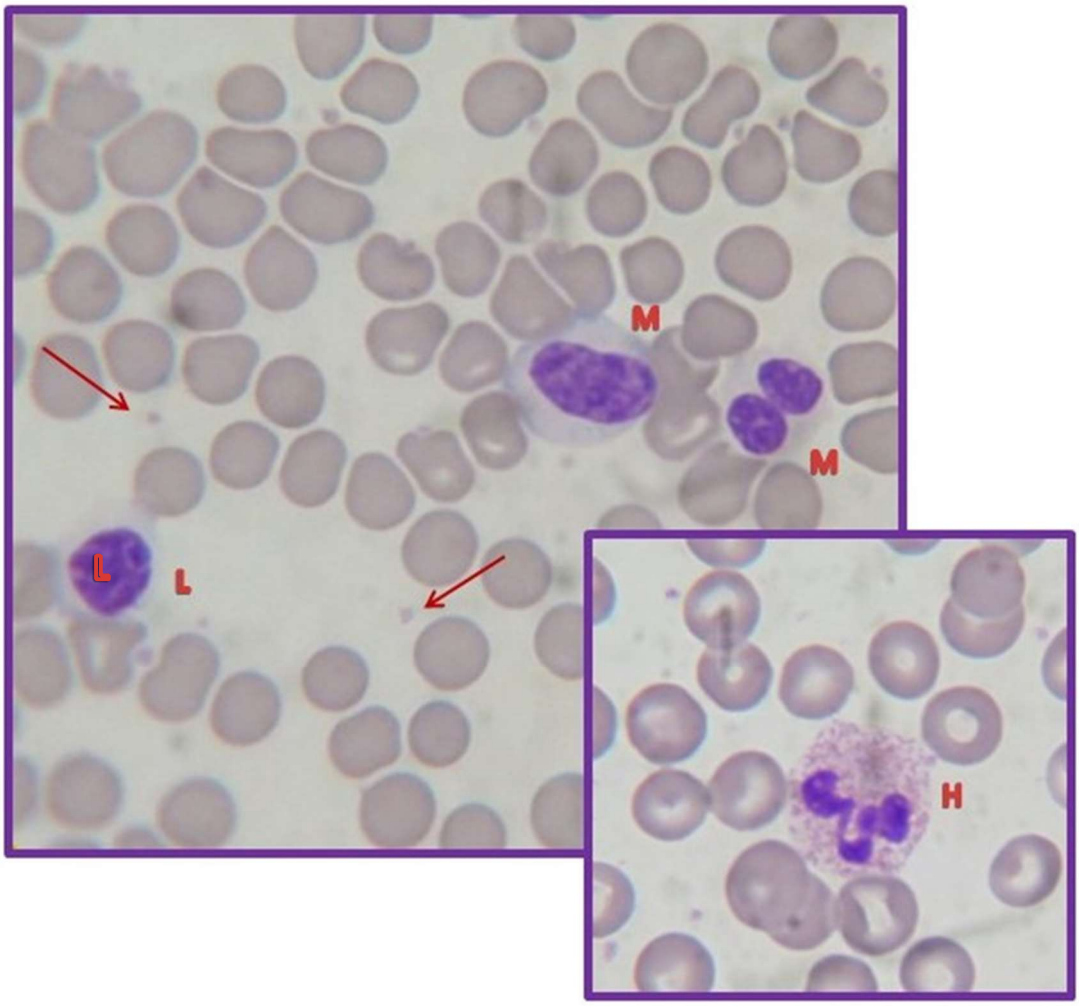


Figure 1: In the early stages of EEHV infection, there will be a relative decrease of monocytes (M) in relation to the heterophils (inset, H). Note that the nucleus of the monocyte can appear bean-shaped or bi-lobed. A lymphocyte can also be seen on this slide (L). The number of platelets (red arrows) will reduce gradually and persistently throughout clinical disease. A rough estimation of their number can be made from following the change in their average number in ten high power fields. All these estimates can be made in a basic veterinary laboratory and give the clinician insight into disease progression and prognosis. A detailed step-by-step guide to in-house elephant haematology can be found in Appendix 1.

Routine monitoring of the viral load, in combination with routine haematology, will provide an early indication of EEHV infection and will thus buy the clinician time to respond before clinical signs will have progressed to a point beyond which treatments will have less effect. Weekly monitoring is recommended.

2.2 Progression of haematological changes and onset of clinical signs

In those case of EEHV infections that lead to HD, the platelets will deplete further, monocytes will remain low and clinical signs will usually follow. Initial clinical signs of EEHV infection can consist of disrupted sleeping and playing patterns, and a mild lethargy. General inappetence is usually seen. For comfort, a calf might appear to be sucking more from its dam than normal. This can last for several days before outward clinical signs become apparent. Initial outward signs will consist of petechiae and bruising, which will be visible on the tongue, gingiva, palate, vulva and preputium. This will progress to subcutaneous oedema, most visible on the head (Figure 2a and 2b) and ventral abdomen (Figure 2c). Joint pain can occur. Urine production will markedly reduce and hematuria can be detected (Figure 2d). At this point, there will be a marked reduction of platelets in the haematological profile.

Disease progression leads to circulatory failure and advanced lung oedema, eventually causing hypovolemia and hypoxia, with severe cyanosis (Figure 2e) usually preceding death. Juveniles can either become moribund several hours prior to death (Figure 2f), or walk around until the final moment when compensatory mechanisms fail and literally 'drop dead'.

If the clinical signs remain mild, for example a mild inappetence and stiffness of the joints, without progression to the oedematous stage of the disease, there is a good possibility of survival.[29] In these cases, excretion of viral DNA can be detected in conjunctival and trunk secretion samples. This excretion usually commences about 7-14 days after the onset of viraemia, and can persist for many months afterwards [44]

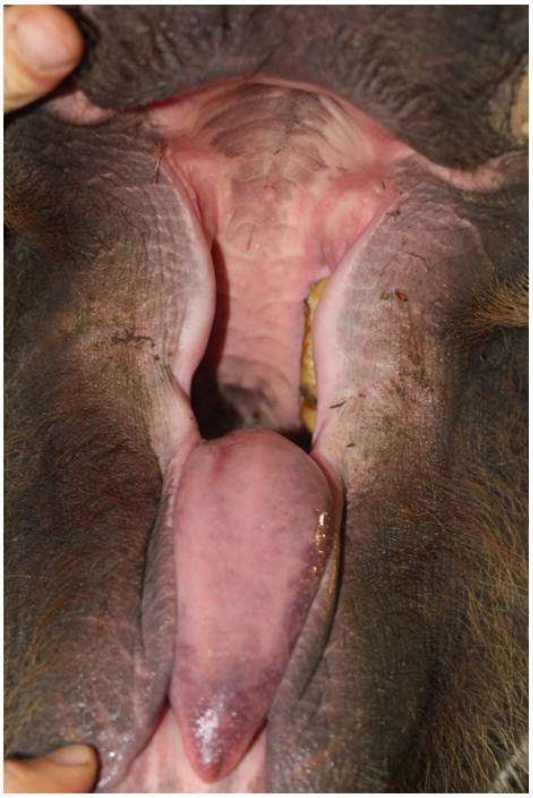
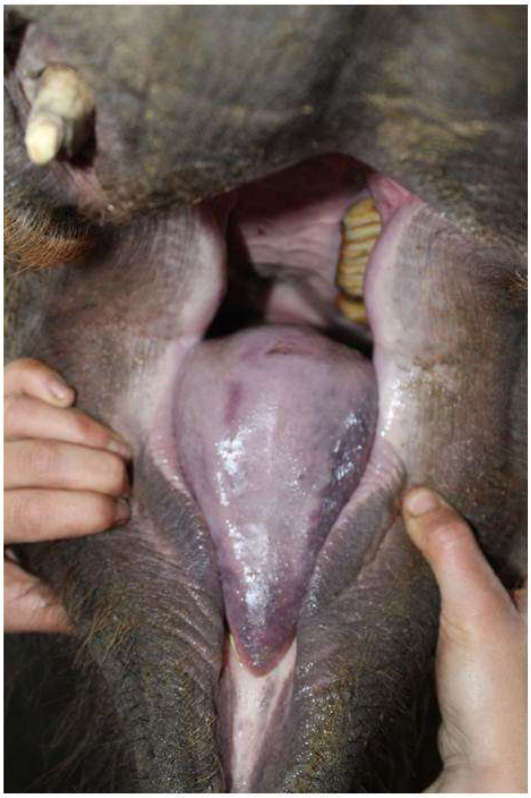
Figure 2: Clinical signs of haemorrhagic disease

2a) Oedema is first visible at the head

2b) Oedema is especially apparent around the eyes

2c) Ventral oedema can be visible; the trunk is flaccid and the calf listless

2d) Urinary output is much reduced and the urine becomes concentrated

2e) 'Bruising' of the tongue, seen on the left, is an early clinical sign. The picture on the right was taken of the same calf, 12 hours later. Note the marked cyanosis, caused by reduced circulating volume, reduced cardiac output and progressive pulmonary oedema.

2f) In the final stages of the disease, some calves become recumbent, as shown above. Other calves can walk around until they literally drop dead.

2.3 Recovery

In cases of immune response recovery, including in those on treatment, a surge in lymphocytes has been observed prior to monocyte recovery.[30,52] Clinical signs may still develop despite treatment and will progress until there is endogenous monocyte recovery.[52] The monocyte/heterophil ratio on a blood smear will prove valuable as a strong prognostic indicator during this time (Figure 3). [7,52,31] In the past, once the significant clinical signs such as tongue bruising and head oedema were detected, death usually followed within 24-36 hours. Since the report of the first treated survivor of severe HD in 2015,[3] the likelihood of survival of HD calves has increased where aggressive treatment protocols have been implemented.[31,14,15,3] Success of treatment may be partly due to early discovery of the viraemia, better interpretation of the immune response, more intensive treatment than previously, and in some cases the trial of novel treatment methods (Dastjerdi, pers comm 2019).[18]

Figure 3: Prognostic value of the monocyte/heterophil ratio

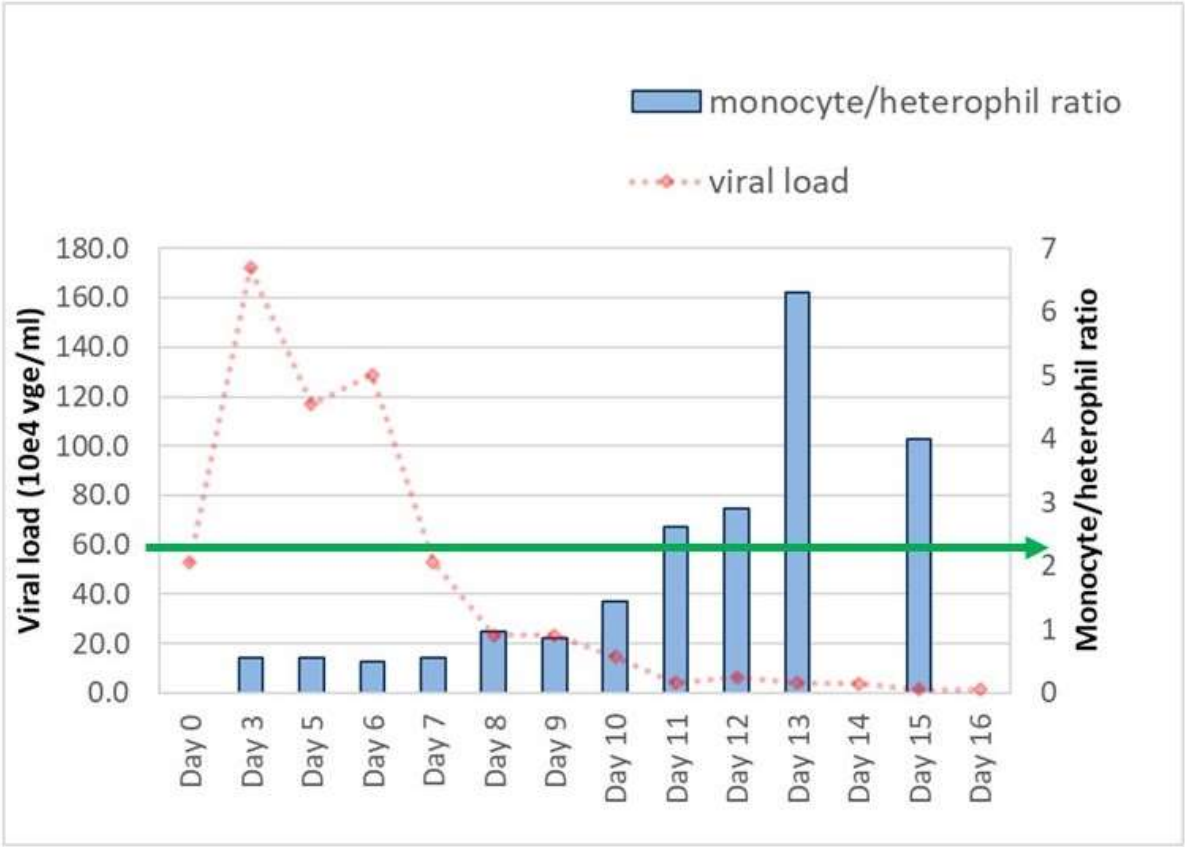
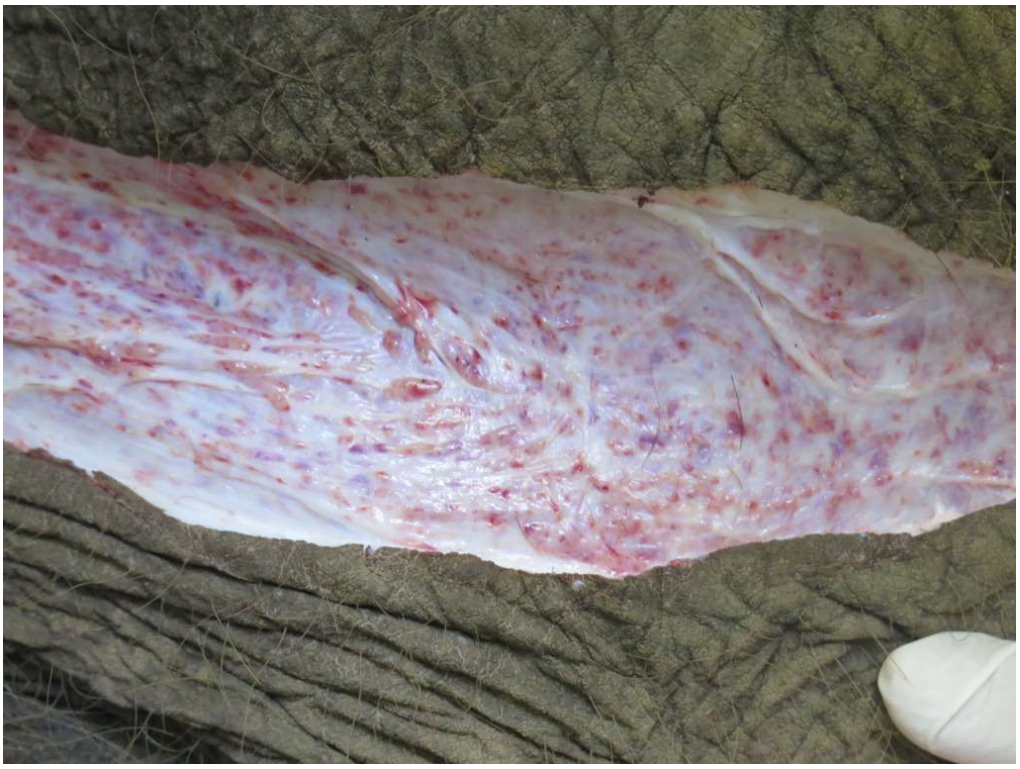


Figure 2: The monocyte/heterophil (M/H) ratio in a clinical EEHV1b case that survived with treatment. Note that the M/H ratio remains low in the run-up to an episode of clinical signs (Day9-10), and clearly shows how the monocytes recover. Resolution of clinical signs coincided with the M/H ratio increasing over this individual's normal value (green arrow).

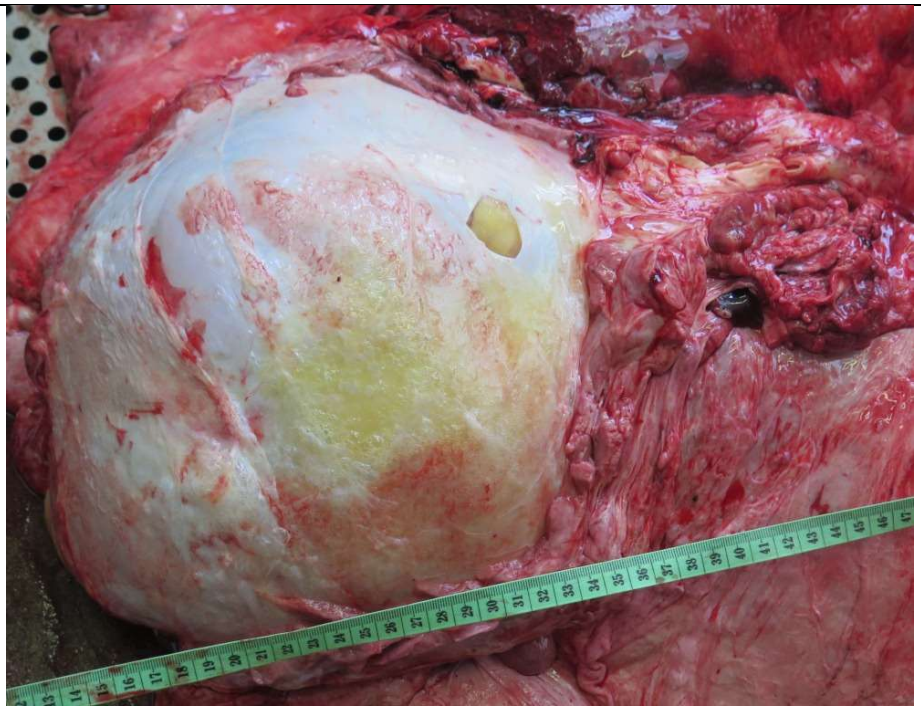
2.4 Gross post-mortem examination

Gross post-mortem findings can vary from case to case, depending on where the most vascular damage has occurred, but there are some general findings that are seen in all cases. Extensive subcutaneous oedema with petechial hemorrhages can be seen (Figure 4a). There can be free hemorrhagic fluid present in the abdominal cavity. The pericardium is usually filled with several liters of hemorrhagic fluid (Figure 4b,c) and myocardial hemorrhages are common (Figure 4d). Oedema and hemorrhages can additionally be seen in the mesentery and intestinal wall (Figure 4e), meninges and brain (Figure 4f).

Figure 4: Common gross post-mortem findings

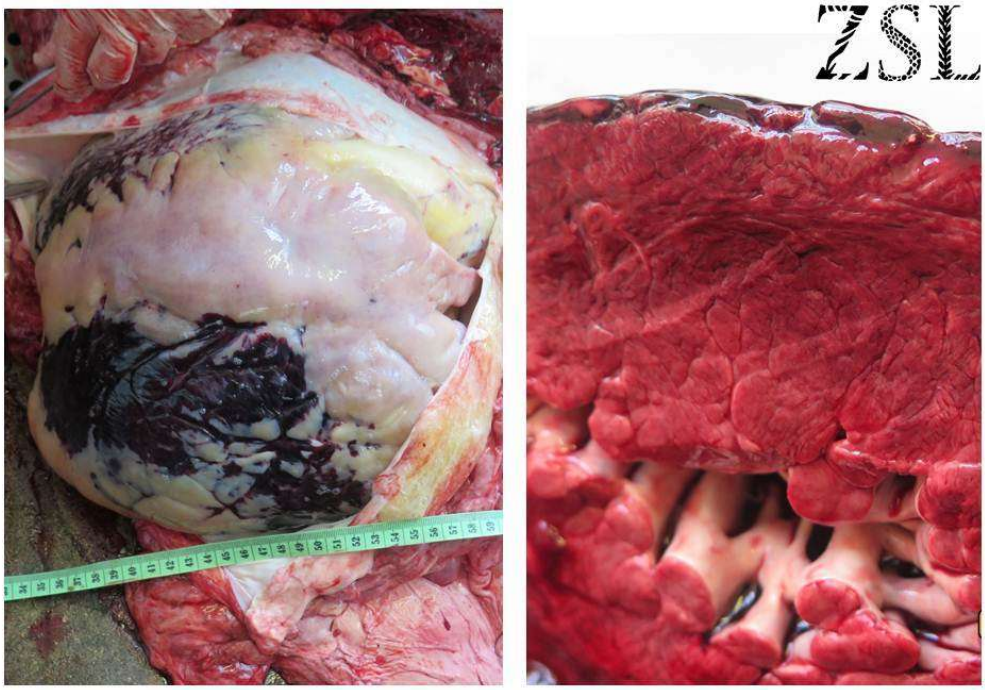


4a) Subcutaneous oedema and petechiae

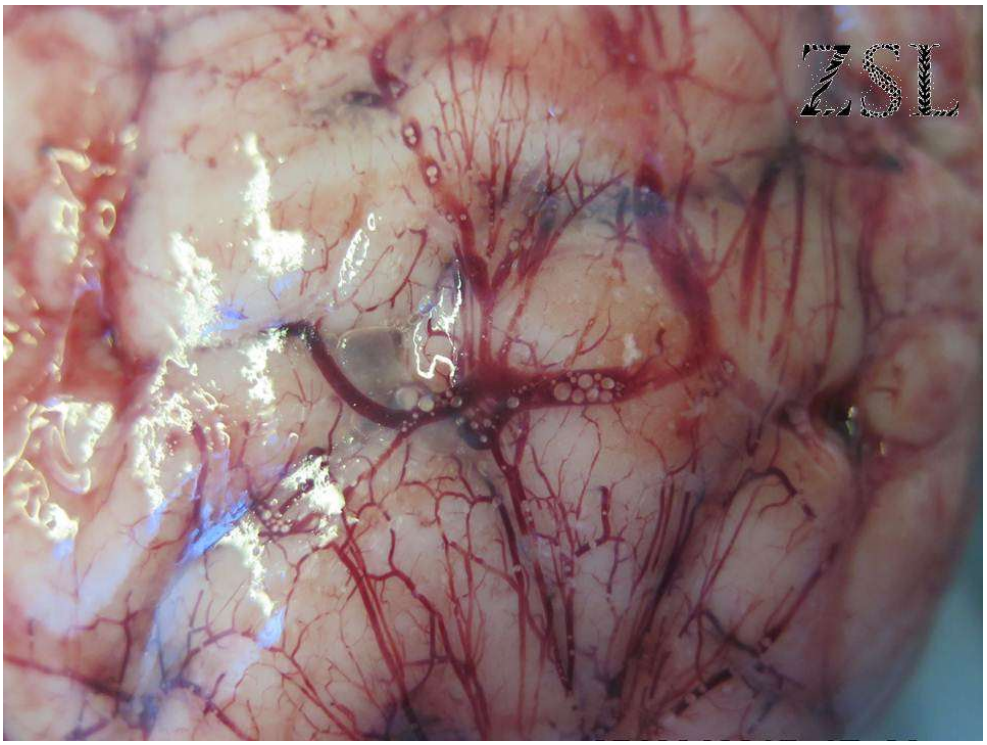


4b) Distended pericardial sac (hydropericardium) and oedematous lungs

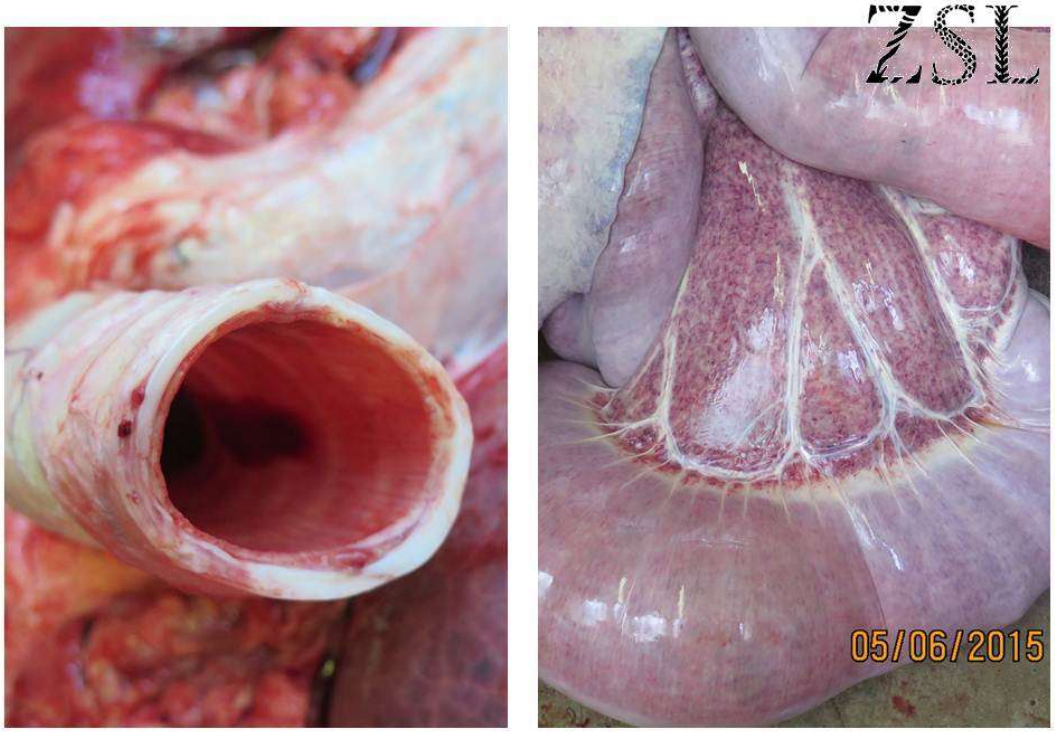
4c) In most cases, several liters of hemorrhagic pericardial fluid is present



4d) Hemorrhages in the heart are evident throughout the epicardium, myocardium and endocardium



4e) Oedema and hemorrhaging in the meningae and brain



4f) Hyperemia, petechiae and hemorrhages can be seen throughout various organs, such as in the tracheal rings in the image on the left and the mesentery and intestinal wall on the right

2.5 Sample collection and storage

It is prudent to store a large selection of tissues both in 10% buffered formalin for histopathology and immunohistochemistry, and a further selection of tissues frozen (preferably at -80°C) for PCR. Any free fluid should also be sampled and stored frozen. Detailed post-mortem protocols for EEHV cases, with the latest information on sample requests for current EEHV research, can be found on www.eehvinfo.org on the EAZA elephant TAG website.

2.6 Histopathological changes and viral distribution

Histopathologically there will be vascular epithelial degeneration and hypertrophy, with capillary endothelial cell necrosis and intranuclear endothelial inclusion bodies throughout multiple tissues. Changes to the heart include multifocal to confluent subepicardial, subendocardial and myocardial haemorrhages and oedema with myofibre degeneration and necrosis. Changes to the liver can include acute perivascular hemorrhage with hepatocellular necrosis and depletion, and the kidneys can show multifocal acute haemorrhages and oedema with tubular epithelial necrosis. Myofibre necrosis of the striated musculature can be seen, and hematoma formation within the spleen. Lymphoid depletion of lymph nodes, spleen and GALT is commonly seen.

Viral DNA has been detected in a number of necropsy tissues of Asian and African elephants. EEHV1 has been found mainly in heart and mesenteric lymphnodes, and to a lesser degree in the lung and spleen. EEHV4 DNA has been detected in the mesenteric lymphnodes, adrenal glands, lung, ileum, spleen and heart, and to a lesser degree in the testis and thymus.[44] In asymptomatic African elephants, EEHV2, EEHV3 and EEHV6 DNA has been detected in lung nodules, bronchioles and spleen, and EEHV7 DNA in lung nodules only.[57] As for excretion, EEHV DNA can be detected in trunk secretions[49] and conjunctival swabs.[16] The exact site of viral replication of the various EEHV species however is as yet unknown.

3. Treatment

There are different moments in time when clinicians decide to commence treatment. At ZSL Whipsnade Zoo, where we have established haematological normal ranges for each individual, we start treatment of calves if one of the following occurs (Appendix 2):

1) High EEHV viral load by PCR in a <u>single</u> blood sample (>50,000 vge/ml)
2) Increasing EEHV viral load by PCR in two <u>consecutive</u> blood samples
3) Relative marked drop in monocytes compared to heterophils and/or continuous reduction of platelets – three consecutive blood samples are required to distinguish a try downward trend from natural fluctuation. Note that leucopenia and monocytopenia are not pathognomonic for EEHV and other infectious diseases should also be considered.

As yet (May 2019) there is no scientifically proven effective treatment for EEHV HD, but that does not mean that every attempt should be made to support the elephant during the stage of active EEHV infection and to trial drugs that might, although not proven, reduce the viral replication and subsequent endothelial degradation and circulatory failure. Unless required for the handling and treatment of the juvenile, it is not necessary to split the affected elephant off from the entire herd. On the contrary, this could create additional social stress, which could lead to immunosuppression that would be deleterious for the affected elephant. It would be prudent to keep adult related herd mates with the juvenile at all times, if at all possible.

3.1 Fluid therapy

Fluid therapy is the one treatment likely to be the most supportive throughout clinical disease caused by EEHV. Initial rectal fluids are essential, as fluid absorption through the rectum is very effective in elephants. Clearance of fecal matter from the rectum and distal colon is a prerequisite, using lubricants and lukewarm water. After this, several liters of lukewarm tap water can be instilled in the distal colon using a meter long hose. Total volume given should be approximately 10-20 ml/kg, two to three times daily.

Please note that if intravenous access has been acquired, any intravenous

crystalloids should be followed up by rectal fluids for the most efficient boosting of the circulating volume, as routinely used commercially available crystalloids are hyperosmotic to elephants.[14]
To place an intravenous catheter, apply local anesthetic cream (e.g. EMLA cream) to the medial aspect of the ear pinna. After one hour, aseptically prepare a selected ear vein. Make a small incision parallel to the vein using a size 15 scalpel blade. Insert a 20G 6cm cannula (or longer, but not shorter) and advance in the vein in the direction of the blood flow. Fix the cannula to the skin using adhesive tape and skin glue. Attach the giving set to the cannula, creating a loop with the i/v line, attaching this to the skin using adhesive tape and skin glue. After infusion of fluids (or plasma, drugs) the line can be disconnected and the cannula flushed with heparin solution, prior to sealing off with a bung (Figure 5).

3.2 Plasma transfusions

Plasma from adult elephants can be used as a source of antibodies, nutrients and, in the case of fresh plasma, platelets. Although the efficacy of plasma transfusions has not yet been scientifically proven, it has been applied in the most recent survival cases. [12,8,31,14,15] Freezing plasma will activate donor platelets, rendering them useless for the recipient. If continuous depletion of platelets is seen in a clinical case, a change to fresh plasma is therefore essential for sustaining the calf through the HD episode.[52]
Plasma transfusion requires cross matching between donors and recipients, screening of the donor plasma for EEHV by PCR and, if storage is required, a -80°C freezer. Plasma can be given at a rate of 0.5-2 ml/kg/day. A detailed plasma collection protocol can be found in Appendix 3.

3.3 Antiviral drugs

None of the antiviral drugs currently used by elephant veterinarians have proven efficacy with regards to slowing down EEHV viral replication. However, all survivors were treated with one drug or another at an early stage of infection. One of the following drugs have all been administered:

- Famciclovir 12-15 mg/kg TID per rectum or per os
- Ganciclovir 5 mg/kg BID intravenous (slow, over one hour, diluted in i/v fluids)
- Acyclovir 15 mg/kg BID p/o, per rectum, i/v

Intravenous delivery, however, is essential in advanced stages of HD, as the circulation of the gastrointestinal tract will be compromised.[7]

One could consider teaming these drugs up with other antiviral drugs that have shown efficacy to herpes viruses in humans, such as foscarnet or codivorir, [45] although no successful reports of their use in elephants exist and potential side effects when used in elephants are unknown.

For rectal administration of antiviral drugs, oral tablets are ground down into powder with a pestle and mortar, and suspended in lukewarm water. Note that the rectum should be cleared of all fecal matter and that the suspension should be

placed at approximately one-meter depth in the distal colon using flexible tubing and a funnel, to ensure adequate absorption of the drug.

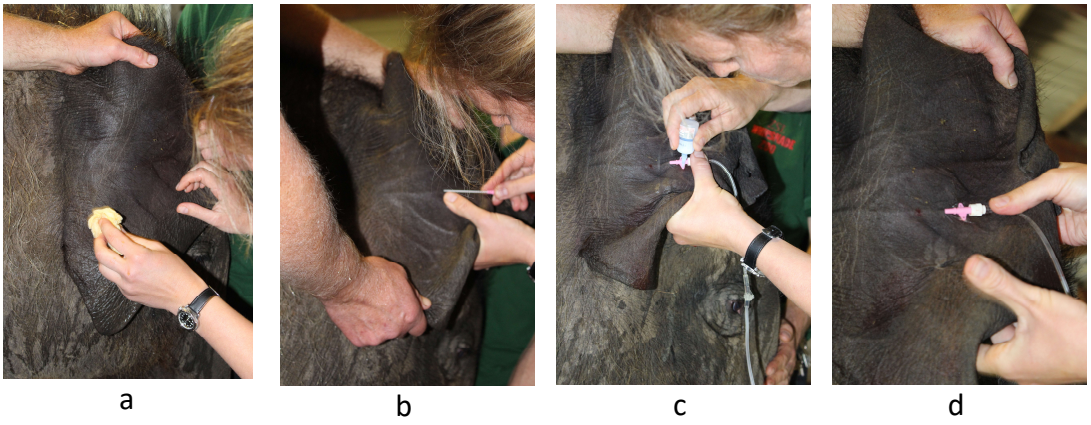
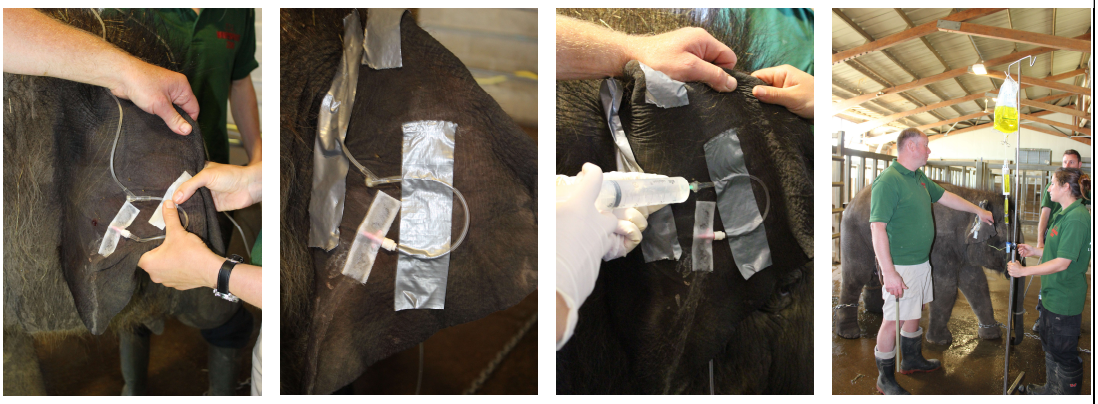


Figure 5: Placement of an ear vein cannula in a juvenile Asian elephant
a) aseptic preparation of the ear pinna after numbing the area with EMLA cream one hour previous, b) insertion of the cannula after cutdown of the skin with a scalpel blade, c,d) fixing the cannula to the skin with skin glue, e) attaching the giving set and creating a loop to prevent removal of cannula on movement of the head, f) fixing the giving set to the head, g) boluses of medication can be given swiftly through giving set ports, e.g. fluids and antibiotics, h) antivirals, fluids and nutraceuticals can be given slowly



3.4 Antibiosis

Although unhelpful in reducing the viral replication, the use of antibiotics has its place in the treatment of EEHV clinical disease: to prevent infection in the severely immunosuppressed calf, and to treat underlying illnesses that could be contributing to the susceptibility of the elephant to the virus. At ZSL Whipsnade Zoo we have

seen EEHV viral replication accelerate when a temporal gland abscess manifested itself. Drugs of choice should be broad spectrum such as penicillins, cephalosporines, trimethoprim/sulfonamides or fluoroquinolones. Administration should ideally be parenteral. If i/v access has already been established, this is the preferred route of administration. Please note that care should be taken to avoid perivascular injection of any substances at all times.

3.5 Gluco-corticosteroids

It has been hypothesized that an excessive response of the immune system, leading to sudden deterioration, further decrease of monocytes and platelets and vascular shock, contribute to the external clinical signs seen in EEHV-HD. In such cases, short-acting corticosteroids have been used and may have had a beneficial effect.[15,52]

3.6 Pain relief

Certain stages of the disease can be associated with various degrees of discomfort, and pain relief should be considered. At ZSL Whipsnade Zoo, the pain relief of choice in such instances would be butorphanol at intramuscular doses of 0.05 mg/kg.

3.7 Immuno-stimulants and nutraceuticals

Immuno-stimulants such as Vitamin C can be considered as additional supportive therapy.[3] Electrolyte, vitamin and amino-acid solutions such as Duphalyte® (FortDodge) can also be used.

3.8 Restraint

In untrained elephants where chemical restraint is required to carry out necessary intervention for monitoring and treatment purposes, a standing sedation can be achieved using one of the following combinations, both including provision of nasal oxygen throughout, where possible:

- Detomidine (0.01-0.02 mg/kg) + butorphanol (0.045-0.075 mg/kg) i/m --- reverse with atipamezole (0.03-0.06 mg/kg) i/m
- Xylazine (0.04-0.08 mg/kg) i/m --- reverse with atipamezole (0.004-0.008 mg/kg) i/m – note that ataxia is common with the use of xylazine standing sedations, and that preparations should be in place in case the calf sits down.

4. Advances in research

The biggest problem researchers struggle with is that as yet the EEHV virus cannot be cultured in vitro. The research group from the Baylor Institute in Houston has been looking into T-cell activation during clinical disease,[11,10] and the findings[9] have been used to develop a trial vaccine. Initial trials of this experimental vaccine will be starting later on in 2019 – in mice.[23] This is very encouraging, but one should consider that, if the initial trials are successful, it will still take many months/years before the vaccine will be suitable for general use in captive elephants.

At the University of Zurich, in vitro research has been taking place on the initial activation of pro-drugs and therefore potential efficacy of famciclovir and ganciclovir.[1] Further trails of antiviral drugs in laboratory circumstances have not yet taken place. Sample collection of all clinical cases is of the utmost importance to increase our knowledge of this complicated virus and its response to treatment regimes.

An antigen capture ELISA has been developed for the detection of IgG antibodies to EEHV.[51,50] The test, in its current state, cannot differentiate between the antibodies against the different species of EEHV, and there is no information available about cross-species protective immunity. Therefore, a high antibody titer does not necessarily correspond with reliable immunity against virulent species like EEHV1a and EEHV1b. The test is not used much currently. However, serological results from this initial test may still appear in the literature. Newer serological assays have now been developed, and have already shown to antibodies against genotype of EEHV1a do not protect against infections with EEHV1b.[13] This assay is not yet available in Europe.

Stemcell research is taking place.[5,6,20] Researchers have also looked into the pathophysiology and possible treatment options of the coagulopathy that occurs in the late stages of the disease.[35,21]

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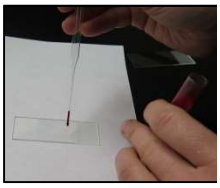
Appendix 1: ELEPHANT BLOOD SMEAR PREPARATION AND EVALUATION

Sample required

Blood collected in an anticoagulant (EDTA or heparin)

Smear preparation

1. Place a small drop (<0.05 ml) near the frosted area on the microscope slide
2. Advance with a second slide, at a 45 degree angle, to just touch the drop
3. Once it forms a line on the slide edge,
4. Move the slide forward in one smooth motion
5. This pulls the blood drop, creating a feathered edge
6. Air dry the slide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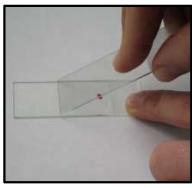
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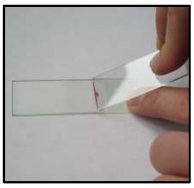
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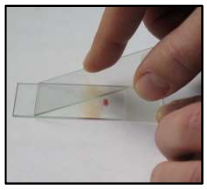
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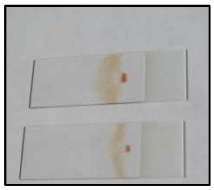

5.

Slide staining (using DiffQuick)

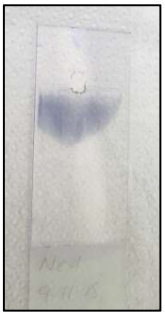
1. Dip the slide, one second at a time, five times in the pale green fixative. Let the fixative slide off each time before dipping again
2. Dip the slide, one second at a time, five times into the red solution. Let the solution slide off each time before dipping again.
3. Dip the slide, one second at a time, five times in the purple/blue solution. Let the solution slide off each time before dipping again.
4. Rinse the slide under tap water
5. Air dry


1.


2.

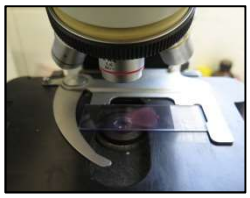
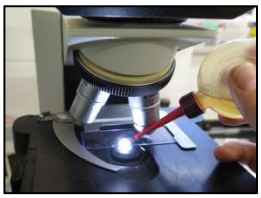

3.


4.


5.

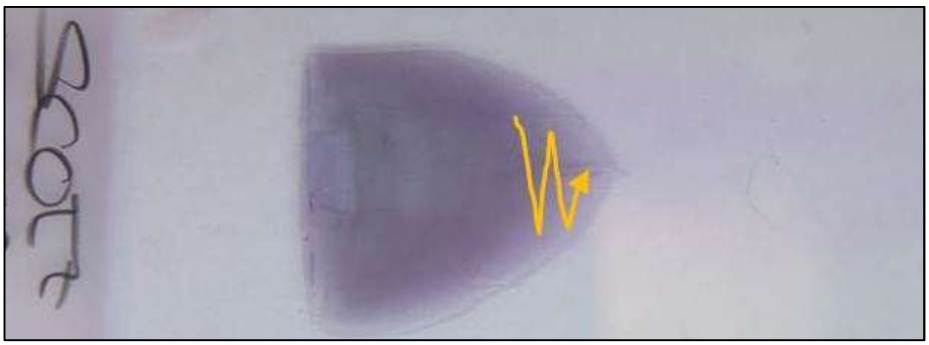
Viewing a blood smear

- Place under microscope, observe at x10 and x20 to assess
    - Staining quality
    - Subjective interpretation of anaemia
    - Subjective interpretation of white blood cell numbers
    - Best location for closer examination (thin layer, red blood cells not overlapping)
- Examine the slide at x40 to select a good area to start
- Place drop of oil on slide
- Examine slide at x100 for a white blood cell ratio count

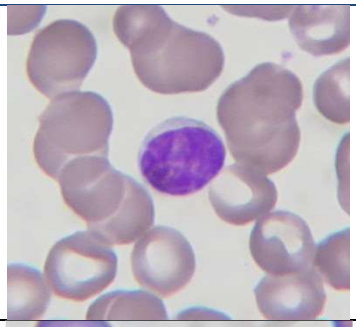
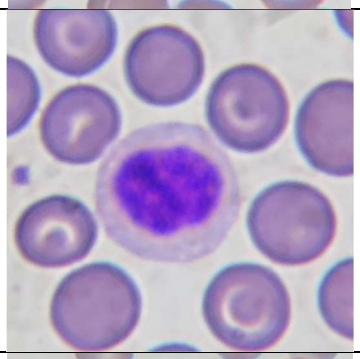
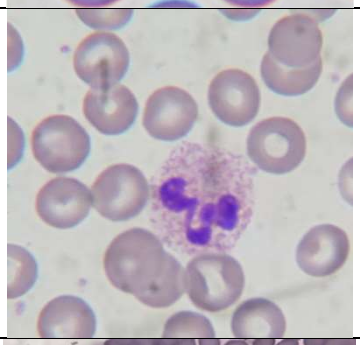
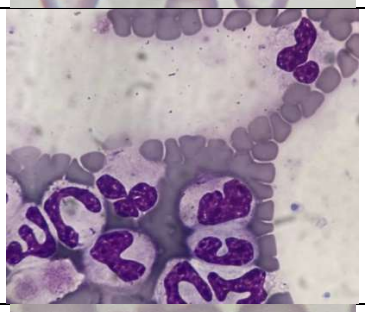
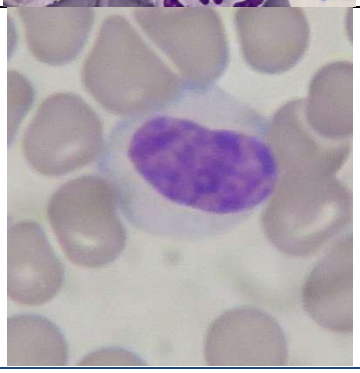



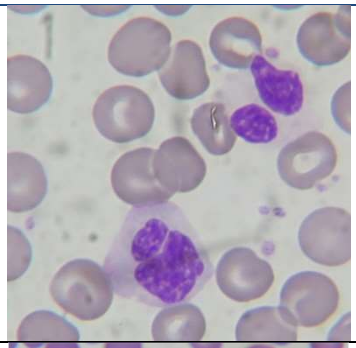
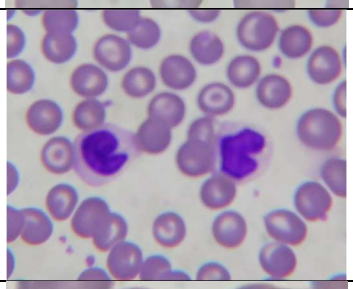
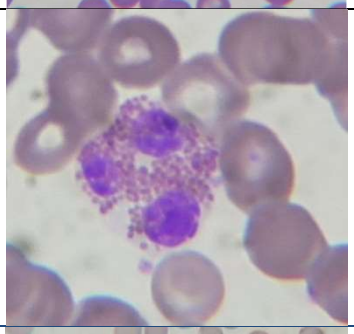
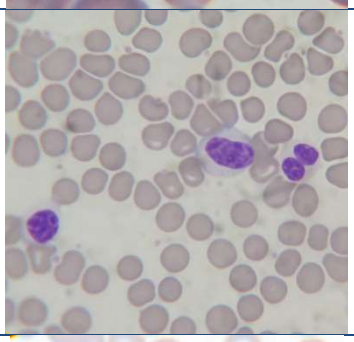
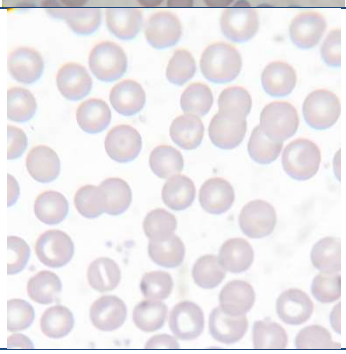

White blood cell ratio

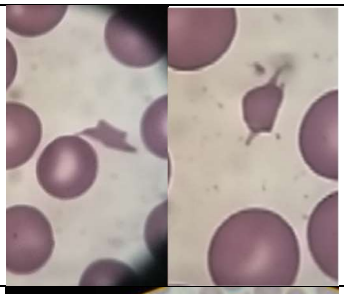
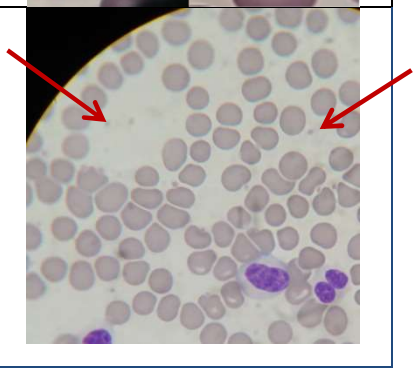
- Identify white blood cells at x100
- Differentiate lymphocytes, heterophils, monocytes and eosinophils
- Zig-zag through the thin, unicellular layer part of the smear (see below)
- Count up to 100 white blood cells in total
- Each count will give the ratio (%) of that particular cell in the entire white blood cell count



Elephant white blood cell identification chart

Lymphocyte Round nucleus Thin rim of cytoplasm, usually only around ¾ of nuclease	
Immature lymphocytes Larger Round nucleus Foamy cytoplasm around nucleus	
Heterophil (cfr neutrophil in other mammals) Irregular, multilobular nucleus Irregular granules, usually eosinophilic	
Band heterophils Immature heterophils entering the circulation Instead of a lobulated nucleus, these heterophils have a straight nucleus They can be numerous in cases of sepsis	
Monocyte – mononuclear Large nucleus, oval to kidney shaped Cytoplasm all around nucleus Vacuoles sometimes visible in cytoplasm activated monocytes (punch out appearance) Note: elephants naturally have a high monocyte count (20-40%)	

Monocyte – binuclear Two nuclei in one large cell Cytoplasm surrounding both Most commonly found monocyte in elephants (60-80% of all monocytes)	
Immature monocytes Small Cytoplasm shows no punctured out vacuoles Become numerous when recovery takes place after monocytopenia	
Eosinophil Multilobular nucleus Similar to heterophil Granules in cytoplasm very eosinophilic and all of them are uniformly round	
Compare lymphocyte to monocytes On the left is a lymphocyte On the right are a mononuclear monocyte and a binuclear monocyte Lymphocytes and mononuclear monocytes can look very similar and are distinguishable because monocytes have got far more cytoplasm, but this is sometimes hard to see depending on staining quality	
Red blood cells Codocytes (target cells): bulls-eye appearance Occur naturally in elephants	

Schistocytes Fragments of red blood cells May Seen in cases of sepsis and/or disseminated intravascular coagulation (DIC)	
Platelets Can be found on each smear Stain differently with each elephant / smear quality Usually pick up some purple stain Different size and number per individual Usual numbers in juvenile Asian elephants are 25-65 per high power field A manual count can be made by counting 10 high-power (x100) fields and multiplying the total by 1.5 to obtain platelets*10^9/L	

ZSL | WHIPSNADE ZOO

Appendix 2: ZSL WHIPSNADE ZOO EEHV TREATMENT PROTOCOL

START TREATMENT IF:		
qPCR positive + Significant haematological changes	EEHV1	10,000 VGE/ml
	EEHV 4, EEHV5	5000 VGE/ml
	Any	Exponentially increasing
	Rapidly/persistently decreasing monocytes and/or platelets (requires 3 consecutive haematology smears to assess)	
Clinical signs + decrease platelets and/or monocytes (if unable to run PCR immediately)	Endotheliotropic, so anything to do with vascular damage - Lethargy, change of sleeping patterns - Petechiae on mucosae – mouth, vulva, penis tip - Oral ulcers, tongue cyanosis - Stiffness of legs (myalgia, bleeding into joints) - Oedema – swelling head/neck	

INITIAL TREATMENT:		
Standing sedation	– Detomidine (0.015 mg/kg) + butorphanol (0.06 mg/kg) i.m. – Reversal: atipamezole (0.045 mg/kg) i.m.	
Intravenous cannula	– 20 G / at least 60 mm long	
Plasma (fresh preferred / frozen stored at -80°C)	– After cross matching – Or once from one non-related adult if cross matching not possible – Large animal filtration set – 300-500 ml per session, slow i.v., BID – Minimum of 3 hours between treatments	
Fluids	– 1 litre per 450 kg BW BID: saline or Hartmanns (hypertonic action in elephants) – Followed by profuse rectal fluids	
Ganciclovir (Cymeven®, Roche) (*on right top shelf marked EEHV in vet parmacy*)	– 5 mg/kg BID i.v. 5-10 days – Mix in 1 litre of saline – Slow , over >1 hour	Supplying pharmacy: Spa Health Care Markyate Contact: "Haw" 01582 840602 Roche contact (Claire): 01707367862

DIAGNOSTIC SAMPLING	- All suspected infected elephants - All elephants 8 years and younger (juvenile)

Sample	**Storage**	**Test**	**Contact**
EDTA blood	Fridge	qPCR	AHVLA Weybridge *Akbar Dastjerdi* 01932 357509 07940 231292 (mobile)
Urine sample – 500 ml	Fridge or freezer		
Dry swab from each nostril (trunk), each eye, vulva	Fridge or freezer		
EDTA blood	Fridge	Total WBC count	RVC
Serum	Fridge after sampling Freezer after separating	Serology	Erasmus Rotterdam, the Netherlands – now Artemis *Byron Martina / Petra* 0031 10 7044052
Blood smear	Air dry, diff quick	WBC count (monocytes, platelets)	In house
PCV	None	HCT	In house
Citrate	-80°C freezer (protocol)	Coagulation	Kathryn Perrin (kap@zoo.dk)
Heparin blood	Separate, freezer	Antiviral drug levels	Dr Sherry Cox (US)

ADVANCED TREATMENT PROTOCOL

If, despite initial plasma therapy, the disease progresses:

CLINICAL SIGN or LABORATORY CHANGE	**TREATMENT on top of continuous INITIAL TREATMENT**		
Further decrease in platelets	Plasma	After cross matching	Change to FRESH plasma
Indwelling catheter use	Antibiosis	Ceftiofur (Naxcel)	1.1 mg/kg SID-BID i.m.
Lethargy / Off food Onset of petechiae and/or oedema + Surge in lymphocytes (2+ days) without recovery of monocytes	Corticosteroid	Dexamethasone	0.05 mg/kg i.v. or i.m. once daily for a maximum of 2 days, unless otherwise directed by haematology
	Antibiosis	Ceftiofur (Naxcel)	1.1 mg/kg SID-BID i.m.
		Marbofloxacin (Forcyl)	2 mg/kg i.v.
Persistent petechiae and/or Progressive oedema and/or PCV <15% (Knowing PME changes, assume that is in pain)	Fluids	Hypertonic saline / Colloids	8 ml/kg over one hour - slow Follow up with rectal fluids
	Analgesia	Butorphanol	0.006-0.01 mg/kg i.v. (repeat every 3-4h) 0.05 mg/kg i.m.
	Diuretic	Frusemide	0.4 mg/kg i.m.
	Blood transfusion	From other adults	2-4 ml/kg/h (human blood transfusion rate)
	Gastric protectant	Omeprazole	1 mg/kg SID
	Oxygen therapy		Nasal cannula
Persistent high viraemia for >3 days after initiating ganciclovir or Rapidly increasing viraemia	Antiviral drug change	Acyclovir	10 mg/kg TID or 15 mg/kg BID i.v. 10 days
		Codifovir	5 mg/kg (once a week in humans) Slow, over >1 hour (dilute in saline) *nephrotoxic* After more than 1 litre of hydration (iv fluids)

Appendix 3: ZSL WHIPSNADE ZOO ELEPHANT PLASMA COLLECTION PROTOCOL

PLASMA COLLECTION	
Frequency	Fortnightly per individual (in case of two donors: alternating weeks for donation)
Suitable individuals	All non-related elephants that are not on medical treatment (including Improvac, NSAIDS, etc) Current suitable donors: – *Name of donor here: _____* – *Name of donor here:_____*
Serum samples	Required for cross matching Once every 4 weeks Red top – CAT – Vacutainer serum tubes – 4 ml Donors and all calves
Equipment required	Serum: Vacutainer red top + 21G 1" vacutainer needles Plasma collection bags: 450 ml unit CPDA 16G with 150 ml transfer bag
Useful veins	Ear vein Medial leg vein
Volume required	Depending on blood flow One bag per donor per fortnight (equivalent of 300 ml plasma/month per donor)
Storage	Stand overnight prior to manual separation - store -80°C freezer – 5 years max -20°C freezer – 2 months max

CROSS MATCHING	
When	Once every 4 weeks With the aim to be able to use stored plasma without delay
Preparation of sera	- Fresh sera, not stored - Separate the serum from the clot - Re-suspend the red cells in saline to wash
Major cross match	mix 2 drops of donor RBCs with 2 drops of recipient serum
Minor cross match	mix 2 drops of donor serum with 2 drops of recipient RBCs
	Centrifuge after mixing
Interpretation	– Supernatant: haemolysis = incompatibility – Tap and re-suspend: agglutination = incompatibility – On microscope slide (x10): agglutination = incompatibility