Elephant Endotheliotropic Herpesvirus Infection in Asian Elephants (*Elephas maximus*): A Review

Triawan Alkausar[1]*, Marlina Kamelia[2], Rani Yosilia[3], Shinta Anisya[4], Yuni Satitiningrum[5]
Department of Biology, Universitas Islam Negeri Raden Intan Lampung
Jl. Letkol Endro Suratmin, Sukarame, Bandar Lampung 35131, Indonesia
e-mail: triawanalkausar@radenintan.ac.id

Abstrak

Gajah adalah hewan eksotik yang karismatik. Sebagai hewan darat terbesar di bumi, berat maksimum mereka bisa mencapai lebih dari 7 ton dan tinggi badan hingga 4 meter. Namun, dibalik keeksotisannya, gajah, khususnya gajah Asia, kini kehilangan lebih dari 70% habitat mereka. Akibatnya, *International Union for Conservation of Nature* (IUCN) telah memasukkan gajah Asia ke dalam daftar merah sebagai hewan yang terancam punah. Berbagai upaya konservasi telah dilakukan, seperti translokasi, pengendalian perburuan liar, kampanye edukasi, dan penelitian. Namun, selama dua dekade terakhir, *Elephant Endotheliotropic Herpesvirus* (EEHV), penyakit infeksius baru, telah menjadi ancaman serius bagi kesehatan gajah Asia di seluruh dunia. Virus ini menyebabkan penyakit hemoragik yang cepat, akut, dan fatal sebagai manifestasi klinis utama pada gajah Asia dewasa dan khususnya pada gajah muda baik di alam liar maupun populasi yang dipelihara. Artikel ini menyediakan tinjauan literatur mengenai infeksi EEHV pada gajah Asia.

Kata Kunci—Gajah asia, Elephant Endotheliotrophic Herpesvirus, patobiologi, histopatologi

Abstract

Elephants are charismatic exotic animals. As the largest land animal on the earth, their maximum weight can reach more than 7 tons and 4 meters in height. Apart from their exoticness, however, elephants, particularly Asian elephants are now losing more than 70% of their habitat. As a result, the International Union for Conservation of Nature (IUCN) has red-listed Asian elephants as the critically endangered animal. Various conservation efforts have been implemented, such as translocation of the elephants, control of poaching, educational campaigns, and research. Nonetheless, over the past two decades, Elephant Endotheliotropic Herpesvirus (EEHV), a newly emerging disease has caused a serious threat to Asian elephants' health worldwide. The virus causes a rapid, acute, and fatal haemorrhagic disease as the major clinical manifestation in adult Asian elephants and particularly in juvenile elephants in both wild and captive populations. This article provides a literature review regarding the EEHV infection in Asian elephants.

Keywords: Asian elephants, Elephant Endotheliotropic Herpesvirus, pathobiology, histopathology.

I. INTRODUCTION

Elephants, recognised for their charismatic and exotic presence, are the largest terrestrial animal on Earth. These majestic creature can attain a maximum weight exceeding 7 tons and height up to 4 metres (Shoshani, 2008). They also have a maximum weight of brain up to 6.5 kg; five times of adult human brain and the largest brain in land mammals (Miller, 2008). Their gestation period of 22 months is the longest gestation among all land animal in the world (Schmitt, 2006; Lueders and Hildebrandt, 2012). Apart from their exoticness, however, elephants, particularly Asian elephants are now losing more than 70% of their habitat (Fernando *et al.*, 2012). Consequently, the International Union for Conservation of Nature (IUCN) has classified the Asian elephant as a critically endangered animal (IUCN, 2010). The approximate estimation of Asian elephants' worldwide population was 35,000 – 50,000 in 2011, which is spread across 13 countries in Asia

(Fernando and Pastorini, 2011; Fernando *et al.*, 2012; Azmi and Gunaryadi, 2011). Human-elephant conflicts (HEC) due to a growing human population and increasing settlement demands are believed to be the factor contributing most to Asian elephants' mortality along with infectious and non-infectious diseases (Leimgruber *et al.*, 2003; Miller *et al.*, 2015). Translocation of the elephants, control of poaching, educational campaigns and research have been conducted as arrays of conservation strategies (Jackson and Santiapillai, 1990). These actions should be carried on and intensified in order to save Asian elephants from extinction. Nevertheless , a newly emerging disease, namely Elephant Endotheliotropic Herpesvirus (EEHV), has caused a serious threat to Asian elephants' health worldwide over the recent two decades. The virus causes a swift, acute and often fatal haemorrhagic disease as the major clinical sign in adult Asian elephants and particularly in young elephants within both wild and captive populations (Ossent *et al.*, 1990). Given the critical impact of EEHV on Asian elephant population, a comprehensive review of current knowledge and treatment is extremely essential for effective conservation and management strategies.

II. METHODS OF RESEARCH

The research was conducted by performing a literature review using vast secondary data. To begin, data collection was conducted by searching for scientific articles and journal, such as *Google Scholar, Science Direct, Research Gate, PubMed* with the relevant topic to EEHV infection in Asian Elephants. Data search and collection were performed with the following keywords: "*Elephant Endotheliotropic Herpes Virus", "*EEHV*"*, "Elephants", and "Asian Elephants". Subsequently, the literature criteria that will be included in this study were literature with primary data or research, in-vitro studies, and English-typed literatures. Since the EEHV is classified as an emerging disease, there are no restriction for publication year.

III. DISCUSSION

A. Aetiology

Elephants Endotheliotropic Herpesvirus (EEHV) is a double stranded DNA virus that belongs to the family *Herpesviridae* and the genus *Proboscivirus*, which is the genus that can infect both African elephants (*Loxodonta africana*) and Asian elephants (*Elephas maximus*) (Azab *et al.*, 2018). Even though EEHV belongs to *Deltaherpesvirinae subfamily*, the virus itself is phylogenetically related to human cytomegalovirus (HCMV), a member of the *Betaherpesvirinae* subfamily (Dastjerdi *et al.*, 2016; Long *et al.*, 2016). Currently there are seven types of EEHV that have been recorded and three of these strains, namely EEHV1, EEHV4 and EEHV5 affect Asian elephants, whereas EEHV2, EEHV3, EEHV6 and EEHV7 affect African elephants. Based on phylogenetic classification, the viruses are divided into 2 groups, namely AT-rich group (EEHV1, EEHV2, EEHV5, EEHV6) and GC-rich group (EEHV3, EEHV4 and EEHV7) (Ackermann *et al.*, 2017). It is hypothesised that the AT-rich group has a more selective organ tropism than GC-rich group (Long *et al.*, 2016). Moreover, the previously mentioned types (EEHV1, EEHV 4 and EEHV5) that infect Asian elephants are the most lethal type. Although in most cases EEHV causes an asymptomatic infection in both adult African and Asian elephants, it can cause a lethal haemorrhagic disease in juvenile Asian elephants from 1 to 8 years of age (Fuery *et al.*, 2016).

B. Pathobiology

First reported in Europe in 1990, EEHV is responsible for up to 85% of young Asian elephants' mortality in 60 reported cases worldwide (33 cases in North America, 20 cases in Europe and 7 cases in Asia) and is considered to be the most significant cause of death in young Asian elephants in Europe and North America (Latimer *et al.*, 2011; Ossent *et al.*, 1990; Fuery *et al.*, 2017). The lesions caused by EEHV in Asian elephants are atypical in that the target organs are usually liver adrenal gland and brain,

whereas EEHV in African elephants shows tropism for vascular endothelial cells. In addition, most herpesviruses in mammals do not cause lethal disease and no other mammalian herpesvirus can produce such a rapid disease progress as demonstrated by EEHV. Subclinical or benign infection is more common in African elephants and haemorrhagic disease is extremely rare (Richman *et al.*, 2000). Several studies have hypothesised that the transmission of EEHV infections by several routes, including trunk, saliva, conjunctiva, and vaginal secretions that contains virus particles. Moreover, some studies have shown that virus particles can be found and isolated from papillomas (figure 1) on the skin in clinically healthy elephants (Dastjerdi *et al.*, 2016; Hardman *et al.*, 2012; Jacobson *et al.*, 1986). However, there are no reports showing that the virus can be transmitted via semen (Azab *et al.*, 2018; Sripiboon *et al.*, 2016).

Figure 1. Proliferative skin papilloma from the trunk of a clinically healthy adult African elephant (Long et al., 2016)

A study conducted by Zachariah *et al* in 2013 suggested that adult elephants can be a reservoir for the virus and potentially transmit it to young elephants. Juvenile Asian elephants from 5 months to 18 years of age are believed to be the most vulnerable for EEHV infection (Sripiboon *et al.*, 2016). In addition, it is hypothesised that close contact between a virus-carrier adult and an immunocompromised young elephant could possibly increase the risk of transmission of EEHV. Psychological and physiological stressors, such as injury or malnutrition could predispose to the risk of fatal EEHV haemorrhagic disease (Kendall *et al.*, 2016).

A study conducted by Richman *et al.* (2000) and Hildebrandt *et al.* (2005) proposed the pathogenesis of EEHV in susceptible elephants, particularly Asian elephants. They state that the virus first replicates in epithelial cells. As a cellular immune response, lymphocytes migrate to the site of infection and engulf the viral particles. When viraemia occurs after the virus has entered the elephant's body and resides in lymphocytes, the virus then replicates in the heart and endothelial cells, causing damage that leads to haemorrhage and oedema of the heart and also other organs, such as the liver. Subsequently, this can lead to heart failure by ischemia, intracardial swelling or metabolic disruption. In addition, heart failure may cause cyanosis, particularly cyanosis seen in the tongue.

On the other hand, EEHV can also produce latent or subclinical infection in Asian elephants. In this instance, viral reactivation may occur and result in intermittent shedding in trunk secretions. Reactivated viral particles remain for weeks in the cells that produce nasal secretions, such as Goblet cells. Subsequently, presumably the virus is inactivated and results in a long-term subclinical infection. A mixed infection between EEHV1 and EEHV4 and *Clostridium perfringens* has been reported by Boonsri *et al.* (2018). The coinfection occurred in two captive elephants less than one year of age in Thailand. The researchers suggested that infection with EEHV impairs immune function. This may then lead to increased susceptibility to *C. perfingens* infection. As a result, *C. perfringens* proliferates and exacerbates the clinical signs. This finding also indicates that EEHV infection can involve more than one strain of the virus.

In summary, the mechanism of EEHV infection and pathogenesis in the elephants is still unclear. One of the factors limiting the research is that *in-vitro* propagation of EEHV in cell culture is still not possible. For these reasons, the vital aspects to understand the pathogenesis of EEHV, such as the viral particles mechanics during the infection and distinctive characteristics for each of virus strains in EEHV infection has not been found yet (Kochagul *et al.*, 2018).

Moreover, laboratory tests to confirm the diagnosis of EEHV are also limited (Ackermann *et al.*, 2017).

C. Clinical Manifestation

In Asian elephants that are infected with the lethal strain of EEHV, non-specific clinical manifestations such as diarrhoea, lethargy, lameness, anorexia, and colic are commonly seen. At the same time, there may be oral ulceration, tongue cyanosis and oedema in the head and limb region which can eventually develop into haemorrhagic disease. Often the infected calves die within 1 to 7 days after the onset of clinical signs (Atkins *et al.*, 2013). The cause of death is mainly hypovolaemic shock and multiple organ failure (Richman *et al.*, 2000). Based on complete blood count (CBC), elephants infected with EEHV show significant leukopenia and thrombocytopenia (Atkins *et al.*, 2013, Fuery *et al.*, 2016).

However, juvenile elephants may survive infection without showing any significant clinical manifestations (Kochagul *et al.*, 2018). Although there are no clear explanations regarding the resistance of EEHV infection in young elephants, according to Long *et al.* (2016), maternal immunity plays a significant role in this phenomenon. Vertically transmitted parental immunity can provide the ability to calves to enhance their immune response and neutralise the infection. It is also suggested that juvenile elephants that are born from different origins (captive or wild) could provide the higher resistance to EEHV infection.

D. Gross Pathology

In most studies, the gross lesions of EEHV infection are similar. The tongue is cyanotic with ulceration and multifocal petechial to ecchymotic haemorrhages. All of the heart layers, namely epicardium, myocardium and endocardium have severe petechial haemorrhage. Multifocal petechial haemorrhages also can be found in various organs including the eyelids, conjunctiva, brain, oesophagus, trachea, stomach, intestine, spleen, liver, and urinary bladder. In addition, more than 500 ml of serous fluid may be collected from the abdominal cavity, which is the indication of ascites (Seilern-Moy *et al.*, 2016; Wilkie *et al.*, 2014; Kendall *et al.*, 2016; Richman *et al.*, 2000).

However, Garner *et al* (2009) in their study revealed a different pathological lesion. Although gross pathologic changes associated with EEHV3 infection showed marked petechial and ecchymotic haemorrhage in all layers of the heart, including epicardium and myocardium and chorda tendineae similar to that noted above (Figure 2.3) (Kendall *et al.*, 2016). In addition, there were haemorrhages and congestion in the liver, spleen, retina, submandibular muscles and cut surface of the kidney (Figure 2.2). Significant oedema was observed in the mesentery and omentum (Figure 2.1). The haemorrhagic lesions in the kidney and retina were considered to be distinctive for EEHV3 infection given these changes have not been recorded in other cases of EEHV infection. (Garner *et al.*, 2009). Variation in pathogenicity of different strains of EEHV is also supported by Kochangul *et al*, who stated that haemorrhages in the heart in EEHV1 infected Asian elephant are more severe than in elephant calves infected with EEHV4. (Kochagul *et al.*, 2018). This indicates that the different strains of EEHV could result in different types of gross morphological changes.

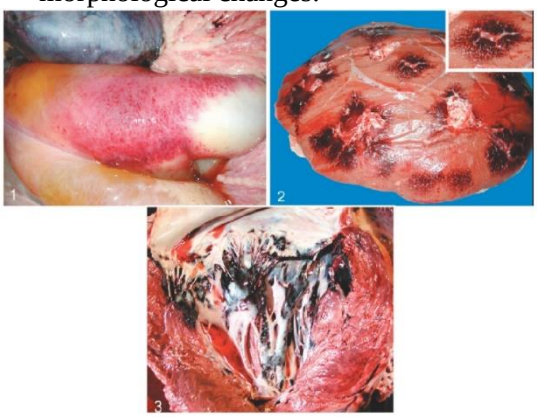


Figure 2. The gross lesion of EEHV infection in Asian Elephant. (1) Significant oedema in mesentery with petechial and ecchymoses haemorrhage. (2) Cut surface of the kidney showing hyperaemia and congestion of the medulla. (3) Left side of the heart, there is a haemorrhage in endocardium, chorda tendineae, myocardium and ventricular septum.(Garner et al., 2009)

E. Histopathology

On histopathological examination, the histological changes are generally consistent with the gross lesions. Specifically, the variation of haemorrhage, necrosis, oedema, and fibrosis with or without thrombus formation are found in blood vessels in visceral organs such as heart, liver, digestive tract, tongue, lungs, lymph node and brain (Latimer *et al.*, 2011; Long *et al.*, 2016). The infiltration of cellular infiltrates, which mainly consist of neutrophils and lymphocytes is found in the heart, tongue and liver accompanied with pyknotic degenerative cells and necrosis (Wilkie *et al.*, 2014). Additionally, Garner *et al* (2009) reported histological lesions, including haemorrhage and perivascular oedema in the arteries of the splenic capsule and medulla of the kidney from two Asian elephants with EEHV3 infection. An amphophilic, smeared intranuclear inclusion body is found in the capillary vessels of the heart, lungs, kidney, liver and tongue in most cases (Wilkie *et al.*, 2013). However, a study conducted by Kendall *et al* (2016) involving three Asian elephants did not reveal any intranuclear inclusion bodies in any of the samples. This suggests that intranuclear inclusion bodies are not always present in all cases of EEHV infection.

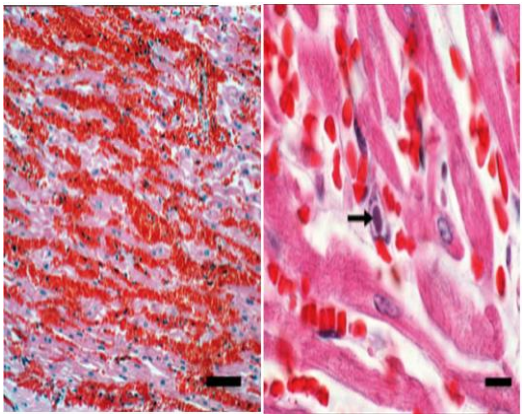
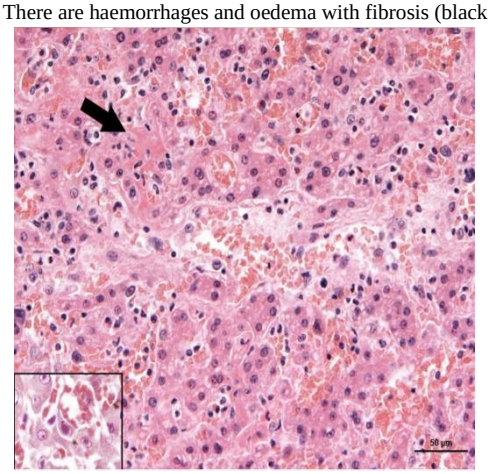


Figure 3. Left: the low magnification of myocardium revealing the extensive haemorrhages; Right: Higher magnification of myocardium showing the intranuclear amphophilic inclusion body (black arrow). Hematoxylin & Eosin (Long et al., 2016).

Figure 4. The liver from an Asian elephant with EEHV5 infection. There are haemorrhages and oedema with fibrosis (black arrow);



Inset: Intranuclear inclusion body associated with EEHV. Hematoxylin & Eosin (Long et al., 2016).

F. Diagnosis

In general, the diagnosis of EEHV relies on pathological findings (gross lesions and histopathology) and detection of virus through *in situ* hybridisation. As mentioned before, amphophilic intranuclear inclusion bodies in tissue samples is pathognomonic for EEHV infection, although not all cases of EEHV infection have inclusion bodies (Hildebrandt *et al.*, 2005). Samples for diagnosis of EEHV may be from organs, trunk wash and whole blood (Pellett, 2014). Samples from internal organs can be used for histopathology examination, immunohistochemistry, Polymerase Chain Reaction (PCR) or *in situ* hybridisation. Samples from trunk wash can be used for molecular analysis including PCR and DNA sequencing, whereas samples from whole blood can be used for serological tests, such as ELISA, immunoblotting, or SDS-PAGE (Humphreys *et al.*, 2015).

In order to get a trunk wash sample, the elephant firstly has to hold its trunk above. Then the keeper carefully pours 100-200 mL of saline into both nostrils. The elephant then raises its trunk upwards to make the saline flow as far as possible into the trunk for 30 seconds. Subsequently, the elephant let down its trunk allowing the fluid into a container. In addition, the keeper must get the elephant to breathe out into a

plastic bag during the collection procedure. Eventually, the sample is relocated into a sterile container to be analysed within at most 8 hours. Trunk wash samples may have a large number of contaminants, such as bacteria and food constituents. Moreover, herpesvirus is considered to be a highly cell-associated virus, hence any contaminant can disrupt the DNA extraction (Stanton *et al.*, 2014). Therefore, to ensure the validity of the sample, amplification of the TNF alpha gene by qPCR (Quantitative PCR) needs to be conducted. Any sample that displays a positive signal with qPCR can be used for DNA extraction (Stanton *et al.*, 2010). Although this procedure is difficult, particularly for wild elephants and needs training, trunk washing is less invasive and highly effective for molecular assays, such as PCR and gene sequencing.

A blood sample is obtained by venepuncture from the auricular vein or saphenous vein using a 19 or 21 G needle and 3 or 5mL syringe. Approximately 2-4 mL of blood is collected. After that, the blood is transferred into an EDTA tube, stored at 4^{O}C and analysed within 24 hours.

There are several reported techniques of diagnosis for EEHV, namely:

1. Molecular Analysis

In most of the studies of EEHV diagnosis, PCR is used for detection of EEHV DNA sequence in various samples, including blood, organs, and trunk wash. In addition, PCR also can be used to compare the genetic similarity to other strains of EEHV (Reid *et al.*, 2006, Sariya *et al.*, 2012). In fact, the first diagnosis of EEHV infection were confirmed by PCR (Ossent *et al.*, 1990). However, a study by Fickel and colleagues revealed that PCR is not appropriate for the screening of animals that are clinically healthy as it works better in clinically ill animals and with necropsy samples (Fickel *et al.*, 2003). Conventional PCR is insensitive when it comes to detect the virus in carrier elephants and cannot measure the concentration of viral loads in samples (Garner *et al.*, 2009).

In order to address the weakness of PCR, a variant of PCR, namely quantitative PCR (qPCR) can be used for measuring the viral loads of EEHV. A study in the UK revealed that the highest concentration of viral particles was found in the liver and heart (Seilern-Moy *et al.*, 2016). In terms of screening and measuring the virus presence in carrier elephants, it was revealed that viral DNA can be detected in a trunk wash sample and reaches its peak 21 days after the initial infection (Stanton *et al.*, 2013). In addition, clinical signs of EEHV are observed when the viral loads reach 10^4 viral genome equivalents per millilitre (VGE/ml) whole blood and in the elephants with fatal case of EEHV showed the viral loads exceed 10^6 VGE/ml whole blood (Stanton *et al.*, 2013).

2. Serological Analysis

In most of the cases, the serological diagnosis for viral disease use the detection of antibodies against the virus. However, since EEHV cannot be cultivated by *in-vitro* culture, the most suitable alternative is to utilise an EEHV protein, such as glycoprotein B (gB) that has a significant role in antibody neutralisation in host. A serological study conducted by van den Doel *et al.* (2015) revealed that the seroprevalence of EEHV in captive and wild elephants was higher than expected. Moreover, antibodies against EEHV were also found in elephants that were clinically healthy. This could be an indication that elephants are the natural host of EEHV without showing any clinical manifestation. It is consistent with the theory that elephants, particularly adult elephants can be the carrier of the virus and intermittently shed the virus to susceptible hosts (Zachariah *et al.*, 2013).

3. *In-situ* Hybridisation

One of the recently developed diagnosis techniques for EEHV infection is *in-situ* hybridisation (ISH). This technique is used to detect and localise the DNA sequence of virus in the tissue sample by exploiting the special feature of nucleic acid. To be specific, the ability of nucleic acids (DNA and RNA) to anneal specifically to their complementary strand and form a hybrid sequence is the characteristic that is utilised in this technique. The hybrid sequence containing viral DNA and probe is then subsequently visualised using a radioactive probe

or chemical staining/chromogen (e.g., fluorescent). The main advantage of *in-situ* hybridisation is that it gives the researcher the ability to analyse the distribution of specific nucleic acids (DNA, RNA, mRNA etc) related to a protein or target gene and their association with cellular structure.

A recent study in Thailand by Kochakul *et al* used in-situ hybridisation as a method to detect EEHV in various organs. In this study, viral polymerase and terminase were used as the target genes in two adult elephants that were clinically infected and died by EEHV1A and EEHV4. Subsequently, the visceral organs from both animals were collected and tested for EEHV by ISH. In this study highest viral load was found in the spleen, liver, and heart. In addition, the virus was found in the lungs, kidney, ileum, and colon.

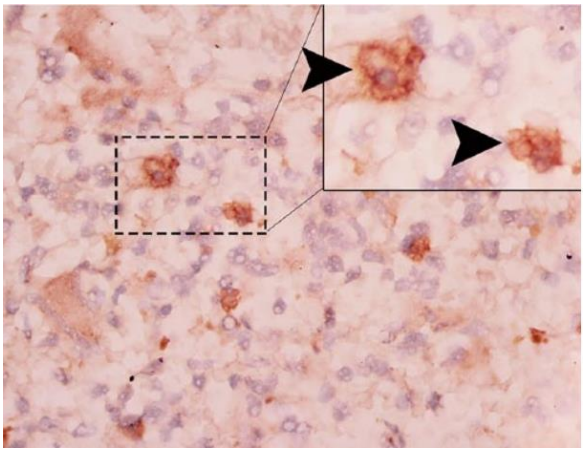


Figure 5. The result of ISH on spleen sample showing the presence of polymerase gene in macrophages (inset with arrowhead)(Kochakul et al., 2018)

G. Treatment

Several studies have reported the usage of these anti-herpesvirus drugs. A study conducted by Richman *et al* that treated two Asian elephants with famciclovir 50-70 mg/kg per-os and per-rectum 3 times a day for I month, found that 5 days after the onset of clinical signs there was a clinical improvement. The elephants showed a reduction of tongue cyanosis and submandibular oedema, a return of appetite and eventually survived the infection (Richman *et al.*, 2000). Moreover, a study in Thailand revealed the same outcome. A 3-year-old elephant with clinical signs of EEHV infection was initially treated with per-rectal acyclovir 12 mg/kg and supportive therapy such as IV fluids, antibiotics, and vitamins. However, because there was no change in the condition, the administration of acyclovir was changed to the intra-venous route. It is also important to notice that the administration of oral drugs in elephants with EEHV infection is often challenging because the infection can cause decreased appetite and intravenous administration is more effective in trained elephants (Sripiboon *et al.*, 2017, Dastjerdi *et al.*, 2016).

Two days after beginning intravenous administration, the condition was improved drastically, for instance, body temperature returned to normal, and there was a reduction of tongue cyanosis. In addition, the improvement was also followed by a decrease in viral load, which proves the theory of study conducted by Stanton *et al.* (2013), which states that the dynamics of viral loads in the blood can affect the clinical condition of an elephant with EEHV infection.

In other words, the antiviral treatment should also be followed by supportive drugs such as IV fluids, antibiotics, and vitamins. IV fluids are beneficial to prevent hypovolaemic shock and maintain the body fluid balance. Antibiotics are administered to provide protection against secondary bacterial infections and vitamins are used to improve immune response (Sripiboon *et al.*, 2017).

H. Vaccination

Currently, vaccination for EEHV is still unavailable. The main obstacle of producing the vaccine against EEHV is the inability of the virus to grow in *in-vitro* culture. In fact, the *in-vitro* culture of virus, particularly EEHV is essential, because it could give the insight about pathogenesis and virulence factors of the virus. Thus, the better understanding of the virus could lead to improved treatment and prevention of the disease. Despite the exertion of cultivating EEHV in cell culture, some researchers have found the

potential candidates for EEHV vaccines, namely glycoprotein B (gB) (Fuery *et al.*, 2017; Humphreys *et al.*, 2015; Griffiths *et al.*, 2015; Fickel *et al.*, 2003). This protein is an important component of herpesvirus envelope that involves in regulating the virus entrance to susceptible hosts (Griffiths *et al.*, 2015). The vaccination strategy for EEHV can be carried out with gB-expressing recombinant virus or expression system. Besides that, a study by Fuery *et al* revealed that elephant EEHV-specific T cell could also be a potential option for the vaccination (Fuery *et al.*, 2016; Fuery *et al.*, 2017).

The most recent study performed by Pursell et al (2022) found the novel vaccine for EEHV, namely Modified Vaccinia Ankara (MVA). The vaccine has proven to produce a protective immunity against EEHV infection with no side effects in Asian Elephants (Pursell *et al.*, 2022). Therefore, further studies are required to enlighten the knowledge of vaccine efficacy in Asian Elephants in various countries.

IV. CONCLUSION

To sum up, Elephant Endotheliotropic Herpesvirus (EEHV) infection is becoming a devastating disease for elephants nowadays, particularly for Asian elephant because of its significant mortality rate. It also exacerbates the campaign of worldwide elephant conservation. With the present research result, scientist has delivered the insight of the disease. However, the further knowledge regarding the pathogenesis, virulence factors, diagnosis, treatment, and prevention needs to be carried on in order to rescue the elephants from extinction.

REFERENCES

Anonymous. 2017. Chapter 9 - Herpesvirales A2 - MacLachlan, N. James. *In:* DUBOVI, E. J. (ed.) *Fenner's Veterinary Virology (Fifth Edition).* Boston: Academic Press.

Ackermann, M., Hatt, J. M., Schetle, N. & Steinmetz, H. 2017. Identification of shedders of elephant endotheliotropic herpesviruses among Asian elephants (Elephas maximus) in Switzerland. *PLoS One,* 12**,** e0176891.

Atkins, L., Zong, J. C., Tan, J., Mejia, A., Heaggans, S. Y., Nofs, S. A., Stanton, J. J., Flanagan, J. P., Howard, L., Latimer, E., Stevens, M. R., Hoffman, D. S., Hayward, G. S. & Ling, P. D. 2013. Elephant endotheliotropic herpesvirus 5, a newly recognized elephant herpesvirus associated with clinical and subclinical infections in captive Asian elephants (Elephas maximus). *J Zoo Wildl Med,* 44**,** 136-43.

Azab, W., Damiani, A. M., Ochs, A. & Osterrieder, N. 2018. Subclinical infection of a young captive Asian elephant with elephant endotheliotropic herpesvirus 1. *Arch Virol,* 163**,** 495-500.

Azmi, W. & Gunaryadi, D. 2011. Current status of Asian elephants in Indonesia. *Gajah,* 35**,** 55-61.

Boonsri, K., Somgird, C., Noinafai, P., Pringproa, K., Janyamethakul, T., Angkawanish, T., Brown, J. L., Tankaew, P., Srivorakul, S. & Thitaram, C. 2018. Elephant Endotheliotropic Herpesvirus Associated with Clostridium Perfringens Infection in Two Asian Elephant (Elephas Maximus) Calves. *J Zoo Wildl Med,* 49**,** 178-182.

Dastjerdi, A., Seilern-Moy, K., Darpel, K., Steinbach, F. & Molenaar, F. 2016. Surviving and fatal Elephant Endotheliotropic Herpesvirus-1A infections in juvenile Asian elephants - lessons learned and recommendations on anti-herpesviral therapy. *BMC Vet Res,* 12**,** 178.

Fernando, P., Leimgruber, P., Prasad, T. & Pastorini, J. 2012. Problem-elephant translocation: translocating the problem and the elephant? *PLoS One,* 7**,** e50917.

Fernando, P. & Pastorini, J. 2011. Range-wide Status of Asian Elephants. *Gajah,* 35**,** 15-20.

Fickel, J., Lieckfeldt, D., Richman, L. K., Streich, W. J., Hildebrandt, T. B & Pitra, C. 2003. Comparison of glycoprotein B (gB) variants of the elephant endotheliotropic herpesvirus (EEHV) isolated from Asian elephants (Elephas maximus). *Vet Microbiol,* 91**,** 11-21.

Fuery, A., Browning, G. R., Tan, J., Long, S., Hayward, G. S., Cox, S. K., Flanagan, J. P., Tocidlowski, M. E., Howard, L. L. & Ling, P. D. 2016. Clinical Infection of Captive Asian Elephants (Elephas Maximus) with Elephant Endotheliotropic Herpesvirus 4. *J Zoo Wildl Med,* 47**,** 311-8.

Fuery, A., Leen, A. M., Peng, R., Wong, M. C., Liu, H. & Ling, P. D. 2017. Asian elephant T cell responses to Elephant Endotheliotropic Herpesvirus (EEHV). *J Virol*.

Garner, M. M., Helmick, K., Ochsenreiter, J., Richman, L. K., Latimer, E., Wise, A. G., Maes, R. K., Kiupel, M., Nordhausen, R.W., Zong, J. C. & Hayward, G. S. 2009. Clinico-pathologic features of fatal disease attributed to new variants of endotheliotropic herpesviruses in two Asian elephants (Elephas maximus). *Vet Pathol,* 46**,** 97-104.

Griffiths, P., Baraniak, I. & Reeves, M. 2015. The pathogenesis of human cytomegalovirus. *J Pathol,* 235**,** 288-97.

Hardman, K., Dastjerdi, A., Gurrala, R., Routh, A., Banks, M., Steinbach, F. & Bouts, T. 2012. Detection of elephant endotheliotropic herpesvirus type 1 in asymptomatic elephants using TaqMan real-time PCR. *Vet Rec,* 170**,** 205.

Hildebrandt, T. B., Hermes, R., Ratanakorn, P., Rietschel, W., Fickel, J., Frey, R., Wibbelt, G., Reid, C. & Goritz, F. 2005. Ultrasonographic assessment and ultrasound-guided biopsy of the retropharyngeal lymph nodes in Asian elephants (Elephas maximus). *Vet Rec,* 157**,** 544-8.

Humphreys, A. F., Tan, J., Peng, R., Benton, S. M., Qin, X., Worley, K. C., Mikulski, R. L., Chow, D. C., Palzkill, T. G. & Ling, P. D. 2015. Generation and characterization of antibodies against Asian elephant (Elephas maximus) IgG, IgM, and IgA. *PLoS One,* 10**,** e0116318.

IUCN. 2010. *The IUCN Red List of Threatened Species* [Online]. Available: http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T7140A12828813.en [Accessed 15/03/2018].

Jackson, P. & Santiapillai, C. 1990. The Asian elephant : an action plan for its conservation. IUCN/SSC Action Plans for the Conservation of Biological Diversity.

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. *J Am Vet Med assoc,* 189**,** 1075-8.

Kendall, R., Howard, L., Masters, N. & Grant, R. 2016. The Impact of Elephant Endotheliotropic Herpesvirus on the Captive Asian Elephant (Elephas Maximus) Population of the United Kingdom and Ireland (1995-2013). *J Zoo Wildl Med,* 47**,** 405-18.

Kochagul, V., Srivorakul, S., Boonsri, K., Somgird, C., Sthitmatee, N., Thitaram, C. & Pringproa, K. 2018. Production of antibody against elephant endotheliotropic herpesvirus (EEHV) unveils tissue tropisms and routes of viral transmission in EEHV-infected Asian elephants. *Sci Rep,* 8**,** 4675.

Kochakul, V., Boonsri, K., Tiwananthagorn, S., Somgird, C., Thitaram, C. & Pringproa, K. 2018. Development of in situ hybridization for detection of elephant endotheliotropic herpesvirus in Asian elephants. *J Vet Diagn Invest***,** 1040638718773810.

Latimer, E., Zong, J. C., Heaggans, S. Y., Richman, L. K. & Hayward, G. S. 2011. Detection and evaluation of novel herpesviruses in routine and pathological samples from Asian and African elephants: identification of two new proboscivirus (EEHV5 and EEHV6) and two new gammaherpesviruses (EGHV3B and EGHV5). *Vet Microbiol,* 147**,** 28-41.

Leimgruber, P., Gagnon, J. B., Wemmer, C., Kelly, D. S., Songer, M. A. & Selig, E. R. 2003. Fragmentation of Asia's remaining wildlands: implications for Asian elephant conservation. *Anim Consv,* 6**,** 347-359.

Long, S. Y., Latimer, E. M. & Hayward, G. S. 2016. Review of Elephant Endotheliotropic Herpesviruses and Acute Hemorrhagic Disease. *ILAR J,* 56**,** 283-96.

Lueders, I. & Hildebrandt, T. B. 2012. Chapter 66 - Female Elephant Reproduction A2 - Miller, R. Eric. *In:* FOWLER, M. (ed.) *Fowler's Zoo and Wild Animal Medicine.* Saint Louis: W.B. Saunders.

Miller, D., Jackson, B., Riddle, H. S., Stremme, C., Schmitt, D. & Miller, T. 2015. Elephant (Elephas maximus) Health and Management in Asia: Variations in Veterinary Perspectives. *Vet Med Int,* 2015**,** 614690.

Miller, M. 2008. Nervous System. *Biology, Medicine, and Surgery of Elephants.* Blackwell Publishing Ltd

Ossent, P., Guscetti, F., Metzler, A. E., Lang, E. M., Rubel, A. & Hauser, B. 1990. Acute and fatal herpesvirus infection in a young Asian elephant (Elephas maximus). *Vet Pathol,* 27**,** 131-3.

Pellett, P. E. 2014. Trunkloads of viruses. *J Virol,* 88**,** 13520-2.

Pursell, T., Clinton, J. L., Tan, J., Peng, R. & Ling, P. D. 2022. Modified vaccinia Ankara expressing EEHV1A glycoprotein B elicits humoral and cell-mediated immune responses in mice. *PLOS ONE,* 17**,** e0265424.

Reid, C. E., Hildebrandt, T. B., Marx, N., Hunt, M., Thy, N., Reynes, J. M., Schaftenaar, W. & Fickel, J. 2006. Endotheliotropic elephant herpes virus (EEHV) infection. The first PCR-confirmed fatal case in Asia. *Vet Q,* 28**,** 61-4.

Richman, L. K., Montali, R. J., Cambre, R. C., Schmitt, D., Hardy, D., Hildbrant, T., Bengis, R. G., Hamzeh, F. M., Shahkolahi, A. & Hayward, G. S. 2000. Clinical and pathological findings of a newly recognized disease of elephants caused by endotheliotropic herpesviruses. *J Wildl Dis,* 36**,** 1-12.

Sariya, L., Chatsirivech, J., Suksai, P., Wiriyarat, W., Songjaeng, A., Tangsudjai, S., Kanthasaewee, O., Maikaew, U. & Chaichoun, K. 2012. Development of a SYBR Green I-based real-time PCR for detection of elephant endotheliotropic herpesvirus 1 infection in Asian elephants (Elephas maximus). *J Virol Methods,* 185**,** 160-5.

Schmitt, D. 2006. Reproductive System. *Biology, Medicine, and Surgery of Elephants.*

Seilern-Moy, K., Darpel, K., Steinbach, F. & Dastjerdi, A. 2016. Distribution and load of elephant endotheliotropic herpesviruses in tissues from associated fatalities of Asian elephants. *Virus Res,* 220**,** 91-6.

Shoshani, J. 2008. Taxonomy, Classification, History, and Evolution of Elephants. *Biology, Medicine, and Surgery of Elephants.* Blackwell Publishing Ltd.

Sripiboon, S., Angkawanish, T., Boonprasert, K., Sombutputorn, P., Langkaphin, W., Ditcham, W. & Warren, K. 2017. Successful Treatment of a Clinical Elephant Endotheliotropic Herpesvirus Infection: The Dynamics of Viral Load, Genotype Analysis, and Treatment with Acyclovir. *J Zoo Wildl Med,* 48**,** 1254-1259.

Sripiboon, S., Jackson, B., Ditcham, W., Holyoake, C., Robertson, I., Thitaram, C., Tankaew, P., Letwatchasarakul, P. & Warren, K. 2016. Molecular characterisation and genetic variation of Elephant Endotheliotropic Herpesvirus infection in captive young Asian elephants in Thailand. *Infect Genet Evol,* 44**,** 487-94.

Stanton, J. J., Nofs, S. A., Zachariah, A., Kalaivannan, N. & Ling, P. D. 2014. Detection of elephant endotheliotropic herpesvirus infection among healthy Asian elephants (Elephas maximus) in South India. *J Wildl Dis,* 50**,** 279-87.

Stanton, J. J., Zong, J. C., Eng, C., Howard, L., Flanagan, J., Stevens, M., Schmitt, D., Wiedner, E., Graham, D., Junge, R. E., Weber, M. A., Fischer, M., Mejia, A., Tan, J., Latimer, E., Herron, A., Hayward, G. S. & Ling, P. D. 2013. Kinetics of viral loads and genotypic analysis of elephant endotheliotropic herpesvirus-1 infection in captive Asian elephants (Elephas maximus). *J Zoo Wildl Med,* 44**,** 42-54.

Stanton, J. J., Zong, J. C., Latimer, E., Tan, J., Herron, A., Hayward, G. S. & Ling, P. D. 2010. Detection of pathogenic elephant endotheliotropic herpesvirus in routine trunk washes from healthy adult Asian elephants (Elephas maximus) by use of a real-time

quantitative polymerase chain reaction assay. *Am J Vet Res,* 71**,** 925-33.

Van den Doel, P. B., Prieto, V. R., van Rossum-Fikkert, S. E., Schaftenaar, W., Latimer, E., Howard, L., Chapman, S., Masters, N., Osterhaus, A. D., Ling, P. D., Dastjerdi, A. & Martina, B. 2015. A novel antigen capture ELISA for the specific detection of IgG antibodies to elephant endotheliotropic herpes virus. *BMC Vet Res,* 11**,** 203.

Wilkie, G. S., Davison, A. J., Kerr, K., Stidworthy, M. F., Redrobe, S., Steinbach, F., Dastjerdi, A. & Denk, D. 2014. First fatality associated with elephant endotheliotropic herpesvirus 5 in an Asian elephant: pathological findings and complete viral genome sequence. *Sci Rep,* 4**,** 6299.

Wilkie, G. S., Davison, A. J., Watson, M., Kerr, K., Sanderson, S., Bouts, T., Steinbach, F. & Dastjerdi, A. 2013. Complete genome sequences of elephant endotheliotropic herpesviruses 1A and 1B determined directly from fatal cases. *J Virol,* 87**,** 6700-12.

Zachariah, A., Zong, J. C., Long, S. Y., Latimer, E. M., Heaggans, S. Y., Richman, L. K. & Hayward, G. S. 2013. Fatal herpesvirus hemorrhagic disease in wild and orphan asian elephants in southern India. *J Wildl Dis,* 49**,** 381-93.