



Research article

Detection of subclinical EEHV infection in captive Asian elephants (*Elephas maximus*) in Peninsular Malaysia through a molecular approach

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Abstract

Elephant Endotheliotropic Herpesvirus (EEHV) is a highly destructive viral disease affecting both Asian and African elephants, posing a threat in captivity and the wild. Despite habitat loss, poaching, and human-elephant conflict being major concerns, EEHV emerges as a significant threat to elephant species' long-term survival. Until now, no reported cases of EEHV have been documented in Peninsular Malaysia. This raises questions about whether the absence of report is due to the virus's actual absence or a lack of awareness. Wildlife disease surveillance programmed initiated EEHV screening in Asian elephant at National Elephant Conservation Centre (PKGK) which is one of establish ex-situ facilities for elephant conservation in Peninsular Malaysia. A total of 222 samples were collected, comprising trunk wash, whole blood, oral swab, and rectal swab samples. Molecular detection approach by utilizing the polymerase chain reaction amplification targeting polymerase U38 gene region revealed 12 individuals out of 33 captive Asian elephants was found EEHV-positive, spanning ages from 2 months to 15 years and both sexes. Phylogenetic analysis revealed distinct EEHV subtypes identified in this study. Despite being EEHV-positive, the captive Asian elephants displayed good health, indicating asymptomatic infections of EEHV1A, EEHV4, and EEHV5. This marks the initial identification of EEHV and subclinical infections in captive elephants in Peninsular Malaysia, emphasizing the need for urgent and enhanced health care management to safeguard these magnificent animals in Peninsular Malaysia for better conservation management strategies in the future

Keywords: Asian elephant, Disease surveillance, EEHV, Hemorrhagic disease, U38 gene

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INTRODUCTION

The Asian elephant (*Elephas maximus*), comprising four extant subspecies, inhabits various regions across the Asian continent, including Bhutan, Bangladesh, Sri Lanka, Nepal, India, Myanmar, Thailand, Cambodia, Laos, Vietnam, China, Indonesia, and Malaysia (Peninsular and Sabah) (IUCN/SSC Asian Elephant Specialist Group, 2017). *Elephas maximus maximus* is a subspecies found in Sri Lanka, while *Elephas maximus indicus* is predominant on the Asian mainland, spanning India, Nepal, Bangladesh, Bhutan, Myanmar, Thailand, Peninsular Malaysia, Laos, China, Cambodia, and Vietnam. In Sumatra, Indonesia, these elephants are identified as *Elephas maximus sumatranus* (Shoshani and Eisenberg, 1982). Notably, the Borneo elephant exhibits molecular distinctions, classifying it as a significant ecological subspecies, *Elephas maximus borneensis* (Sharma et al., 2018).

In Peninsular Malaysia, *E. maximus*, commonly known as the Asian elephant, exhibits a widespread distribution across six states, namely Johor, Pahang, Terengganu, Kelantan, Kedah, and Perak (Saaban et al., 2011). The elephant population in this region is primarily concentrated in three Managed Elephant Ranges (MERs): Belum-Temenggor Complex, Taman Negara, and Endau-Rompin Forest Complex (Saaban et al., 2011). The Department of Wildlife and National Parks (PERHILITAN), serving as the governmental authority for wildlife management, has devised the National Elephant Conservation Action Plan (NECAP 2.0) to oversee comprehensive conservation efforts, including the implementation of an EEHV monitoring program in captive centres (PERHILITAN, 2022). Recognizing the precarious status of the Asian elephant, the International Union for Conservation of Nature (IUCN) Red List categorizes it as endangered globally (Williams et al., 2020). In Peninsular Malaysia, *E. maximus* has attained the 'Totally protected' status under the Second Schedule, Wildlife Conservation Act 2010 (Act 716). Despite the global 'Endangered' classification, the Red List of mammals for Peninsular Malaysia designates these elephants as 'Vulnerable', highlighting a concerning 50% population reduction in the last decade, as opposed to the 'Endangered' classification, which requires a 70% reduction (PERHILITAN, 2017).

Ongoing challenges for survival of Asian elephant populations in Peninsular Malaysia involve habitat loss and degradation, human-elephant conflicts, and the illicit trafficking of wildlife. (Saaban et al., 2011; Mahmood et al., 2021). Another escalating threat to the Asian elephant is infectious disease, which holds equal significance with other potential dangers. One of the most devastating viral infections in elephants is Elephant Endotheliotropic Herpesvirus (EEHV). This disease is a herpesvirus specific to both Asian and African elephants, occurring in those under human care as well as in the wild (Fickel et al., 2001; Hoornweg et al., 2022). This disease can cause fatal haemorrhagic disease (EEHV-HD) in elephant species especially young elephants (Richman et al., 1999; Latimer et al., 2011) and affect overall health and survivability of elephants. To date, eight genotypes of EEHV have been identified so far and two of it has caused the most frequently fatal outcome especially in young calves (Richman, 1999; Reid et al., 2006; Stanton et al., 2010; Latimer et al., 2011; Stanton et al., 2012; Atkins et al., 2013; Fuery et al., 2016a; Fuery et al., 2016b; Sripiboon et al., 2016; Oo et al. 2020).

The significance of continuous monitoring and the implementation of proactive conservation strategies is underscored by the potential threat that diseases pose to elephant populations. In Malaysia, there is a lack of documented cases of elephant infectious diseases, including EEHV. However, back to 2016 in Sabah, Borneo Malaysia, there is finding by Lee et al. (2021) found that three cases of sudden lethal occurred due to EEHV-HD infectious. The findings raise concerns of the potential infection of Asian elephants in Peninsular Malaysia by this disease, prompting a call for comprehensive screening and monitoring efforts. The surveillance of EEHV in Malaysia remains inadequate, and it is crucial to establish early detection capabilities for infections within the Asian elephant population in Peninsular Malaysia. Early detection is important and the data can be used as baseline database for elephant captive management. Hence, this study aims to detect and determine the EEHV subtypes in Asian elephant in Peninsular Malaysia through molecular detection. The study was carried out as part of the Wildlife Disease Surveillance Programme (WDSP) by the Ex-Situ Conservation Division, Department of Wildlife and National Parks (PERHILITAN), Peninsular Malaysia.

MATERIALS AND METHODS

Study sites and Sample collection

Two sampling occasions were conducted on captive elephants at National Elephant Conservation Centre (PKGK), Kuala Gandah, Lanchang, Pahang in February and September 2020. A total of 222 samples from 33 individuals (17 adults, nine subadults, six juveniles, a calf) were collected from both sampling occasions. Four types of samples were collected which included trunk wash ($n = 51$), whole blood ($n = 57$), oral swab ($n = 57$), and rectal swab ($n = 57$). The sampling process in this study received approval under the Wildlife Conservation Act 2010 (Act 716) and was assisted by veterinary officers and mahouts. This study addresses the challenges encountered in sample collection for EEHV screening at a conservation centre including most of elephant in PKGK are untrained for trunk wash procedures and difficulties in approaching certain individuals for blood sampling. Hence, this study implemented an opportunistic sampling approach by collecting additional samples from other shedding areas of elephants, like the oral (saliva) and rectal areas. This allowed us to gather diverse samples for EEHV screening despite the challenges. Notably, not all individual elephants could provide all four types of samples due to these constraints in this study.

Trunk wash samples collected (Stanton et al., 2010) following the standard method recommended for collection samples for *Mycobacterium tuberculosis* testing by the USDA. A total of 50 mL of sterile saline (0.9% NaCl) solution was poured into the nares of each elephant and the trunk was elevated for 20 to 30 seconds. Then, the elephant was then instructed to blow the instilled saline solution into clean plastic. The effluent from the trunk will be transferred to a sterile 50 mL-falcon tube and then chilled on ice until processed. Trunk wash was centrifuged at 1,500 X g for 10 minutes to collect the cell pellet then stored at -80°C for further processing.

Blood sampling in elephants typically involves collecting approximately 2-3 mL of blood from the ear vein. This method is preferred due to the accessibility and ease of the ear veins in elephants. To prevent clotting, the collected blood is immediately placed in tubes containing ethylenediaminetetraacetic acid (EDTA). Meanwhile, swab sampling was conducted carefully collected from oral and rectal areas ensuring thorough contact with the respective areas to obtain sufficient biological material. Subsequently, each swab was placed in a tube containing TRIzol reagent. To maintain sample integrity and to prevent sample degradation during field sampling, all samples were kept in an icebox. Upon arrival at the laboratory, the blood and swabs samples were transferred to -80°C for a long-term storage.

Molecular work and sequence analysis

Blood and trunk wash were extracted the genomic DNA by using DNeasy Blood & Tissue Kit (Qiagen, Germany) while for the swab samples were extracted using NucliSens easyMAG (bioMérieux, The Netherlands) following the manufacturer protocols. DNA was eluted in volume of 60 µL and all DNA samples were stored in -20°C prior to the nested PCR process. The quality and concentration of nucleic acid were measured using a spectrophotometer (Implen NanoPhotometer® N60/N50) and all samples resulted concentration of minimum 10 ng/µL.

This study screened all samples using five sets of primers. Both the first and second rounds of nested PCR were carried out for each primer set, regardless of whether the PCR product showed a negative or positive result when visualized using 1.5% agarose gel electrophoresis. The implementation of the nested PCR technique, employing primers targeting the DNA polymerase gene (U38/POL) (Latimer et al., 2011) as shown in Table 1. All samples were tested Each individual PCR was in volume of 20 µL contained of 2 µL of DNA, 10 µL GoTaq® Colorless Master Mix (Promega, USA), 1 µL of each primer and 6 µL nuclease free water. All first and second round amplification were performed for each 35 cycles using following profile: First round, initial denaturation at 94°C for 2 minutes, then 35 cycles for denaturation at 94°C for 30 s, annealing temperature (Ta) as demonstrated in Table 1 for 30 s, and extension at 72°C for 45 s, followed by a final extension step at 72°C for 7 minutes. Second round, after 2 minutes of initial denaturation, the PCR proceed with denaturation at 94°C for 15 s, followed by annealing process (Table 1) for 15 s, and extension at 72°C for 30 s, and final extension step for 7 minutes at 72°C. Gel electrophoresis was performed to visualise the PCR products using 1% agarose gel. Successful PCR products were subjected for DNA sequencing at a local sequencing service provider (Apical Scientific Sdn. Bhd. Malaysia). The DNA sequences obtained underwent quality checking and alignment using Geneious Prime® 2022.1.1 software (Biomatters 2020). Subsequently, the sequences were subjected to BLAST analysis against the GenBank database (NCBI) to confirm the EEHV genotype. A total of 34 reference sequences of EEHV from a previous study were retrieved from GenBank for the construction of the phylogenetic tree (Figure 1). Pairwise geneious alignment was employed for sequence alignment. The Neighbour-joining (NJ) phylogenetic tree, generated using the Tamura-Nei model of nucleotide substitution, was conducted using Geneious Prime® 2022.1.1 (Biomatters 2020).

Table 1 List of primers used in this study (Latimer et al., 2011).

EEHV Subtype	Primers	Primer Sequences	Size (bp)	Ta (°C)
EEHV 1, 2, 4, 5 and Specific EEHV6	PAN-EBP(A1)	–ACAAACACGCTGTCRGTRTCYCCRTA–	500	58
	PAN-EBP(B1)	–GTATTTGATTTYGCNAGYYTGTAYCC–		
	PAN-EBP(B2)	–TGAAAYGCCGTNTAYGGATTYACCGG–	250	62
	EBP6P(A2)	–CATCGATTTTGAACCTCTCATGGTC–	470	60
EEHV3 or 4	EB3/4P(A1)	–CGTTGAAGGTGTCGCAGAT–	390	55
	EB3/4P(B1)	–CAGCATCATCCAGGCCTACAAC–		
	EB3/4P(B2)	–ATCCTGGCGCAGCTGCTGAC–	250	64
EEHV5	EB5P(A1)	–CTACATCTATACAGAACTTTCC–	500	50
	EB5P(B1)	–GTACCTTAGTTACGGACGAGAC–		
	EB5P(B2)	–CGCTGTATATGGATTACCGG–	230	58
EEHV7	R1 LGH7906B	–GCTTCACGAAGACCTTGTCTACC–	670	60
	L1 LGH7924	–CTTCGTCAAGGCCACGTACGCACC–		
	L2 LGH7925	–GCCGTTTCGGGAGAACTCAAGGT–	630	56

RESULTS

A total of 222 samples from blood, trunk wash and swabs (oral and rectal) were tested for EEHV1 to EEHV7. Among the 222 total samples collected, 19 samples tested positive for EEHV infection, comprising blood samples (n = 1), trunk washes (n = 5), oral swabs (n = 7), and rectal swabs (n = 6). The positive samples were identified through the use of the primers viral DNA polymerase genes for EEHV-PAN, EEHV3/4, and EEHV5. Notably, these positive findings were associated with 12 Asian elephant individuals in PKGK. Based on the blast findings, three individuals were found to have EEHV1A, exhibiting a match of 99-100% similarity with EEHV references in GenBank. Additionally, EEHV4 was detected in ten elephants, and EEHV5 was found in one individual (Table 2).

The result showed the absence of EEHV in adult elephants, five out of nine subadult individuals were tested positive, meanwhile all juveniles and one calf exhibited infection with the virus. These results signify a substantial decrease in EEHV infection prevalence among elephant individuals relative to their age classification. The age class used in this study are according to Arivazhagan and Sukumar (2008). Adult elephant were more than 15 years old, subadult elephant were 5–15 years old, juveniles were 1–5 years old and calf that were less than one year old. In addition, two young elephants named Poi and Kelaik were found to harbour multiple EEHV strains. Poi was detected with EEHV4 in the trunk wash sample and EEHV1A in both the trunk wash and blood samples. Meanwhile, Kelaik was found to have EEHV4 and EEHV5 in the trunk wash sample. Notably, during the sampling, all the captive elephants were clinically healthy.

Table 2 Sequencing result of EEHV-positive in Asian elephant in PKGK.

Elephant name	Sex	Age (years)	Age class	Sample type	Sample ID	Sequencing result
Lanchang	M	6	Subadult	Rectal swab	WDSP/20/120SR	EEHV4
				Oral swab	WDSP/20/120SM	EEHV4
Elly	F	4	Juvenile	Rectal swab	WDSP/20/21SR	EEHV4
				Oral swab	WDSP/20/123SM	EEHV4
Amoi	F	3	Juvenile	Oral swab	WDSP/20/22SM	EEHV4
				Rectal swab	WDSP/20/22SR	EEHV4
Dara	F	8	Subadult	Oral swab	WDSP/20/19SM	EEHV4
Siput	F	14	Subadult	Oral swab	WDSP/20/126SM	EEHV4
Poi	M	2	Juvenile	Trunk wash	WDSP/20/23TW	EEHV4, 1A
				Whole blood	WDSP/20/23WB	EEHV1A
Jes	M	1	Juvenile	Rectal swab	WDSP/20/19SR	EEHV4
				Oral swab	WDSP/20/128SM	EEHV4
Pes	M	4	Juvenile	Rectal swab	WDSP/20/20SR	EEHV4
Ani	F	8	Subadult	Trunk wash	WDSP/20/131TW	EEHV1A
Kelat	M	14	Subadult	Trunk wash	WDSP/20/16TW	EEHV1A
Panti	M	2	Juvenile	Trunk wash	WDSP/20/148SM	EEHV4
				Trunk wash	WDSP/20/148TW	EEHV4
Kelaik	F	< 1	Calve	Trunk wash	WDSP/20/149TW	EEHV4, 5

A phylogenetic tree of polymerase U38/POL gene was constructed using the NJ methods (Figure 1). The phylogenetic tree showed the relationship of all EEHV-positive samples in this study with the other reference sequences. The NJ tree also showed a clearly distinct clustered EEHV-positive sample in this study with reference sequences EEHV1A instead of EEHV1B with strong supported bootstrap value (86%). EEHV1A positive was found in blood and trunk wash samples from three Asian elephants in this study. In EEHV1 clade, EEHV1-positive samples in this study found to be formed different clade from previous reported EEHV cases in Borneo Malaysia (Lee et al., 2021). The pairwise geneious alignment of variability of EEHV1 sequence shown in Figure 2.

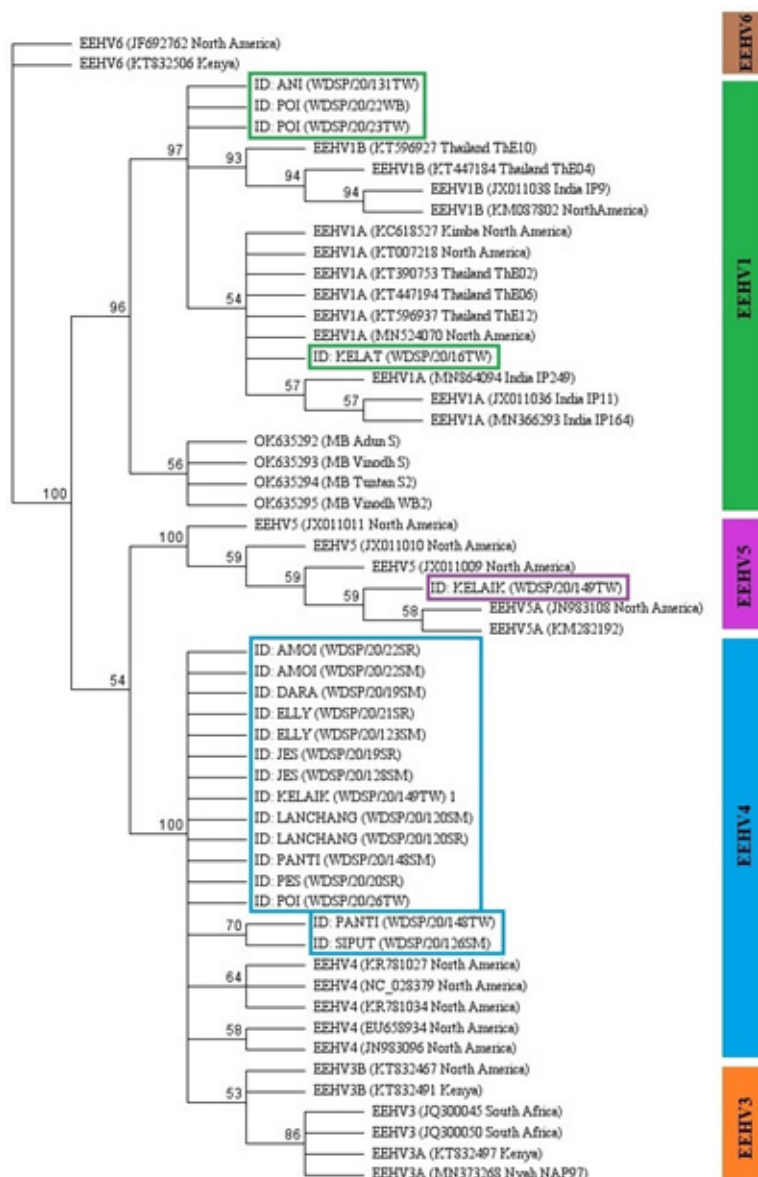


Figure 1 A phylogenetic tree of polymerase U38 gene constructed using the neighbour-joining Tamura-Nei method with 1000 bootstrap replications. Bootstrap value is indicated in each branch. The samples labelled in square boxes in the figure represent samples utilized in this study

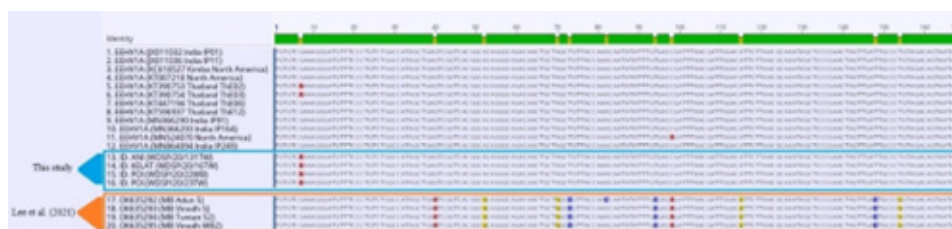


Figure 2 The pairwise alignment variability of EHV1. Samples highlighted in blue square box represent those sequences utilized in this study, while those in orange box are samples from Borneo (Lee et al., 2021)

DISCUSSION

EEHV is known as deadly virus that affects elephants, particularly young elephants. This herpesvirus is highly contagious and can cause severe hemorrhagic disease, leading to rapid death in affected animals (Richman et al., 1999; Reid et al., 2006; Latimer et al., 2011; Dastjerdi et al. 2016). Early detection through regular screening protocols is crucial for implementing timely interventions and better husbandry practices. Efforts to understand, detect, and combat this virus are essential for the conservation of elephants. Therefore, this study initiated with the screening of elephant in Peninsular Malaysia, highlights the necessity for proactive measures in EEHV management to ensure the safeguarding of elephant populations.

In this study, captive Asian elephant in PKGK had been selected for EEHV screening. PKGK is responsible of rescuing injured elephants and relocating those involved in conflicts to suitable forest areas. The centre is actively engaged in the retrieval and care of orphaned or lost young elephant calves. The majority of elephants at PKGK were segregated based on their age groups. Adult elephants were typically housed in designated adult areas, while juvenile elephants were accommodated in separate juvenile zones. Additionally, certain elephants were segregated based on their individual health conditions. Furthermore, the PKGK centre facilitates public visitation, serving as an avenue for enhancing exposure and awareness regarding the primary threats confronting the Asian elephant population in Malaysia.

Lack of information awareness about EEHV in Malaysia and there is no comprehensive screening for infectious diseases, including EEHV, has been carried out for Asian elephants in Peninsular Malaysia. EEHV is known as global problem that's effects young elephant in wide range of countries such as Thailand, Myanmar, Laos and Cambodia (Reid et al., 2006; Bouchard et al., 2014; Sripiboon et al., 2016; Oo et al., 2020). In Malaysia, the major threat and death cause of elephant were reported due to illegal poaching and elephant-human conflict cases such as roadkill and poisoned cases. This is because there have been no reported cases of sudden or related deaths due to haemorrhagic problems or symptoms in elephants until 2020.

In 2016, a case of EEHV infectious disease was reported in three Bornean elephant calves in Sabah, Malaysia (Lee et al., 2021). The finding of lethal cases of EEHV-HD in Lee et al. (2021) study raise concerns of the potential infection of Asian elephants in Peninsular Malaysia by this disease. The absence of documented cases of EEHV among Asian elephants in Peninsular Malaysia rise to concerns about the of whether EEHV is genuinely absent or if potential infections remain undetected due to a lack of comprehensive surveillance and understanding. Clinical signs of EEHV may resemble those of other hemorrhagic diseases, making diagnosis challenging and resulting in few identified clinical cases in Peninsular Malaysia. Thus, conducting EEHV surveillance in Asian elephants is crucial for early detection of infections. This study represents the first detection of EEHV in captive Asian elephants in Peninsular Malaysia, facilitating an early diagnosis of potential diseases.

Off 33 individuals of captive Asian elephant in PKGK, 12 individuals were found EEHV-positive by using molecular detection from five primer specific to eight EEHV subtypes. Collecting multiple samples additional

shedding areas of Asian elephants in PKGK beyond blood and trunk wash, can help in ascertain the likelihood of detection and provides data on potential shedding areas of EEHV. In addition, this study notes that both young elephants named Poi and Kelaik exhibited shedding of more than one strain. Existing multiple strain in one elephant in this study in agreement with previous study which indicates that individual elephant can experience multiple EEHV infections (Latimer et al., 2011; Richman et al., 2014). However, this study does not investigate the prevalence level and infection rate. To provide overview of EEHV presence in Asian elephant in this study, a phylogenetic tree analysis was conducted to further confirm the EEHV subtypes in each positive samples. EEHV4 was the predominant subtype detected in the samples collected, primarily from swabs. Meanwhile, EEHV1A was identified in four samples obtained from blood and trunk wash.

According to phylogenetic tree analysis, EEHV-positive samples were genetically distinct subtype of EEHV1A, EEHV4 and EEHV5 in Figure 1. The primer sets EB3/4P was designed by previous study (Latimer et al., 2011) to amplified both EEHV subtype 3 and 4. Most of the samples, primarily obtained from swabs, indicated the presence of EEHV4. They exhibited a distinct clustering with high support value when compared to EEHV4 reference sequences. This observation is noteworthy, considering that EEHV3 is predominantly associated with African elephants (Zong et al., 2015). Furthermore, NJ phylogenetic tree constructed in this study found EEHV1 sequences were observed to form a distinct cluster, separated from the sequences detected in Sabah Bornean elephant by Lee et al. (2021). They were found to be closer to other EEHV1 sequences from different regions which Thailand, North America and India. The bases highlighted in different colours indicate sequence differences between the EEHV1 strains obtained in this study and those in Lee et al. (2021) study (Figure 2). This sequence difference likely due to variations in elephant population regions and also genetic disparities among elephant subspecies.

During the sample collections conducted in 2020, there were no observed clinical signs of illness in the Asian elephants at PKGK that tested positive for EEHV. Consequently, all positive EEHV results indicated asymptomatic infections with EEHV1A, EEHV4, and EEHV5 in these elephants. The results notably indicate a prevalence of EEHV virus infection among young Asian elephants, including sub-adult, juveniles and calf. The positive findings of this study are potentially correlated with the age demographics, as the majority of elephants testing positive are predominantly from the calf to sub-adult age categories. The detection of infection suggests potential transmission from recently arrived young elephants within the facility could be the factor of the potential pathway for the introduction of the virus into the facility, subsequently leading to its transmission among other individuals via shedding.

The youngest elephant detected with EEHV, namely, Kelaik, aged two months old (in 2020), was rescued from the wild when it was found abandoned or lost from herd. The sampling was conducted shortly after Kelaik's arrival at PKGK in 2020. Kelaik was tested to have EEHV4 and EEHV5 in trunk wash sample. Nevertheless, Kelaik was found deceased, after a few days displaying clinical symptoms of EEHV in the year 2021. This suggest Kelaik may develop maternal antibodies, however, the presence of maternal antibodies

may decrease due to it has been separated from the mothers at the stage of early age, and unable to control the developing EEHV-HD infection. According to [Hoorweg et al. \(2022\)](#), young elephants that are infected during the early stage of life with high levels of maternal antibodies and living in large herd are likely to develop a strong immune system and manage to control the infection without developing EEHV-HD. It is probable that other infected juvenile Asian elephants in PKGK tested in this study have developed such an immune system, enabling them to recognize and activate a protective immune response when exposed to this virus introduced by newly rescued young elephant from the wild. Moreover, this study believed that the development of a good immune system in other Asian elephants could be attributed to the excellent management and husbandry of captive elephants in PKGK.

Based on these results, PKGK isolated the EEHV-positive elephants as a precautionary measure to prevent virus transmission to EEHV-negative individuals. Consequently, PKGK has been consistently conducting basic health checks, administering antiviral medications, and deworming every three months for all elephants. Additionally, close monitoring is performed monthly for EEHV-positive individuals. [Dastjerdi et al. \(2016\)](#) stated that currently no specific treatment currently exists for EEHV, and the prevailing assumption is that once an elephant contracts the virus, it becomes a latent carrier for life. Nevertheless, the use of antiviral medications and the implementation of effective husbandry practices in captive environments may have a substantial impact on treatment. Despite this, it is vital to initiate early health monitoring measures to prevent the dissemination of EEHV infection in captivity, particularly to avert further fatalities, especially among young elephants. Therefore, the effort of early EEHV detection and immediate intervention with supportive care are critical to the success of treating an elephant affected by EEHV.

CONCLUSIONS

The data gathered from this study establish a fundamental database for the ongoing monitoring and routine surveillance of EEHV infectious diseases. Understanding the prevalence of subclinical infections in Asian elephants in Peninsular Malaysia is crucial for making informed decisions regarding the health management and conservation strategies for both captive and wild Asian elephants in the region. Additionally, this research suggests and recommend the use of quantitative PCR (qPCR) in future studies to identify EEHV infection in Asian elephants throughout Peninsular Malaysia. qPCR is renowned for its superior sensitivity and specificity compared to traditional PCR methods ([Hoffmann et al., 2009](#)). Unlike conventional PCR, qPCR exhibits heightened sensitivity in EEHV detection, irrespective of the presence of clinical signs or shedding. It is essential to further investigate the presence of EEHV in the wild in future studies related to infectious diseases in Asian elephants in Peninsular Malaysia.

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AUTHOR CONTRIBUTIONS

MG, KVK, MFAAR conducted field sampling. MG conducted laboratory work and data analyses. MG wrote and prepared the manuscript. MG, KVK, AAM revised the intellectual content. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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