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REVIEW ARTICLE

Elephant endotheliotropic herpesvirus haemorrhagic disease of Asian elephants: An updated mini review

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ABSTRACT

Asian elephants *(Elephas maximus)*, the National Heritage Animal of India and classified as endangered by the IUCN. Their existence is threatened by habitat loss, poaching, deforestation and emerging diseases such as elephant endotheliotropic herpesvirus (EEHV), which has become a major cause of mortality among elephant calves over the past three decades. First reported in North America in 1995, EEHV has since caused severe losses in both captive and wild populations throughout the world. In India, the first case was recorded in Kerala in 2013. The virus is mainly transmitted through direct mucosal contact, trunk secretions or fomites and may also spread *via* saliva or intestinal contents. Latent nature of EEHV permits to establish a carrier status in adult Asian elephants and intermittent shedding of the virus without associated clinical disease. EEHV infection classically targets endothelial cells, resulting in widespread haemorrhage, DIC and cardiovascular failure. Clinical signs range from lethargy and facial oedema to acute death. Diagnosis is primarily based on nucleic acid detection methods like both conventional PCR and qPCR methods. The serological method like ELISA is developed to assess antibody status. Despite numerous attempts, EEHV could not be isolated in the cell culture system to date. Therapeutic management includes anti-herpes viral drugs like famciclovir, ganciclovir or acyclovir combined with intensive supportive care. Recent research on EEHV vaccines (viral vector and mRNA vaccines) shows promising results. High fatality rate and widespread occurrence, enhanced surveillance, rapid diagnostic capabilities and development of effective vaccines are crucial for mitigating the impact of EEHV on the conservation of Asian elephants.

Keywords: Asian elephant, endotheliotropic, glycoprotein B, haemorrhage, India, latency

INTRODUCTION

India harbours a rich diversity of wildlife that plays a vital role in maintaining ecological balance and the stability of natural ecosystems. Wildlife plays a vital role in regulating populations, facilitating nutrient cycling and maintaining energy flow within food webs, thereby supporting overall ecosystem stability and environmental health[1]. However, increasing anthropogenic pressures such as habitat loss, fragmentation, pollution and climate change have made elephant more vulnerable to several health-related problems, including nutritional and physiological stress, exposure to toxic substances and infectious diseases[2]. Among India's diverse megafauna, elephants hold a place of profound ecological significance and enduring cultural and religious reverence. The Asian elephant *(Elephas maximus)*, belonging to the family *Elephantidae* under the order *Proboscidea*, is the largest living terrestrial mammal. India supports nearly 60% of the global wild Asian elephant population[3]. Despite this, the species is listed as endangered by the IUCN due to habitat degradation, poaching and disease threats[4,5]. Like other mammals, elephants are susceptible to several infectious diseases such as tuberculosis, haemorrhagic septicaemia, rabies, foot and mouth disease and parasitic infestations[6,7]. In recent years, Elephant Endotheliotropic Herpesvirus (EEHV) infection has emerged as one of the most serious diseases affecting elephant populations worldwide. EEHV was first identified in African elephants, and the earliest fatal case in Asian elephants was reported[8]. Several subtypes of EEHV have been identified, among which EEHV-1A and EEHV-1B are predominantly associated with fatal haemorrhagic disease in Asian elephants[9,10]. The disease, termed Elephant Endotheliotropic Herpesvirus Haemorrhagic Disease (EEHV-HD), is an acute and highly fatal condition that primarily affects calves and juvenile elephants under 15 years of age[11]. In India, the first confirmed EEHV fatality was reported in 2013, and subsequent studies have documented EEHV-HD in Indian elephant populations[12-14]. Over the past three decades, Asian elephants have faced a serious threat from EEHV infection, which has emerged as one of the leading causes of mortality among juvenile elephants worldwide, including in India.

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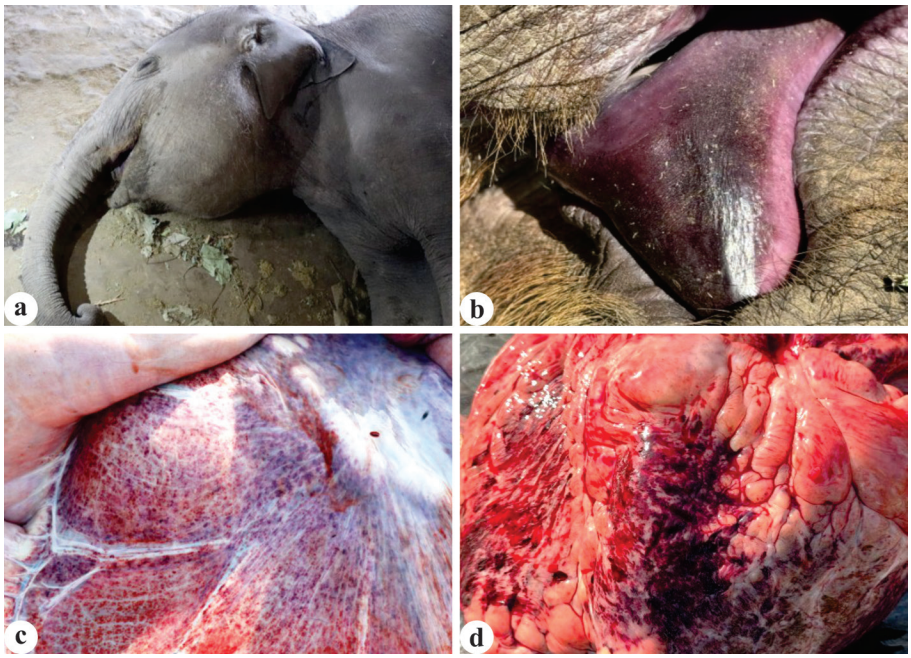
Elephant endotheliotropic herpes virus

The novel elephant herpes



Necropsy findings of EEHV-HD. a. Carcass of elephant calf showing marked swelling of the face due to severe subcutaneous oedema (Kalagarh Range, Jim Corbett National Park). **b.** Diffuse cyanosis of tongue. **c.** Diffusely congested mesenteric blood vessels with haemorrhages. **d.** Diffuse epicardial haemorrhages.

virus is currently classified within the genus Proboscivirus of the subfamily *Betaherpesvirinae*. However, recent genomic analyses of EEHV have led to the proposal of a new subfamily, *Deltaherpesvirinae*, to accommodate these viruses[15]. However, the proposed classification has not yet been officially adopted by the International Committee on Taxonomy of Viruses (ICTV). The EEHV cause a spectrum of infections in elephants, ranging from subclinical or localized lesions to acute, often fatal, haemorrhagic disease. The first lethal case of an acute haemorrhagic disease of unknown aetiology in an Asian elephant was reported from a circus in Switzerland in 1988[8]. Later, characteristic intranuclear inclusion bodies in vascular endothelial cells of a 16-month-old Asian elephant calf that died at the Smithsonian's National Zoo, USA confirming the involvement of a novel herpesvirus, subsequently named EEHV-1[9]. To date, several subtypes of Probosciviruses have been identified, including EEHV-1A, EEHV-1B, EEHV-2, EEHV-3, EEHV-4, EEHV-5, EEHV-6 and EEHV-7[10,16]. Asian elephants are primarily affected by EEHV-1, EEHV-4 and EEHV-5, which exhibit variable pathogenicity, whereas EEHV-2, EEHV-3, EEHV-6 and EEHV-7 have been identified in African elephants, with EEHV-2, EEHV-3 and EEHV-6 associated with fatal infections[10,16,17]. These viruses are believed to have co-evolved with their host species[18]. Although cross-species transmission from African to Asian elephants was once proposed, genomic analyses revealed that EEHV-1 is endemic to Asian elephants, while African elephants harbour different EEHV genotypes[10]. Genomic characterization has shown that EEHVs diverged independently from other mammalian herpesviruses millions of years ago[19]. The EEHV genome (-180 kb) encodes about 115-120 genes, including 35 conserved core genes and nearly 60 novel genes not found in other herpesviruses[10,15]. EEHV-1 and EEHV-2 form an A + T-rich branch (-42% GC), whereas EEHV-3 and EEHV-4 are G + C-rich. At the nucleotide level, EEHV-1 and EEHV-2 differ by -25%, while EEHV-1A and EEHV-1B show 15-40% variation in several glycoprotein genes[20]. Hypervariability in glycoprotein-H and vGPCR1 regions of EEHV-1 suggests long-term host-virus co-evolution[13]. Among the currently recognized Probosciviruses, EEHV-1 is the most pathogenic and prevalent[10]. The disease, termed EEHV-HD, is now recognized as a major cause of mortality in juvenile Asian elephants[11]. Calves between one and five years of age are most susceptible, while adults often remain asymptomatic carriers that intermittently shed the virus[21]. Protection in calves below one year of age is attributed to maternal antibodies[10]. Molecular genotyping of EEHV cases indicates that infections are sporadic rather than epidemic, with no evidence of inter-facility spread among captive herds[10,16].

Epidemiology of EEHV

The EEHV-HD has caused significant mortality in captive Asian elephants, with up to 65% of young, captive-born calves in Europe and North America succumbing to the disease[22]. The disease has also been reported across Asia, including India, Myanmar, Malaysia, Cambodia, Laos, Thailand, Nepal and Sumatra[11,17,23-27]. EEHV is primarily transmitted through direct contact with infected

bodily secretions, including saliva, trunk secretions and intestinal contents, with aerosol or droplet spread likely in close-contact situations[20,28]. Vertical transmission *via* placenta or breast milk is suspected but not fully understood. Latent infections can reactivate under stressors such as pregnancy, weaning or husbandry changes, leading to viral shedding without clinical signs. Pregnant elephants, particularly in the third trimester, show higher viral shedding[20,29] though shedding can occur independently of stress hormone levels.

Young, seronegative calves are most susceptible to EEHV-HD, with trained or recently weaned individuals disproportionately affected. EEHV-1 is associated with higher mortality than other subtypes. Cases occur in both captive and wild populations, across sexes and are more frequent during the rainy season[10,30]. Viral shedding is linked to pregnancy and elevated oxidative stress markers such as ROS and MDA[31]. Subclinical infections, documented in Asia, Europe and North America enable elephants to carry and disseminate EEHV without showing symptoms[32,33]. Latent infections allow the virus to persist in host cells, reactivating under stress or external stimuli[34]. Endothelial cells of umbilical cord vessels are suspected latency sites for EEHV-1.

Pathogenesis of EEHV-HD

The pathogenicity of EEHV depends on multiple host factors like[20,29]:

1. Age at exposure to the virus
2. Presence of passively transferred protective maternal antibodies obtained through nursing or during gestation
3. Presence of any immunocompromising disease states
4. Primary infection of EEHV is said to result in severe infection of EEHV, as most elephants found dead due to EEHV-HD were sero-negative for specific antibodies against EEHV
5. Young elephant calves are most susceptible to infection
6. Majority of positive cases are reported in trained or weaned calves
7. More number of cases are reported in rainy season
8. EEHV1 causes severe mortality in Asian elephants than other EEHV subtypes
9. Increased viral shedding is documented in pregnant animals

During the acute phase of infection, EEHV exhibits tropism for monocytes/macrophages, endothelial cells and epithelial cells of the elephant's alimentary tract, which serve as the primary sites for viral replication[35]. The virus mediated endothelial cell injury leads to increased vascular permeability and leakage with severe, widespread oedema and haemorrhage.

Virus entry and dissemination

In most herpesvirus, the binding of virion to host cell involves the interaction of Glycoprotein B (gB) with cell surface heparan sulphate proteoglycans and the virus enter either through fusion of the virion envelope with the plasma membrane or by endocytosis. The gB present in most herpesvirus is cleaved by cellular furin and such a conserved furin cleavage site is also found in EEHV-1 gB protein which helps to cleave the protein in the centre[36]. The specific cellular receptor for EEHV has not yet been identified.

A study on antibody production against EEHV demonstrated that salivary glands and gastrointestinal epithelial cells are the main target tissues for EEHV-1A and EEHV-4 infections. The presence of EEHV gB antigen in salivary glands has been confirmed through immunohistochemical analysis, suggesting that these glands may serve as the primary sites of viral replication for both EEHV-1A and EEHV-4[28]. The virus spreads throughout the elephant's body via infected blood monocytes[37]. EEHV gB antigens have been detected in PBMCs within the blood vessels of internal organs[28]. This pattern is similar to betaherpesviruses like cytomegalovirus, which replicate in salivary glands and spread through monocytes and macrophages[38].

Apoptosis of PBMCs is commonly seen in fatal EEHV-1A-HD cases[37]. EEHV viral particles present within circulating monocytes are transferred to endothelial cells of the small blood vessels through monocyte adhesion to specific molecules expressed on the endothelial surface[39]. The interactions between the leukocytes and endothelial cells are initiated by a variety of chemical mediators derived from inflamed tissues, and the entire process of adhesion is regulated by the sequential activation of different families of adhesion molecules expressed on endothelial cells and the surface of leukocytes. The expression of Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1), a leukocyte adhesion receptor and endothelial ligand, has been demonstrated by immunohistochemistry. PECAM-1-positive cells were found to be significantly more prominent in EEHV-HD-positive tissues compared to EEHV-negative controls, indicating their potential role in the pathogenesis of the disease[39].

Endothelial damage

Endothelium is considered as an endocrine organ which plays a major role in the maintenance of homeostasis in the body. The name of the virus itself suggests its endotheliotropic nature. EEHV shows strong tropism for endothelial cells of the tongue, heart and liver[40]. Intranuclear inclusion bodies indicate severe infection of endothelial cells in large myocardial arteries and the endocardium[41]. EEHV preferentially infects endothelial and smooth muscle cells of small

vessels such as arterioles, venules and capillaries. This may be due to unique surface antigens and lower blood pressure in small vessels, which favour viral adhesion and infection[39]. The exact mechanism of endothelial damage in EEHV-HD remains unclear, though viral-mediated injury is considered the most likely cause, with immune-mediated damage and endothelial apoptosis also possible contributors[10]. Infection of EEHV-1A/B. EEHV-2, EEHV-4 and EEHV-5 primarily affect capillary endothelium, whereas larger vessel changes were mostly seen in EEHV-3 affected elephants[42]. As affected organs number in EEHV positive cases is more, viral dissemination can potentially happen through circulating EEHV infected endothelial cells[41]. The endothelial cells may also act as sites of latency of EEHV virus like Human cytomegalovirus[41].

Although endothelial cells play an important role in EEHV pathogenesis, viral load found more in liver and heart, which is less vascular than lungs, suggest that hepatocytes or cardiac myocytes may also support *in vitro* replication of virus[41]. The elevated level of Cardiac troponin in high viraemic elephants indicates cardiomyocytes damage[43]. Viral DNA is widely distributed across organs, with the highest loads in the liver for EEHV-1A and in the heart for EEHV-1B and EEHV-5 fatal cases[41]. Another study reported higher viral loads in the heart and lower in the brain[44]. Immunohistochemistry using gB protein showed positive signals in salivary gland epithelium, PBMCs in the spleen and heart and lymph node follicles[28]. Increase in the vascular permeability results in leakage of intravascular fluid through the endothelial gaps and results in oedema. Intranuclear inclusion bodies detected in endothelium in all the EEHV-HD cases, which demonstrates direct viral damage to the endothelium[18].

Formation of thrombus and DIC

Endothelial injury and dysfunction play a key role in thrombus formation. Normal endothelium allows smooth blood flow, but when damaged, it exposes collagen and releases procoagulants like von Willebr and factor (vWF). vWF forms a bridge between collagen and platelet receptor GpIb, promoting clot formation. It is also a marker of endothelial dysfunction and is produced by both endothelial cells and megakaryocytes[45]. In EEHV-1A HD and EEHV-4 HD, vWF antigens are mainly detected in the heart, intestine and lungs[39]. Activated platelets release granules and produce thromboxane to enhance haemostasis. Excessive platelet uses leads to DIC, characterized by bleeding and microthrombi[46].

Thrombocytopenia in EEHV is due to platelet consumption rather than destruction or decreased production, as bone marrow megakaryocyte numbers remain unchanged[35]. Cyanosis of the tongue, considered a pathognomonic lesion of EEHV-HD, represents a late clinical manifestation of overt disseminated intravascular coagulation (DIC). The cyanotic appearance results from severe oedema and intramuscular haemorrhage within the lingual tissues[18]. However, because of the absence of predominant ischaemic lesions, thrombosis is not likely to be the primary cause of EEHV-HD coagulopathy[18]. The DIC and irreversible damage to blood vessels subsequently leads to failure of organs or hypovolemic shock followed by the death of the elephant[47]. Systemic inflammation in EEHV is also hypothesized due to increase in the proinflammatory cytokine mRNA expression and this results in destruction of smaller blood vessels and followed by disseminated intravascular coagulopathy[48]. Upregulation of cytokines like IL-1, IL-2, IL-4, IL-8 and TNF-α were observed in EEHVHD infected cases[39]. In addition to severe tissue damages produced by cytokines, it is also suggested that cytokines are also important in EEHV infection control in young elephants by mediating innate immune response[18]. Recent study, using RNAscope® *in situ* hybridization, EEHV-1A DNA polymerase and terminase genes were detected in multiple archival tissues, including the heart, lung, tongue, spleen, liver, kidney, lymph node, gastrointestinal tract, salivary gland and central nervous system. The virus was found to replicate exclusively within capillary endothelial cells, with the highest viral loads observed in the heart and liver, suggesting that tissue-specific endothelial heterogeneity contributes to variation in viral replication and lesion severity[49]. Furthermore, significantly elevated expression of IFN-γ, IL-6 and IL-10 in tissues with high viral loads indicates a cytokine storm-like response as a key pathogenic mechanism in EEHV-HD, underscoring the potential benefit of incorporating anti-inflammatory or immunomodulatory therapies in clinical management[50].

Pathomorphological alterations of EEHV-HD in Asian elephants

Clinical findings

EEHV-HD among Asian elephants develops in a rapid progression and results in death within 1-5 days[51]. The mortality rate in calves that exhibits clinical signs is about 85%[52]. In most cases the disease starts with general clinical signs like lethargy, anorexia and dullness[51,53]. Early clinical signs of EEHV-HD often begin with non-specific symptoms such as lethargy, fever, changes in sleep patterns, reduced appetite and water intake, colic and stiffness[10,18]. Affected elephants may show subcutaneous oedema of the head, neck and limbs, along with swelling of the temporal glands and scleral injection[40,53]. As the disease progresses, severe manifestations like generalized oedema, oral ulceration, tongue and tracheal cyanosis develop, often leading to death within a week[40,54]. Diarrhoea is a common clinical symptom observed in EEHV infections[51,53]. In addition to

these signs abdominal colic was also noticed in positive cases[44]. The reactivation of the virus in reservoir animal results in non-pathogenic clinical signs which includes ulceration and vesicle formation in oral and vaginal mucous membranes[11].

A consistent haematological hallmark of EEHV-HD is acquired thrombocytopenia, reflecting the disease's association with thrombosis and haemorrhage[53]. It represents the most prominent and diagnostically significant haematological alteration observed in infected elephants[39]. Other common findings include leukopenia, anaemia, dehydration and systemic inflammation characterized by heterophilia with left shifting and toxic leukocyte changes[11,39]. EEHV-HD calves typically show anaemia, thrombocytopenia, monocytopenia and reduced plasma protein levels[15]. EEHV viremia in juvenile elephants is characterized by mild to moderate thrombocytopenia and leukopenia and moderate to severe monocytopenia[55]. Elevated acute phase proteins such as serum amyloid A (SAA) and haptoglobin (HP) are also reported in EEHV-HD[33]. Their levels are significantly higher in viraemic elephants and correlate with viral load, while TNF-α and IL-2 show minimal variation between infected and non-infected animals[56].

Gross lesions

Prominent gross lesions include subepicardial and myocardial haemorrhages, cyanotic tongue, oedema and extensive petechial to ecchymotic haemorrhages across multiple organs, notably the heart, lungs, liver, mesentery and gastrointestinal tract[12,40,57]. Additional findings such as pericardial effusion, ascites, hepatomegaly, intestinal ulceration and oedema of the trunk, limbs and head are commonly observed[10,40]. Coinfections with EEHV subtypes (e.g., EEHV-1A and EEHV-4) produce similar lesions, with more severe cardiac haemorrhages noted in EEHV1A cases[28,41]. Gross gastrointestinal lesions may resemble bacterial infections such as *Clostridium perfringens* or *Salmonella* spp. and cardiopulmonary changes can mimic *Citrobacter freundii* infection, highlighting the need for differential diagnosis[55].

Microscopical findings

Histopathological lesions in fatal EEHV-HD cases are characterized by severe vascular damage and endothelial involvement. Typical findings include extensive extravascular haemorrhage, myocardial fibre separation and intranuclear inclusion bodies within capillary endothelial cells[40,53]. In some cases marked vascular and intestinal oedema, haemorrhages and inflammatory infiltration by heterophils and lymphocytes were reported, along with basophilic inclusions in hepatic endothelial cells[39,40].

Co-infections with EEHV-1 and EEHV-4 demonstrated large intranuclear inclusions in myocardial arteries and endocardium[28]. The lesions predominantly involve small blood vessels and arterioles, characterized by varying degrees of haemorrhages and oedema[40]. Endothelial cells of the tongue, liver and heart often display apoptosis, swelling and inclusion bodies, consistent with immunohistochemical detection of viral antigens in these sites and splenic macrophages[58]. Additional vascular changes such as necrosis, hypertrophy, fibrinoid degeneration and oedema are frequently observed, with viral particles confirmed in endothelial cells of the tongue, spleen and heart[42,51].

Diagnosis of EEHV-HD

Diagnosis of EEHV-HD can be performed using clinical signs, gross lesions and histopathological lesions. Immunohistochemical detection using antibody against pan-EEHV antigen or gB is also commonly used for the diagnosis purpose[28,39,40]. Culture and isolation of viruses are considered as the gold standard method of diagnosis of any viral infection. The attempts for culturing and propagating the EEHV in different cell culture systems like primary fibroblasts, white blood cells and placental epithelial cells and umbilical cord vascular endothelial from Asian and African elephants was carried out in various parts of world[36,41,51]. All these efforts to develop the EEHV in cell culture settings were ineffective. EEHV cultured in elephant endothelial cells obtained from Asian elephant umbilical cord survived up to eight passages[59]. Elephant fibroblast cell lines showed growth in four passages when inoculated with EEHV infected elephant tongue along with infected PBMC[36]. Viral particles are also detected in the endothelial cells of tongue, spleen and heart[42,51]. Electron microscopy revealed accumulation of non-enveloped viral particles within the endothelial cells of the liver, associated with perinuclear cytoplasmic electron-dense bodies in EEHV-positive cases[36].

Polymerase chain reaction (PCR) remains the most widely used method for the confirmatory diagnosis of EEHV infection. First detected EEHV DNA in tissues of affected elephants using conventional PCR in 1999[51]. Later subtype specific PCR was developed using universal primer targeting the polymerase gene locus for broad EEHV detection, followed by type-specific primers for genotyping, enabling sensitive and specific diagnosis[52]. However, conventional PCR cannot reliably detect latent infections with low viral loads[29]. To improve sensitivity a quantitative real-time PCR (qPCR) assay was developed for capable of detecting EEHV-1 DNA in blood and trunk wash samples from both symptomatic and asymptomatic elephants[20]. qPCR enables the detection of early viraemia in clinically asymptomatic elephants, thereby facilitating timely therapeutic intervention and potentially reducing mortality[20,40,51]. Although qPCR enables quantification, it cannot determine viral infectivity, as *in vitro* culture of EEHV remains unsuccessful. The highest EEHV-1 viral

loads in bone marrow, heart, liver and lymphoid tissues of fatal cases were reported[36]. Loop-mediated isothermal amplification (LAMP) is another rapid nucleic acid amplification method used for EEHV detection in blood. This assay targets the POL gene of EEHV-1 and offers a detection limit about 100-fold higher than conventional PCR[60]. Additionally, RNAscope® *in situ* hybridization (ISH) has been employed to confirm EEHV1A infection in formalin-fixed tissues[49]. Recently, targeted enrichment of EEHV using ultracentrifugation to concentrate viral copies for complete genome sequencing were attempted[61].

Even though no commercially available diagnostic kits are available for detection of EEHV, several inhouse diagnostic kits are developed like ELISA to detect IgG antibodies of EEHV in serum or plasma samples collected from 125 captive Asian elephants[62]. The antigen used for developing the ELISA is gB, an envelope protein present in herpes virus. Recently, another ELISA was developed using the non-structural protein DNApol with the aim to detect EEHV shedders[21]. Indirect immunofluorescence is also used to view the viral proteins expression like gB protein in infected cells[36]. A serological test developed to differentiate infections with different EEHV, is Luciferase immunoprecipitation system (LIPS)[63]. This method helps in determining whether EEHV infection associated illness and fatalities occurred due to primary infection or reactivation of the latent virus. This method is also employed for assessing the susceptibility of elephant calves to EEHV infections and monitoring immune reactions to anti-EEHV vaccines. More recently, a 10% sero-prevalence of EEHV-1 among captive Asian elephants in India using inhouse developed ELISA was observed in the randomly collected samples[64].

Treatment of EEHV-HD

Treatment of EEHV-HD is very challenging and mostly antiviral medications often used in human medicine to treat herpesvirus infections, with a poor and unreliable success rate are used in this disease. Herpesvirus infections can be treated using nucleoside analogues such as famciclovir, acyclovir and[65,66]. The commonly used drug for EEHV treatment is famciclovir, which requires oral or rectal route of administration and it was found to be ineffective in clinical cases due to the increase in the viral load even after the administration of the drug. Intravenous route of administration is more preferred for the drugs such as acyclovir and ganciclovir[65,66].

Numerous cases of non-survival have been reported among elephants treated with anti-herpes viral drugs; therefore, early detection followed by prompt symptomatic and supportive therapy is considered more effective than antiviral treatment alone. Loss of vascular integrity led to fluid loss and fluid therapy is recommended for EEHV treatment[65]. For counteracting secondary bacterial infection antibiotics and to reduce pain due to inflammation analgesics can also be used. Due to the cardiovascular damage, intranasal oxygen therapy is also suggested in EEHV-HD[65,66]. The successful treatment of an EEHV-1 infected elephant calf with Zelnate also documented[67].

Vaccination

The non-availability of vaccines is also a major problem facing the control of this disease. In recent years, vaccine development efforts have primarily focused on identifying immunogenic viral proteins and optimizing delivery systems capable of inducing robust protective immune responses. The major viral envelope glycoproteins gB, gH and gL have been recognized as key targets due to their roles in viral entry and their ability to elicit neutralizing antibodies. Recombinant protein-based vaccine trials using EEHV-1A gB and gH/gL antigens have shown promising induction of virus-specific humoral responses *in vitro* and in preliminary elephant studies[30,68]. Further advancements include a multivalent EEHV1A mRNA vaccine encoding glycoproteins gB, gH, gL and gO, which demonstrated safety and the ability to elicit strong humoral and T-cell immune responses in mice, representing an important preclinical step toward an effective EEHV vaccine[69]. Similarly, a recombinant EEHV-1A gB subunit vaccine containing gBF1 and gBF2 fragments formulated with Montanide™ ISA 206 VG or incomplete Freund's adjuvant induced strong humoral and $CD4^+$ T-cell (Th1 and Th2) responses in mice, suggesting the potential of gB-based vaccines for EEHV prevention[70]. Although significant progress has been made, no fully protective or commercially available EEHV vaccine exists yet, underscoring the need for continued research to define correlates of protection, optimize adjuvant systems and evaluate vaccine efficacy in elephants.

Major conservation challenges in the management of EEHV-HD in India

The major challenges in management of EEHV include :

1. High mortality in juvenile elephants
2. Lack of detailed knowledge on the prevalence of EEHV and its subtypes circulating among Asian elephants in India
3. Lack of routine screening and monitoring procedures
4. Lack of knowledge of disease among forest officials and veterinarians
5. Elephants are not trained for collection of samples for routine clinical examination
6. Lack of well-developed diagnostic infrastructures in captive facilities
7. No commercially available vaccines/diagnostic kits

8. Non availability of effective anti-viral drug for treatment

There is an urgent need of standard national guidelines for EEHV-HD management in India, as current practices vary significantly across states. Differences in diagnostic protocols**,** including sampling methods and qPCR testing capacity, result in inconsistent detection and delay in confirmation. Additionally, data sharing between states, zoos, research institutions and forest departments remains fragmented, limiting the development of a robust national database. As a result, a cohesive and comprehensive national EEHV management framework is still evolving, highlighting the need for unified guidelines and coordinated implementation across the country.

CONCLUSION

Asian elephants in India now face conservation challenges due to the frequent outbreaks of EEHV-HD, a disease that excessively affects young elephants. Although significant progress has been made globally in understanding the molecular characteristics and diversity of EEHV strains, research in India remains limited. Intensive and widespread screening is urgently required to determine the true burden of the disease in both wild and captive populations, along with the development of point care diagnostic tools capable of detecting infections at subclinical stages. The continued absence of vaccines or targeted antiviral therapies underscores the need for enhanced research efforts. To address these challenges, a coordinated national strategy including qPCR-based screening, regional diagnostic laboratories, treatment protocols and real time surveillance systems are essential. Ultimately, safeguarding India's endangered Asian elephants will require sustained scientific research, strengthened institutional collaboration especially involving Project Elephant, MoEF & CC and leading research institutes with quick, well integrated conservation actions.

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