Veterinary Microbiology 208 (2017) 159–163

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

Incidence of elephant endotheliotropic herpesvirus in Asian elephants in India

Nagendra N. Barman[a], Bhaskar Choudhury[b], Vishnu Kumar[c], Monika Koul[c], Sophia M. Gogoi[a], Elina Khatoon[a], A. Chakroborty[a], P. Basumatary[b], B. Barua[d], T. Rahman[a], S.K. Das[a], Sachin Kumar[c,*]

[a] College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India
[b] Wildlife Trust of India, India
[c] Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati, Assam 781039, India
[d] Veterinary officer, Government of Assam, India

ARTICLE INFO

Keywords:
Herpesvirus
Pathogenicity
Elephant
Phylogenetic
Sequencing

ABSTRACT

Elephant endotheliotropic herpesviruses (EEHVs) are the cause of acute hemorrhagic disease in endangered Asian and African elephants. In the present study, we report the incidence of EEHV infection and associated mortality in the captive elephant of Assam, India. Our result showed the gross morphology and histopathological changes of EEHV infection in the elephant. Moreover, the phylogenetic analysis of the polymerase, helicase, and GPCR genes from the infected tissue samples suggested the presence of EEHV1A virus.

Elephants are living mega-vertebrates. Elephants are protected in many National Sanctuaries in North Eastern States of India. However, the population of Asian elephants is declining in recent years. The Asian elephant (*Elephas maximus*) is listed as "endangered" by the International Union for Conservation of Nature red list. Among the infectious agents, elephant endotheliotropic herpesviruses (EEHV) are identified as the cause of acute hemorrhagic disease in endangered Asian and African (*Loxodonta africana*) elephants. The infections have an acute onset of lethargy, the generalized oedema of the head and limbs, oral ulceration and cyanosis of tongue, trachea, and death within 7 days (Kendall et al., 2016). The mortality rate reaches up to 85% (Fuery et al., 2016). The first fatal case from India was reported in the year 1997 (Zachariah et al., 2013). The disease is known to affect juvenile elephants predominantly, but not exclusively.

The EEHV belongs to the subfamily *Betaherpesvirinae* under genus *Proboscivirus* (Richman et al., 1999; Wilkie et al., 2013). Currently, eight probosciviruses namely, EEHV1A, EEHV1B, EEHV2, EEHV3, EEHV4, EEHV5, EEHV6, and EEHV7 have been identified (Long et al., 2016; Richman et al., 2014). All the EEHVs are believed to infect elephant and EEHV-1 appear to be the most pathogenic and frequently isolated virus from Asian elephants (Long et al., 2016). The prevalence of EEHV in captive Asian elephants in North America and Europe has been well characterized, with an estimated mortality rate of 70%. It has been shown that the overall frequency of detection of EEHV within the Asian elephant herd was 31% (Stanton et al., 2010). There is no information about the prevalence and impact of EEHV on captive as well as wild elephant populations in Assam, which is the hotspot for Asian elephants.

The present study, to the best of our knowledge, is the first laboratory confirmed report on the incidence of EEHV-1A infection in the captive elephant of Assam, India. Extensive pathological and molecular characterization was performed for the confirmation of EEHV infection.

In 2014 to 2016, high mortality was recorded in captive-bred elephants in Kaziranga national park in Assam, India. Moreover, wild elephant calves and ailing adults were rescued during the flood and other natural calamities. Dead carcasses of thirteen wild and four captive elephants reported by the forest department, Government of Assam were attended. Appropriate blood and tissue samples were collected in ice for microbiological investigation. A comprehensive post-mortem procedure following elephant necropsy protocols was carried out within 24 h. Gross changes in the external body as well as in various internal organs were recorded. For histopathological examination, representative tissues from various organs like tongue, heart, lung, spleen, liver, kidneys and all segments of intestine, oral mucosa, lymph nodes were collected. After proper fixation, the samples were processed in 4–5 μ thick sections and stained with routine haematoxylin and eosin

* Corresponding author at: 1N 103, Department of Biosciences and Bioengineering Indian Institute of Technology Guwahati, Guwahati - 781039, Assam, India. Tel: +(91) 3612582229; Fax: +(91) 3612582249.
E-mail address: sachinku@iitg.ernet.in (S. Kumar).

http://dx.doi.org/10.1016/j.vetmic.2017.08.001
Received 21 April 2017; Received in revised form 30 July 2017; Accepted 1 August 2017

Table 1
Detail of the animals used in the study. The age, sex, gross and microscopic changes of the animals are listed.

Age group	Lab ID/ (Sex F/ M)	No of cases	Gross changes	Histo-pathological changes	Organisms detected			Diagnosis
					Bacteria	Tissue sample	Viruses	
1 month	EL0007(F)	1	Swollen face, temporal region, cyanotic tongue, extensive haemorrhage on cardiac muscle and tongue, congested lungs, haemorrhages in Intestine, kidney, spleen,	Cellular infiltrates in the heart, tongue and lung. In liver cytoplasmic vacuolation, pyknotic, necrosis. Characteristic single, variably sized, eosinophilic intranuclear inclusion bodies within sinusoidal endothelial cells of the liver.	Staphylococcus	Spleen	EEHV (1)	EEHV infection
3 months	EL0003(M)	1		Cellular infiltration in the lung. In liver cytoplasmic vacuolation, pyknotic, necrosis. No. intranuclear inclusion bodies seen.	E coli (1)	Heart, Tongue		Not Confirmed
3 years	EL0066 (F)	1			Staphylococcus (1)	Heart blood		Not Confirmed
4 months	EL0001(M)	1	Extensive haemorrhage on cardiac muscle and tongue, Congested lungs, haemorrhages in Intestine, kidney, spleen,	Kidney showed haemorrhages in cortico-medullary regions, interstitial nephritis. In spleen haemorrhages in red pulp areas, follicular atrophy, the heart showed sub-epicardial and myocardial haemorrhages. Congestion of central vein in liver, degenerative changes with focal areas of necrosis in the hepatocytes, in the lung interstitial pneumonia with infiltration of mononuclear cells.	Pneumococci (1)	Lung Kidney		Cardiac arrest
1 month	EL0069(M)	1			E. coli (4)	Tongue		Not Confirmed
3 months	EL0004 (M)	1			NAD	Heart		
1 month	EL0036 (M)	1			Pasteurella (1)	Heart		Cardiac arrest
6 months	EL0026(M) EL0041(M) EL0076('F) EL0078(F) EL0065(F) EL0039(M)	6	Consolidated lung, cyst in lung, Congested liver and pin point haemorages in intestine	The lung showed lesions of interstitial pneumonia with infiltration of mononuclear cells and congestion of alveolar capillaries, alveoli had undergone emphysematous changes. The liver showed congestion of central vein and sinusoidal spaces. The villi showed necrotic changes, haemorrhage in the submucosa and hypertrophy of the goblet cells, infiltration of neutrophils, mononuclear cells.	Staphylococcus (3) E coli (1)	Heart, Liver		Pneumonia
1 year	EL0076(F)	1	Intestinal suppurative nodular lesion	The villi showed focal multiple suppurative lesions characterized by central areas of caseous necrosis with degenerated neutrophils and mononuclear cells.	Candidiasis (1)	Heart		Not Confirmed
4 years	EL0067(F)	1	Haemorrhagic gastritis and haemorrhagic enteritis	Desquamation and focal areas of necrosis in the mucosa, haemorrhage in the submucosa were noticed in stomach as well as in the intestine. The villi structures in small intestine showed necrotic changes with aggregation of cellular debris.	*S. typhimurium* (1)	Tongue, Liver	Rotavirus (1)	Gastro-enteritis
7 years	1EL0068(F)	2	Involved muscles were of the neck, fore legs and back.	Haemorrhages and necrosis in muscle along with vacuolation and aggregation of mononuclear cells, loss of myofibrils.	*Cl. septicum* (1)	Heart		Not Confirmed