Long-term, intermittent, low-level elephant endotheliotropic herpesvirus 1a viremia in a captive Asian elephant calf

Kendra L. Bauer,[1] Erin Latimer, Mitch Finnegan

Smithsonian Institution National Zoological Park, Smithsonian Conservation Biology Institute, Wildlife Health Sciences, Washington, DC (Bauer, Latimer); Oregon Zoo, Portland, OR (Finnegan).

[1]Corresponding author: Kendra L. Bauer, Smithsonian Institution National Zoological Park, Smithsonian Conservation Biology Institute, Wildlife Health Sciences, 3001 Connecticut Avenue, Washington, DC 20008. bauer.kendra@gmail.com

Running head: EEHV1A viremia in Asian elephant calf

Abstract. A 2-y-old male Asian elephant (*Elephas maximus*), with an elevated platelet count ($1,100 \times 10^9$/L [$1,100 \times 10^3$/mm^3]), tested positive for elephant endotheliotropic herpesvirus 1A (EEHV-1A) on conventional PCR (cPCR) of EDTA whole blood. No clinical signs were ever reported and no treatment was administered, but low-level viremia persisted for 2.5 y based on results of cPCR and/or real-time PCR (rtPCR). Sequencing confirmed that the EEHV-1A detected was identical at the beginning through the end of the time period. No other elephants in the herd tested positive for EEHV-1 during this time period. Platelet counts remained elevated throughout the viremia and throughout the animal's life, and direct correlation between the elevated platelet counts and EEHV-1A viremia could not be confirmed. We document long-term, intermittent, low-level viremia of EEHV-1A and provide additional information to consider when determining if treatment is warranted in a case of EEHV infection.

Key words: Elephants; *Herpesviridae*; platelet count; PCR; viremia.

Elephant endotheliotropic herpesviruses (EEHVs; type species *Elephantid betaherpesvirus 1*) are implicated in cases of fatal hemorrhagic disease in Asian elephants (*Elephas maximus*) and African elephants (*Loxodonta africana*).[9] EEHVs are members of genus *Proboscivirus* classified as of 2017 in subfamily *Betaherpesvirinae*; there is a proposal to create a new subfamily, *Deltaherpesvirinae*, and reclassify the viruses accordingly.[9] To date, 12 virus subtypes have been identified (EEHV-1A, -1B, -2, -3A, -3B, -4A, -4B, -5A, -5B, -6, -7A, -7B), and deaths have been associated with EEHV-1A, -1B, -2, -3A, -4A, -5A, and -6. Evidence suggests that EEHV-1A, -1B, -4A, -4B, -5A, and -5B are endogenous in Asian elephants worldwide.[9]

In mid-May 2011, a 2-y-old male Asian elephant at a North American zoo was noted to have increased platelets, and a manual platelet count confirmed an elevated value ($1{,}100 \times 10^9$/L; [$1{,}100 \times 10^3$/mm^3]; reference interval: $142–914 \times 10^9$/L).[14] Due to the abnormal platelet count, blood was submitted for EEHV testing. Blood was collected from auricular veins into EDTA anticoagulant tubes, refrigerated, and sent to the testing laboratory on wet ice overnight. Banked recent routine samples from late April–mid-May 2011 stored at –80°C were also submitted. The calf tested positive for EEHV-1A on conventional PCR (cPCR) on both current and retrospective samples, with the earliest positive sample recorded on April 28, 2011. Additional blood samples were collected approximately weekly, banked at –80°C, and sent for EEHV testing approximately once per month during 2011 and periodically during 2012–2013.

At the testing laboratory, 200 µL of whole blood was processed for DNA as described previously and also per the protocol for column prep (Generation capture column kit, cat. 159916, Qiagen, Valencia, CA).[13] Conventional PCR was completed as described previously (Table 1).[8] Real-time PCR (rtPCR) was performed with previously described methods.[13] All DNA sequencing was carried out by direct cycle sequencing on both strands of purified PCR

DNA products from 2 rounds of nested or semi-nested PCR amplification. The correctly sized PCR products were purified after agarose gel electrophoresis (QIAquick gel extraction kit, Qiagen). Sequencing reactions were carried out and analyzed (ABI PRISM BigDye terminator v.3.1 cycle sequencing kit, ABI 310 DNA sequencer, Applied Biosystems, Foster City, CA) or sequenced at Macrogen (Rockville, MD) and Genewiz (South Plainfield, NJ). All DNA sequence editing, analysis, and manipulation was performed using Sequencher (Gene Codes, Ann Arbor, MI) and Clustal-Omega Multiple Sequence Alignment (EMBL-EBI, Wellcome Genome Campus, Hinxton, Cambridgeshire, UK) together with BLASTn nucleotide comparison program (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Thirty-seven blood samples were collected over a period of ~2.5 y, and 22 of the samples were positive for EEHV-1A based on cPCR. Real-time PCR was performed on all of the samples to quantify the level of viremia (Fig. 1). Several genes were sequenced periodically throughout the 2.5 y; a total of 35 sequencing runs were conducted for all of these genes. All sequences were identical at 5 loci tested over the 2.5 y, which is consistent with the low mutation rates of other herpesviruses, calculated to be 1.8×10^{-8} mutations per nucleotide, per genomic replication.[5] However, variation in nucleotides in parts of the genome not included in the 5 loci tested cannot be ruled out.

Complete blood count and serum biochemistry was evaluated in concert with all samples that were tested for EEHV. No abnormalities were detected other than elevated platelet counts, which persisted throughout the period of EEHV-1A viremia and after EEHV-1A was no longer detected (mean: 879×10^9/L [879×10^3/mm^3], range: 339–$2{,}040 \times 10^9$/L [339–2040×10^3/mm^3], $n = 168$). Clinical signs are usually not seen with viremias $<10^4$ virus genomic equivalents (vge)/mL.[12] The highest level seen in this animal was ~2×10^3 vge/mL.

At the time of the positive EEHV-1A result, the elephant herd consisted of 3 adult bulls and 3 adult cows in addition to the male calf. The male calf was born at the same institution where the viremia was detected, and there were no new elephants introduced to the herd or outside contacts from the time of the male calf's birth to the time of EEHV viremia detection. No animals exhibited any clinical signs of disease, and no treatment was administered. Also, no other animals ever tested positive for EEHV-1, including a female calf born in November 2012, during the period of viremia. In addition, no EEHV testing was completed on the male calf for any dates prior to April 28, 2011. As of fall 2018, the elephant in our case is a 9-y-old healthy bull elephant residing at the same zoo with no evidence of EEHV viremia on routine rtPCR testing.

It is not known if the elevated platelet counts in our case were related to the EEHV viremia. Typically, EEHV clinical infections are associated with decreased platelet counts.[7,11] However, there have been reports of platelet count rebounding to higher-than-normal values following resolution of clinical signs associated with EEHV.[2,6] It is possible that a decreased platelet count occurred in this individual at the time of initial infection with EEHV-1A, with a subsequent transient increase to higher-than-normal platelet counts. However, no manual platelet counts were completed at the time of the earliest EEHV viremia detection, and the timing of initial infection with EEHV is unknown. An incidental finding or normal variation in platelet counts for this individual cannot be ruled out, especially given that juvenile animals of other species have been reported to have increased platelet values in comparison to adults.[1,4] Also, elevated platelet counts have been associated with other clinical diseases in elephants including *Mycobacterium tuberculosis* (Harr et al. Clinicopathological findings in *Mycobacterium*

tuberculosis culture-positive elephants (*Elephas maximus*) in comparison to clinically normal elephants. Proc Am Assoc Zoo Vet; Sept 2001; Orlando, FL).

Although there have been previous reports of low-level EEHV-1 shedding in trunk samples,[3] our report documents of long-term, intermittent, low-level EEHV viremia. To date, latent forms of EEHV have not been detected by PCR from whole blood, and the level detected in this male calf is suspected to similarly represent continuous reactivation or persistence rather than latency.[10] As has been recommended previously, treatment decisions for EEHV-positive animals should involve the evaluation of multiple factors including the leukogram, the level of viremia, and the clinical signs of the elephant.[6,7] Platelet counts should additionally be evaluated to continue to add to the knowledge base of this important viral disease.

Acknowledgments

We thank the animal care and veterinary staff at the Oregon Zoo for the care of the elephant in this report.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

References

1. Aroch I, et al. Hematology and serum biochemistry values of trapped, healthy, free-ranging rock hyraxes (*Procavia capensis*) and their association with age, sex, and gestational status. Vet Clin Pathol 2007;36:40–48.

2. Atkins L, et al. 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 2013;44:136–143.

3. Azab W, et al. Subclinical infection of a young captive Asian elephant with elephant endotheliotropic herpesvirus 1. Arch Virol 2017:1–6.

4. Chin SC, et al. Hematologic and serum biochemical parameters of apparently healthy rescued Formosan pangolins (*Manis pentadactyla pentadactyla*). J Zoo Wildl Med 2015;46:68–76.

5. Duffy S, at al. Rates of evolutionary change in viruses: patterns and determinants. Nat Rev Genet 2008;9:267–276.

6. Fuery A, et al. Clinical infection of captive Asian elephants (*Elephas maximus*) with elephant endotheliotropic herpesvirus 4. J Zoo Wildl Med 2016;47:311–318.

7. Fuery A, et al. Clinical infection of two captive Asian elephants (*Elephas maximus*) with elephant endotheliotropic herpesvirus 1B. J Zoo Wildl Med 2016;47:319–324.

8. Latimer E, et al. Detection and evaluation of novel herpesviruses in routine and pathological samples from Asian and African elephants: identification of two new probosciviruses (EEHV5 and EEHV6) and two new gammaherpesviruses (EGHV3B and EGHV5). Vet Microbiol 2011;147:28–41.

9. Long SY, et al. Review of elephant endotheliotropic herpesviruses and acute hemorrhagic disease. Inst Lab Anim Res J 2016;56:283–296.

10. Richman LK, et al. Elephant endotheliotropic herpesviruses EEHV1A, EEHV1B, and EEHV2 from cases of hemorrhagic disease are highly diverged from other mammalian herpesviruses and may form a new subfamily. J Virol 2014;88:13523–13546.

11. Richman LK, et al. Novel endotheliotropic herpesviruses fatal for Asian and African elephants. Science 1999;283:1171–1176.

12. Stanton JJ, et al. Kinetics of viral loads and genotypic analysis of elephant endotheliotropic herpesvirus-1 infection in captive Asian elephants (*Elephas maximus*). J Zoo Wildl Med 2013;44:42–54.

13. Stanton JJ, et al. 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 2010;71:925–933.

14. Teare JA, ed. Elephas_maximus_No_selection_by_gender_All_ages_cmbined_Conventional_American_units_2013_CD.html. In: ISIS Physiological Reference Intervals for Captive Wildlife: A CD-ROM Resource. Bloomington, MN: International Species Information System, 2013.

158 **Table 1.** Primers used for detection and sequencing of elephant endotheliotropic herpesvirus 1A

159 in a juvenile male Asian elephant (*Elephas maximus*).

Name	Gene target	Orientation	Sequence	GenBank accession
L1/R1 = 530 bp; L1/R2 = 250 bp				
6710	pol-pan herpes	L1	ACAAACACGCTGTCRGTRTCYCCRTA	MG763755
6711		R1	GTATTTGATTTYGCNAGYYTGTAYCC	
6712		R2	TGYAAYGCCGTNTAYGGATTYCGGG	
R1/L1 = 910 bp; R1/L2 = 750 bp				
7506	vGPCR1	R1	GATTGTGAACGCTGTATGTC	MG763756
4963B		L1	GACTTTCTTCGTGTAGCCCTCGTCTT	
5200A		L2	CGTGATACGCTTCCAAACATACAGC	
R1/L1 = 340 bp; R1/L2 = 310 bp				
2429	ter	R1	GTGTCGGCTAAATGTTCTTG	MG763757
2430		L1	GTACGTCCTTTCTAGCTCAC	
3024		L2	AATGTGATATCCTACGTATG	
R1/L1 = 685 bp; can repeat for 2nd round				
6742	hel	L1	CACAGMGCGTTGTAGAACC	MG763759
6743		R1	GCAAGGTRGAACGTATCGTCG	
R1/L1 = 730 bp; R2/L1 = 710 bp				
6749	U71/gM	R1	CTATGGGATCCGAACTTTC	MG763758
6750		R2	CTTTCTAAGGGGGTTTGTTGC	
6752		L1	CTACATGCCCATGCAGATAGG	
R1/L1 = 530 bp; R2/L1 = 490 bp				
7445	pol	R1	GATTTTGCGAGYCTGTAYCC	MG763755
7446		L1	CACGCTGTCAGTATCTCCGTA	
7447		R2	CCCAGTATCATTCAAGCATAC	

160

161

Figure 1. PCR of elephant endotheliotropic herpesvirus 1A in a juvenile male Asian elephant (*Elephas maximus*) in 2011–2013. The upper y-axis indicates viral load detected via real-time PCR (rtPCR). The presence of a bar on the lower graph indicates a positive conventional PCR (cPCR) result corresponding to that date.