



NOTE

Wildlife Science

Successful treatment of elephant endotheliotropic herpesvirus infection in an Asian elephant (*Elephas maximus*) calf by oral acyclovir medication: Case report

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ABSTRACT. Elephant endotheliotropic herpesvirus (EEHV) is a major cause of death in Asian elephant (*Elephas maximus*) calves. A 2-year, 11-month-old female, captive Asian elephant presented with facial edema and a mild fever. Blood samples were collected and showed EEHV1A positivity with a high viral load by real time PCR. Heterophil toxicity also was reported for the first time in this case. The calf was treated orally with acyclovir, 45 mg/kg tid for 28 days, which reduced the EEHV1A viral load to undetectable levels within 9 days and the calf survived. A successful outcome with oral acyclovir administration provides another and affordable option to treat EEHV hemorrhagic disease in Asian elephants, and one that is easier to administer in untrained calves.

KEY WORDS: acyclovir, Asian elephant, elephant endotheliotropic herpesvirus (EEHV), heterophil toxicity, oral medication

Elephant endotheliotropic herpes virus (EEHV) is an acute and highly fatal hemorrhagic disease (HD) of young Asian elephant (*Elephas maximus*) calves, 1–8 years of age. It belongs to the genus *Proboscivirus* in the family *Herpesviridae* and consists of several viral subtypes that differ between Asian (EEHV1A, 1B, 4, and 5) and African (*Loxodonta africana*) (EEHV2, 3, 6, and 7) species. EEHV1A is highly pathogenic in Asian elephant calves and responsible for up to 65% of deaths globally [6, 11, 18, 34]. Death frequently occurs within 48 hr after clinical signs are observed. EEHV viremia causes generalized capillary endothelial cell damage leading to hemorrhage in major internal organs and hypovolemic shock [19]. Typical clinical signs of EEHV-HD include lethargy, anorexia, facial edema, oral ulcerations, and a cyanotic tongue. Early diagnosis by polymerase chain reaction (PCR) and administration of antiviral drugs and supportive treatments, including fluids, adjunctive therapies (NSIADs, antibiotics, analgesics) and plasma transfusion are critical to saving the life of an infected animal [3, 6, 11, 20, 26, 27, 33]. Thus far, EEHV-HD survivors have been treated with antiviral drugs [20], including famciclovir [7, 26], ganciclovir [33], and acyclovir [2, 27]. However, the only pharmacokinetic study in elephants has been conducted with famciclovir [4].

Acyclovir is available in both injection and tablet forms, the latter of which is more useful for treating young, as yet untrained, calves. In range countries, acyclovir is an additionally attractive choice for treating EEHV as it is available in local pharmacies and cheaper than other anti-viral drugs. In Thailand, intravenous acyclovir was successful in treating EEHV-HD and reducing blood viral loads in a 3-year-old male calf [27]. Oral acyclovir also has been used to treat several Asian elephant calves in Thailand with successful and unsuccessful outcomes (S.K., personal observation), but there are no pharmacokinetic data on this antiviral drug in elephants and few published reports of its efficacy [2, 27]. This case report describes the successful treatment of a female Asian elephant calf diagnosed with EEHV1A with oral acyclovir in conjunction with the measurement of acyclovir blood concentrations.

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The calf, 2 years and 11 months old and weaned, was housed in a tourist camp in northern Thailand and exhibited slight facial edema and mild fever (37.5°C; normal range 36–37°C), but no other clinical signs. Blood was collected from an ear vein in ethylenediaminetetraacetic acid-coated tubes and submitted to the diagnostic laboratory at the Faculty of Veterinary Medicine, Chiang Mai University to confirm EEHV by real-time polymerase chain reaction (qPCR) analysis [18]. The day of the first positive qPCR test to EEHV1A was designated as Day 0 [29, 31]. Acyclovir 800 mg tablets (HERPENON 800; Polipharm, Samut Prakan, Thailand) were then administered in a banana starting 24 hr later (Day 1, or 3 days after clinical signs), and continued daily for 28 days. Due to the poor bioavailability observed in horse acyclovir pharmacokinetic studies (10–20 mg/kg oral acyclovir had only 1.5–2.8% bioavailability [1, 8]), a dose of 45 mg/kg, tid PO, was calculated taking into consideration the digestive system of the elephant (hindgut fermentation) with interspecies allometric scaling as described by Hunter [13]. Flunixin meglumine (50 mg/ml, 2 mg/kg i.m.) (NIGLUMINE; Calier, Barcelona, Spain) was administered when the calf's temperature exceeded 37°C (Days 4, 9, 12) to reduce fever. Vitamin C 500 mg tablets (ZEE-500; Patar Lab, Pathum Thani, Thailand; 5,000 mg/day) had been given orally to the calf for 2 years prior to EEHV diagnosis and was continued throughout the study. Manual complete blood counts, blood chemistry tests using an Auto Hematology Analyzer (Mindray BC5300; Mindray Medical, Shenzhen, China) and Biochemical Analyzer (Vitalab Flexor XL; Vital Scientific NV., Dieren, The Netherlands), and qPCR assays were conducted every 1–2 days during treatment. On the final day of treatment (Day 28), when plasma acyclovir concentration was assumed to reach steady-state, blood was collected and the acyclovir concentration determined by high-pressure liquid chromatography, mass spectrometry (LC-MS/MS) (API 3200 AB, Sciex, Framingham, MA, USA) at the Pharmacy Service Center, Faculty of Pharmacy, Chiang Mai University as described for horses [1, 8, 32]. Pharmacokinetic data to base the dose and frequency of administration are lacking for antiviral drugs in elephants. Thus, the decision to stop treatment on Day 28 was based on the viral load having been low or undetectable for 7 consecutive days and over concern about possible adverse side effects, like kidney damage, of longer-term administration [12].

The initial EEHV1A load was 2.96×10^4 viral genome copies (vgc)/ml, with a 22.21 cycle threshold (CT), which increased through Day 7 to a peak of 1.15×10^6 vgc/ml and 14.74 CT (Fig. 1), although day-to-day variability was high with no apparent explanation. The viral load was then undetectable on Day 8 and remained at a low level (<1,000 vgc/ml with CT value >30) throughout the remainder of the treatment period. The plasma acyclovir concentration on Day 28 was 3.56 µg/ml, which was assumed to represent a pharmacokinetic steady state based on data in humans. For example, acyclovir concentrations reached a steady state at 4–5 times after repeating doses [10], and exceeded the 50% maximal inhibitory concentration (IC₅₀) during treatment of herpes simplex virus (HSV) HSV-1 (0.02–0.9 µg/ml) and HSV-2 (0.03–2.2 µg/ml) in human *in vitro* studies [24].

Nephrotoxicity, which is a major side effect of acyclovir treatment in humans [16, 23, 25], was not observed in this calf based on blood chemistry analyzes. No discernable changes in blood urea nitrogen (mg/dl) or creatinine (mg/dl) were observed over the 28-day treatment period in our study. All of these parameters were within the normal range [14]. Moreover, long-term use of acyclovir in humans prevented the recurrent of HSV-1 and HSV-2, with no adverse clinical drug reactions noted [9, 21]. Packed cell volume and platelet counts monitored to assess disease progression also fell within normal ranges throughout the evaluation period (Fig. 1). Heterophil left-shifting and toxicity were observed on the day of clinical sign onset and when the viral load was high (Day 0) (Table 1). Heterophils were scored as moderate based on Stacy *et al.* [28], showed a lack of nuclear constrictions and smooth parallel nuclear walls, with a single clear vacuole and presence of moderate cytoplasmic basophilia (Fig. 2). Toxic neutrophils and band cells also have been reported in association with systemic inflammatory responses in horses [17]. Thus, scoring of heterophil toxicity was useful for evaluating the progression of EEHV and treatment efficacy in this calf.

Administration of 5,000 mg Vitamin C both prior to and during treatment may have increased the calf's immune response. Vitamin C has been used as an immunomodulatory agent [22] in treatment of a number of viruses, including poliovirus, Venezuelan equine encephalitis, human lymphotropic virus type 1, human immunodeficiency virus, parvovirus and rabies due to positive effects on leucocytes, lymphocytes, and macrophages. Vitamin C also increases neutrophil phagocytic capacity and oxidative killing, and supports lymphocyte proliferation and function by improving chemotaxis [5]. The fact that this calf had been treated prophylactically with Vitamin C for nearly 2 years prior may have improved its outcome.

This is the first report to confirm oral acyclovir treatment reduced a high EEHV1A viral load to concentrations below the cut-off level by Day 5, although it increased again on Day 7, which may have been related to detection of dead viruses or viral gene fragments [15], and so should be investigated more in the future. The viral load pattern in our study was similar to that of Stanton [30], where clinical signs were in accordance with the pattern reflecting a true infection. In a previous case [27], successful treatment of EEHV1A was achieved using oral acyclovir (24 mg/kg) for 2 days and then intravenous acyclovir (12 mg/kg) twice a day, with supportive antibiotics (penicillin G), vitamin C, and fluid therapy. In that study, the viral load was decreased after 2 weeks of treatment and reached undetectable levels within 1 month. In another report [26], loading doses of oral famciclovir were given to two EEHV positive calves in the first few days of infection, which were then reduced during subsequent days and resulted in recovery 3–4 weeks following. Thus, loading dose regimens for oral acyclovir may be more therapeutic by reaching high circulating drug concentrations more quickly. In cases of well-trained calves, an intravenous route should be considered to reach rapid therapeutic blood concentrations, whereas an oral route may be more appropriate for wild or untrained elephants. However, plasma acyclovir concentrations, an important parameter for determining appropriate dose regimens, were not determined in either of those studies. And although not noted in this study, side effects and toxicity can occur during and after antiviral treatment and should be carefully monitored. The successful outcome in this case undoubtedly was due to early diagnosis and treatment, as that is known to be key to survival in EEHV-HD cases [2, 12, 20, 27], while unsuccessful cases often are related to late diagnosis and treatment even when antiviral drugs are administered.

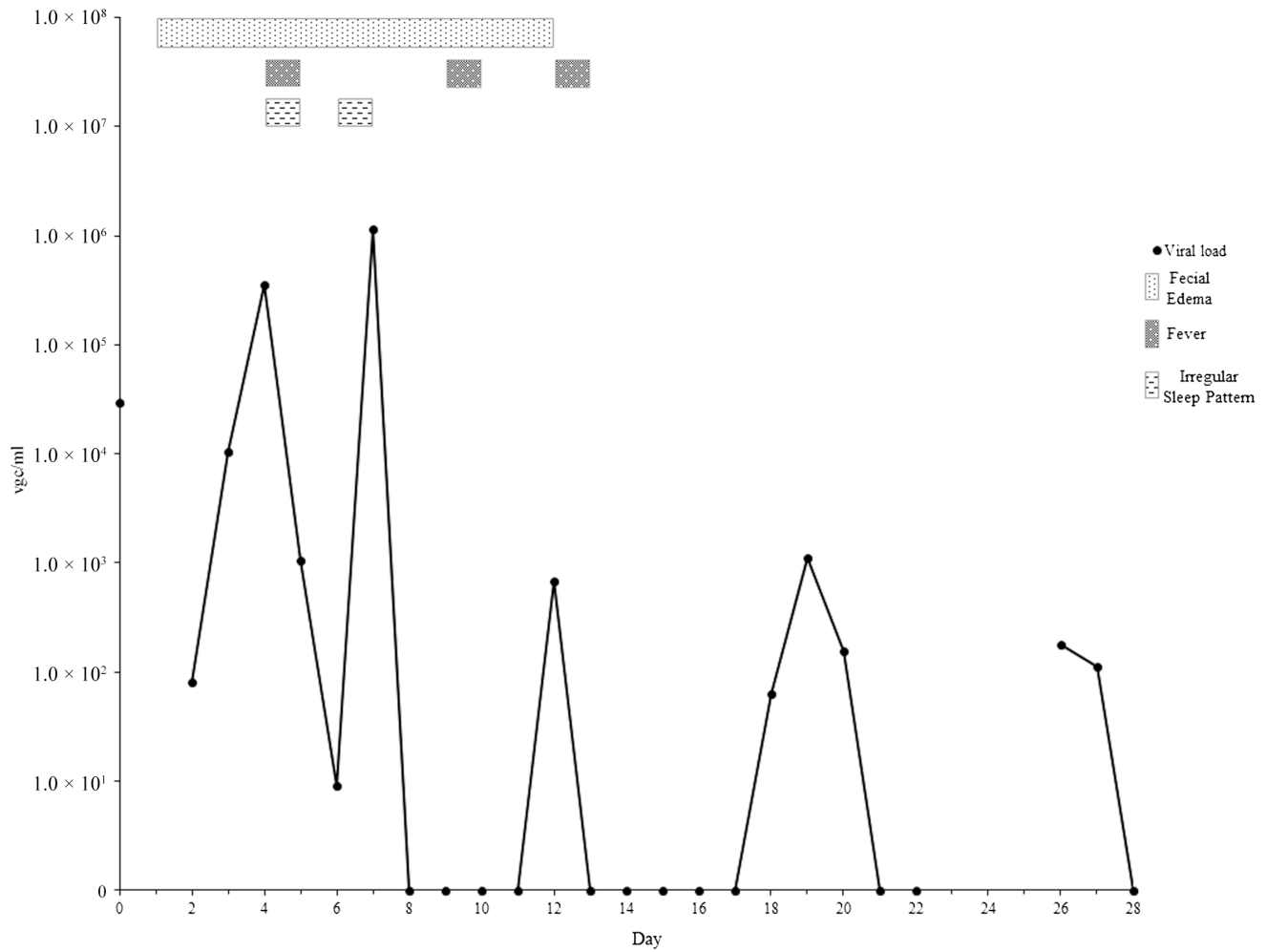


Fig. 1. Viral load of an elephant calf during elephant endotheliotropic herpesvirus1A-hemorrhagic disease (EEHV1A-HD) infection and treatment with oral acyclovir (45 mg/kg/d) for 28 days, Note log scale for viral loads. Undetectable viral load was showed in the dot at the baseline. The clinical signs were showed above the figure.

Table 1. Blood analysis results indicating heterophil left-shifting and toxicity during (Days 1–27) and after (Day 36) treatment

Day	Heterophil left-shifting	Heterophil toxicity
1	√	Mild
7	√	Moderate
12	√	Mild
27	√	Mild
36	-	-

Clinical signs of elephant endotheliotropic herpesvirus (EEHV) were observed 3 days before acyclovir treatment was initiated on Day 1. Heterophil toxicity categorized; Mild toxicity (>3%), Moderate (3–10%), Severe (>10%).

Finally, this was the first report of heterophil toxicity associated with EEHV in Asian elephants, which provided additional data on the health status of this calf. More work is needed to fully characterize the pharmacokinetics of oral acyclovir treatment using frequent blood samples over time, and different treatment dose and frequency regimens to establish effective protocols. Overall results suggest an oral form of acyclovir may be an effective treatment for EEHV, and an alternative to injectable forms to improve calf compliance.

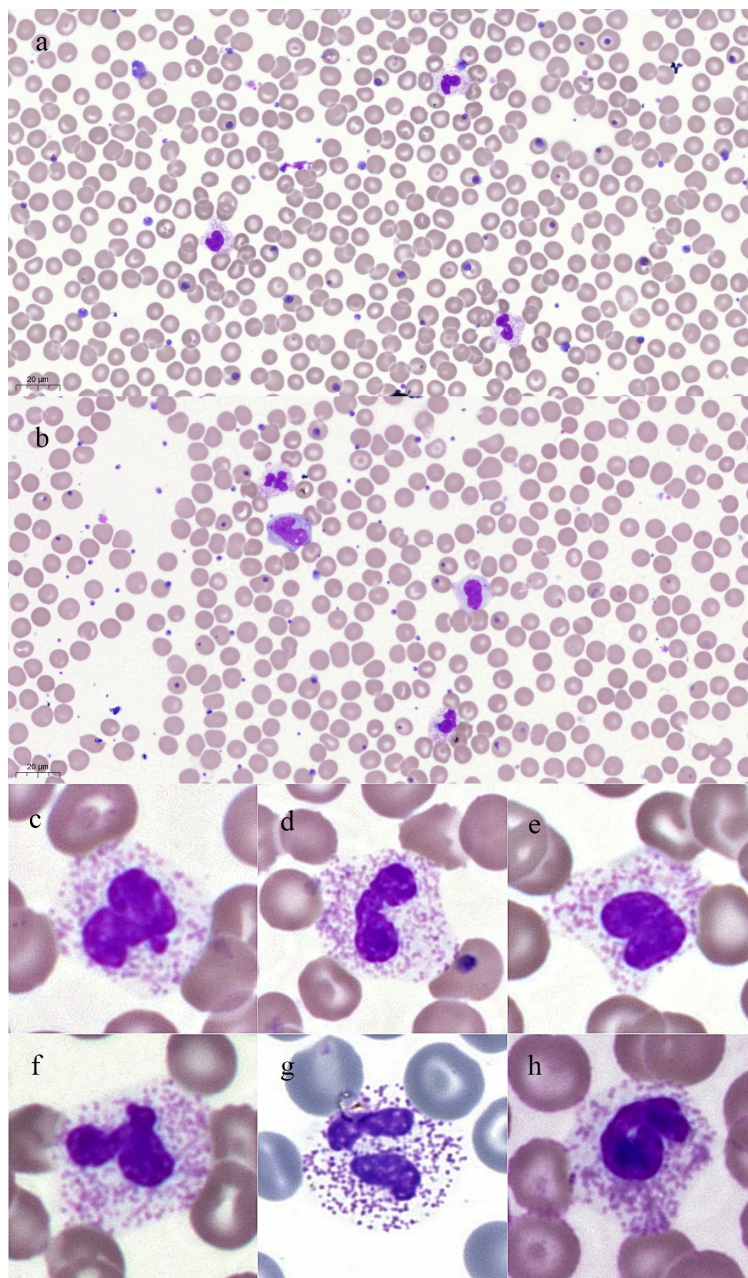


Fig. 2. Heterophil left-shifting and toxicity with a monocyte and lymphocyte in a blood smear collected, $\times 40$ objective (a, b). A band heterophil that lacked nuclear constrictions and smooth parallel nuclear walls (c, d, e) and a heterophil with moderate cytoplasmic basophilia, $\times 100$ objective (f, g, h). Wright Giemsa stain.

CONFLICT OF INTEREST. We confirm there are no conflicts of interest.

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