

THE IMPACT OF ELEPHANT ENDOTHELIO TROPIC HERPESVIRUS ON THE CAPTIVE ASIAN ELEPHANT (*ELEPHAS MAXIMUS*) POPULATION OF THE UNITED KINGDOM AND IRELAND (1995–2013)

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Abstract: Elephant endotheliotropic herpesvirus (EEHV) is one of the most devastating infections and causes of mortality in captive Asian elephant (*Elephas maximus*) populations. Eight confirmed fatal EEHV cases have occurred since 1995 within the captive Asian elephant population of the United Kingdom and Ireland. This report aims to review the impact of EEHV on the captive Asian elephant population in the United Kingdom and Ireland, document and compare fatal cases, and recommend a framework of monitoring within the United Kingdom and Ireland to increase the success of treatment of EEHV hemorrhagic disease (EEHV HD) in the future. Six zoologic institutions (which include zoos, safari parks, and wildlife parks) that currently house or have previously housed a captive Asian elephant group were included in this report. Medical records and postmortem results were collected from four of these institutions for each confirmed fatal case. EEHV HD was found to be responsible for 29.6% of fatalities in Asian elephants born in captivity in the United Kingdom and Ireland between 1995 and 2013. Following a review of all the cases, it is shown that although clinical signs may be associated with specific EEHV species, the swiftness of disease progression means that most body tissues are impacted 1–6 days following the presentation of visible clinical signs and treatment is less likely to succeed. Therefore, EEHV monitoring should consist of conducting regular polymerase chain reaction analysis of whole blood samples from at-risk, young Asian elephants aged 1–8 yr in order for subclinical viremia to be identified early and treatment to be started before the appearance of visible clinical signs.



Key words: Case studies, EEHV, elephant calves, health monitoring, hemorrhagic disease.

INTRODUCTION

One of the most significant causes of captive Asian elephant (*Elephas maximus*) mortality is elephant endotheliotropic herpesvirus (EEHV).⁴ The virus is named for its specific targeting of the endothelial cell lining of capillaries, resulting in vascular dysfunction and hemorrhaging.¹⁴ The first identified case for the disease in elephants occurred in 1995 in North America.¹³ Since then, over 80 cases of EEHV-associated disease have been identified in North America, Europe, and Asia.^{7,18} Although EEHV is also detected in African elephants, it is found to be far less virulent than in the Asian species.^{13,18}

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There are seven distinct species of EEHV (belonging to the *Proboscivirus* genus), three of which are associated primarily with Asian elephants (EEHV1, EEHV4, and EEHV5),^{1,5,7,14,15,17} Pathogenicity varies, with EEHV1 (which has two subtypes: EEHV1A and EEHV1B) being responsible for 90% of EEHV-associated fatalities in Asian elephants.¹⁹ Approximately 85% of the time, the onset of clinical signs culminates in fatality after a very short observable clinical disease course of 1–7 days; death is due to vascular damage inducing organ failure.^{7,17}

Although viral transmission is still not fully understood, it is clear that young Asian calves between 1 and 8 yr old are most susceptible to fatal EEHV hemorrhagic disease (EEHV HD).¹⁴ The majority of adult Asian elephants in captive groups worldwide appear to carry latent EEHV infections (most commonly EEHV1) with occasional asymptomatic reactivation and shedding.^{14,17} It is theorized that intimate contact between an asymptomatic adult shedding the virus and a naïve juvenile or immunocompromised group mate could lead to the transmission of a possibly fatal primary infection.⁶ Stress, caused by various psychological or physiological

pressures, could be a factor that increases the risk of developing EEHV HD in elephants by compromising their immune systems; this theory may be relevant for fatal EEHV infections.¹⁴

The majority of published reports on EEHV cases and EEHV-related research originate from North America, mainland Europe, and Asia.^{1,3,4,6-8,11-15,17} Although the captive Asian elephant population in the United Kingdom and Ireland is relatively small, a number of fatal EEHV cases have occurred in the past decade, and this is of importance to the field. The purpose of this report is to characterize EEHV cases in the United Kingdom and Ireland, specifically aiming to 1) summarize the impact of EEHV in the United Kingdom and Ireland between 1995 and 2013, 2) document and compare the confirmed EEHV cases that have occurred in the United Kingdom and Ireland between 1995 and 2013, and 3) provide a recommendation for an EEHV monitoring strategy to be used in captivity in the United Kingdom and Ireland. These data will enhance the contribution of the United Kingdom and Ireland to the EEHV field and hopefully increase the success of treatment of EEHV HD in these countries in the future.

MATERIALS AND METHODS

The impact of EEHV on the population

The European Association of Zoos and Aquariums European Endangered Species Programme studbook for Asian elephants (R. Belterman, unpubl. data) current to November 2008 was used to determine population statistics for the captive Asian elephant population of the United Kingdom and Ireland in order to assess the impact of EEHV. Supplemental information for studbook data post-November 2008 was accessed from zoo records encompassing the entirety of the captive Asian elephant population of the United Kingdom and Ireland. Studbook data and zoo records used for analysis in this report included the period 1 January 1995 through 31 December 2013. This timeline was chosen based on the initial report of the EEHV index case in North America, which occurred in 1995.¹³

Case studies

Zoologic institutions within the United Kingdom and Ireland that currently house or have previously housed Asian elephants since 1995 were approached with a request for data. Although EEHV-associated disease cases and latent infections did precede 1995, when the index case

was first documented,^{9,10,13} knowledge of EEHV was limited before this time, which is why the case studies analyzed in this report are subsequent to 1995. Zoologic institutions were approached based on the following inclusion criteria: 1) a zoologic institution could include a zoo, wildlife park, or safari park, but not a circus or private collection; and 2) the collection must currently or have previously held a breeding group consisting of three or more elephants (because of the predilection of EEHV-associated disease for young calves and a lack of evidence within the literature that fatal EEHV cases occur in singularly held adults). These criteria resulted in the inclusion of six collections. Of these six, four institutions with a history of EEHV fatalities were approached for data regarding EEHV case studies. The remaining two institutions had no history of EEHV fatalities and were not included in the study. To ensure confidentiality, collections are numbered rather than named and EEHV cases are listed alphanumerically, beginning with the collection number that the deceased elephant belonged to, followed by a letter based on chronologic order of case occurrence.

Clinical and pathology records were obtained for eight deceased Asian elephants that had been infected with EEHV. The records were obtained through communication with researchers and/or veterinarians associated with each of the four collections and included personal communication as well as assessment of zoo records. The respective research and/or veterinary departments at each collection that provided data reviewed and approved the objectives described in this report.

Data points used for comparison between case studies included aspects of the following: 1) patient signalment, 2) group composition and contact management type during the elephant's lifetime, 3) possible stressors that may have increased the risk of a fatal EEHV infection, 4) the date of death, 5) the EEHV species (and subtype, where applicable) responsible for the fatal infection, 6) clinical signs presented during the disease course, 7) treatments used during the disease course, 8) gross pathology results upon postmortem examination, and 9) histologic results upon postmortem examination. These data points can be seen in Tables 1 and 2 and Figure 1.

Summarized data points were labeled as *predictors* if they included management practices, the presence of concurrent EEHV HD cases within the collection, and stressors, including previous illnesses and injuries. Management practices

Table 1. Case descriptions for the fatal elephant endotheliotropic herpesvirus (EEHV) cases that have occurred within the captive Asian elephant (*Elephas maximus*) population of the United Kingdom and Ireland between 1995 and 2013, including signalment, herd composition during the elephant's lifetime, contact type, possible stressors that may have attributed to an increased risk of EEHV infection, the date of death, and the EEHV species responsible.^a

Case No.	Signalment	Age at death	Weight at death	Herd mates	Contact type in breeding herd	Possible stressors	Date of Death	EEHV Species
1A	Captive-born female	2 yr 9 mo	835 kg	Adults: 1 bull, 6 cows (1 euthanized 10 mo before 1A death) Calves: 1 male Adults: 1 bull, 5 cows Calves: 1 male, 1 female	FC	From birth: poor nutrition and growth due to suckling issues	17 Dec 2006	EEHV1B
1B	Captive-born male	1 yr 3.5 mo	500 kg	Adults: 1 bull, 5 cows Calves: 1 male, 1 female	FC	From birth: umbilical hernia	02 May 2009	EEHV1A
1C	Captive-born female	2 yr 4 mo	833 kg	Adults: 1 bull, 5 cows Calves: 2 males (1 died 15 days before 1C [case 1B])	FC	18 days old: hind leg stepped on by adult 11.5 mo old: ulcer near rectum 22 mo old: fluid-filled pocket near rectum	17 May 2009	EEHV1A
2A	Captive-born male	2 yr 8 mo	975 kg	Adults: 1 bull, 6 cows Calves: 1 male, 1 female	PC	14 mo old: traumatic injury delivered by aggressive adult cow, treated with NSAIDs, healed quickly 23 mo old: lame right hind limb from traumatic injury, treated with NSAIDs, recovered	23 Jul 2009	EEHV1A
2B	Captive-born female	2 yr 5 mo	910 kg	Adults: 2 bulls (present at different times), 5 cows Calves: 2 males, 1 female	PC	7 mo old: two red, 1-mm elevated spots on hard palate, one larger spot with a centralized ulcer on right hard palate, and one 1-mm spot further back; treated with famciclovir for 7 days (suspected EEHV case), no other symptoms, resolved 11 mo old: ulcer on right side of mouth, no other symptoms, untreated 15.5 mo old: lame in hind legs, no other symptoms, given NSAIDs, resolved	03 Jul 2013	EEHV1A

Table 1. Continued.

Case No.	Signalment	Age at death	Weight at death	Herd mates	Contact type in breeding herd	Possible stressors	Date of Death	EEHV Species
2C	Captive-born male	3 yr	1,092 kg	Adults: 2 bulls (present at different times), 5 cows Calves: 1 male, 2 females (1 died 26 days before 2C [case 2B])	PC	13 mo old: ulcers in oral cavity and increased temperature; treated with famciclovir for 10 days (suspected EEHV case), resolved; samples sent to AHVLA: EEHV1 negative 17 mo old: 0.5–1-cm ulcer in center of hard palate, no other symptoms, resolved 17.5 mo old: toxicosis in oral cavity from toxic plant; treated with antibacterial, anti-inflammatory, and NSAIDs, resolved 19.5 mo old: onset of partial anorexia, lethargy, stiffness, dehydration, and elevated temperature (suspected EEHV infection); treated with famciclovir for 2 days, NSAIDs, and antibiotics, resolved 22 mo old: onset of lameness in left forelimb and localized swelling in left elbow due to injury from adult; treatment with NSAIDs 2 yr old: small, localized swelling on ventral abdomen, resolved	29 Jul 2013	EEHV1A
3A	Captive-born male (via AI)	1 yr 8 mo	N/A	Adults: 4 cows	PC	16 mo old: lesions on tongue 19 mo old: lesions on tail base (tested negative for EEHV1, healed)	13 Apr 2011	EEHV5

Table 1. Continued.

Case No.	Signalment	Age at death	Weight at death	Herd mates	Contact type in breeding herd	Possible stressors	Date of Death	EEHV Species
6A	Captive-born male	3 yr 1.5 mo	N/A	Adults: 2 bulls (1 transferred out of collection 2 yr 2.5 mo after 6A was born), 9 cows (3 transferred out of collection 4.5 mo after 6A was born) Calves: 2 males transferred out of collection 4.5 mo after 6A was born, 1 female	N/A	2 yr 4 mo old: fractured left tusk exposing nervous pulp, treated with NSAID, became infected, washed out and surgically cleaned, remained prone to infection several weeks later 2 yr 7.5 mo old: sedated for treatment of devitalized right ear (due to i.v. administration of NSAIDs during surgery), required washing and antibiotic treatment for over 1 mo 2 yr 8.5 mo old: loose feces for several weeks, given probiotic, mostly resolved 2 yr 10 mo old: structure of herd changed daily, causing relationship strains	12 Aug 2005	Unknown

^a FC indicates free contact (keepers have barrier-free contact with the elephants of a captive herd); PC, protected contact (keepers work with the elephants of a captive herd through a secure barrier); NSAID, nonsteroidal anti-inflammatory drug; AHVLA, Animal Health and Veterinary Laboratories Agency, Surrey, United Kingdom; and AI, artificial insemination.

Table 2. Description of the clinical signs present and treatments used during the observable course of disease in the fatal elephant endotheliotropic herpesvirus cases that have occurred in the captive Asian elephant (*Elephas maximus*) population of the United Kingdom and Ireland between 1995 and 2013.^a

Case No.	Disease course	Clinical signs	Treatment	
			Antiviral	Other
1A	Day 1	Morning: lethargic, some hyperemic spots on vulva mucosa, no ulcers Evening: purple vascular structures on lateral tongue tip		Antibiotic (amoxicillin) and probiotic
	Day 2	Morning: depressed, increased petechiae on edges of tongue Afternoon: more depressed, tongue darker over larger area Evening: very depressed, excessive salivation, increased petechiae and cyanosis on tongue, head pressing against bars		i.v. fluids, edema treatment (furosemide), NSAID (carprofen), and antibiotic (enrofloxacin)
	Day 3	Morning: collapsed into right lateral recumbency but got up again, tongue extremely cyanotic, immense salivation, greatly increased heart rate, mild ventral edema developed Afternoon: fell into right lateral recumbency and could not get back up, mild ataxia Evening: began mouth breathing on inhalation (suspected edema of pharyngeal tissues), stood once more but condition continued to deteriorate Death: unassisted, 2.5 days following onset of clinical signs	Famciclovir orally (13 mg/kg twice) Veterinary antiviral therapy (ω interferon) (unknown amount)	Intranasal oxygen, i.v. fluids, edema treatment (furosemide), and morphine
1B	Day 1	Morning: acting slightly off, purple tongue developed Afternoon: tongue slightly darker	Famciclovir rectally (12 mg/kg 3 times)	Antibiotic (marbofloxacin) and NSAID (meloxicam)
	Day 2	Morning: tongue appeared to improve but then worsened with presence of petechiae Afternoon: tongue darker purple, edema developing above both eyes	Famciclovir rectally (12 mg/kg twice)	Antibiotic (marbofloxacin), edema treatment (furosemide), and NSAID (metacam). In the afternoon, sedated, given analgesic (butorphanol) and plasma i.v.
	Day 3	Morning: more edematous around eyes, tongue darker at tip, collapsed suddenly and went into cardiac and respiratory arrest Death: unassisted, 2 days following onset of clinical signs		

Table 2. Continued.

Case No.	Disease course	Clinical signs	Treatment	
			Antiviral	Other
1C	Day 1	Morning: acting slightly off, stood hunched, tip of tongue slightly purple Afternoon: tongue more purple with cyanotic tip, dry mouth and tongue, behavior and eating habits still normal Evening: tongue worse with demarcation line on each side and more cyanotic tip, but still drinking and eating	Famciclovir orally (12 mg/kg twice) Ganciclovir i.v. (5 mg/kg once)	NSAID (metacam) and antibiotic (marbofloxacin)
	Day 2	Afternoon: tongue more purple, edema above and around eyes Evening: began head pressing, edema worsened substantially, mouth breathing, very wobbly on feet	Ganciclovir i.v. (4 mg/kg once) Famciclovir orally (4 mg/kg once, 12 mg/kg twice)	10 L fluid per rectum, NSAID (Metacam), antibiotic (marbofloxacin), and morphine
	Day 3	Morning: tongue severely purple, very wobbly, mouth breathing followed by shallow, abnormal breathing, twitching legs, discomfort in weight bearing Death: euthanized, 2 days following onset of clinical signs	Famciclovir orally (12 mg/kg once)	5 L fluid per rectum, morphine, and 15 L nasal oxygen
2A	Day 1	Morning: lethargic, lame left foreleg, stiff, temperature normal, partial anorexia, tongue slightly paler than normal, 1 focal ulcer (5 × 7-mm diameter) on hard palate, more lethargic and not responding to keeper commands later in morning Afternoon: some improvement in stiffness and responsiveness after treatment		NSAID (Finadyne)
	Day 2	Morning: no change		NSAID (Finadyne)
	Day 3	Death: sudden death, 2 days following onset of clinical signs		
2B	Day 1	Afternoon: acting slightly off		
	Day 2	Morning: depressed, lethargic, tongue discolored Afternoon: temperature higher than normal, 2 areas of discoloration on tongue, slight swelling of foreleg joints, briefly straining and gasping, dry feces; later in afternoon more depressed, anorexic, lying down often Evening: leaning on fence line, motionless, eyes closed, very depressed	Famciclovir orally (15 mg/kg once)	Analgesic (suxibuzone) and NSAID (Finadyne)
	Day 3	Death: found dead, 1.5 days following onset of clinical signs		

Table 2. Continued.

Case No.	Disease course	Clinical signs	Treatment	
			Antiviral	Other
2C	Day 1	Morning: decreased alertness, higher temperature than normal throughout week, no lesions in oral cavity, no swelling of joints, not as interested in food	Famciclovir orally (unknown amount, refused further attempts)	Antibiotic (trimethoprim)
	Day 2	Morning: no change and then possible improvement, not depressed or anorexic		
	Day 3	Morning: very high temperature, lethargic, reluctant to eat		Antibiotic (amoxicillin)
	Day 4	Morning: lying down, cannot stand for long periods of time, edema of face and head Death: collapsed suddenly and died several minutes later, 3 days following onset of clinical signs		
3A	Day 1	Initially lethargic and slow to eat	Veterinary antiviral therapy (interferon) (unknown amount)	Antibacterial (Trimediazine)
	Day 2	Anorexia		
	Day 6	Edema of head		
	Day 7	Death: euthanized, 6 days following onset of clinical signs		
6A	Day 1	Not quite himself, feces normal but appetite suppressed		
	Day 2	Death: found dead in paddock, 1 day following onset of clinical signs		

^a NSAID indicates nonsteroidal anti-inflammatory drug.

^b Drainage of a pericardial effusion under ultrasonographic guidance.

include free contact (barrier-free access to the elephants) and protected contact (working with the elephants through a secure barrier). Summarized data points were labeled as *observations* if they included a symptom that was consistently reported over all case studies, including tongue discoloration, facial edema, lethargy, appetite suppression, gasping, and hemorrhagic conjunc-

tiva. Oral ulcers and raised body temperatures were not included in the analyses, as they were not consistently documented in all case studies. Two k-means cluster analyses were conducted in Matlab (MATLAB and Statistics Toolbox Release 2010a, The MathWorks, Inc., Natick, Massachusetts 00000, United States) using the summarized data points coded into integers from the case

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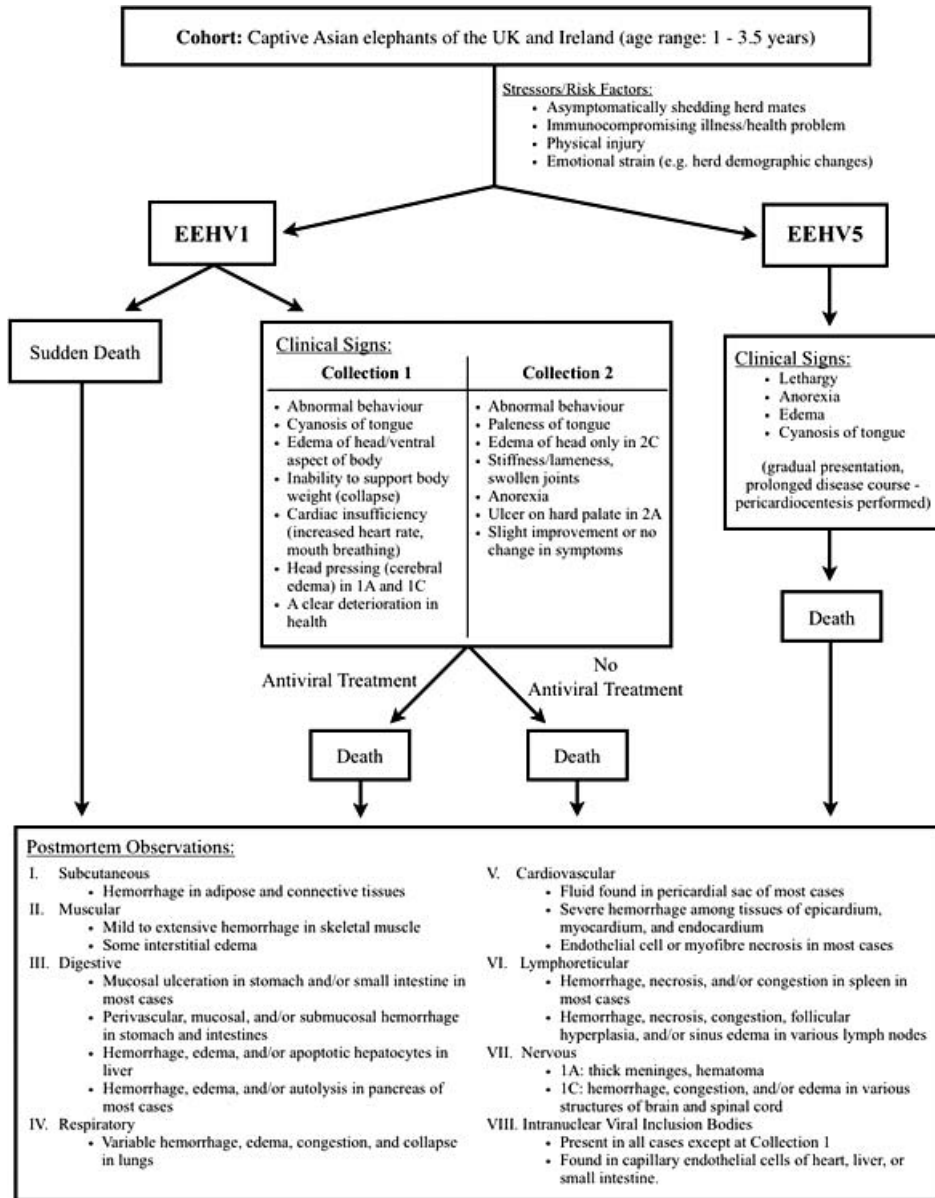


Figure 1. A comprehensive clinicopathologic comparison between the fatal elephant endotheliotropic herpesvirus cases that have occurred within the captive Asian elephant (*Elephas maximus*) population of the United Kingdom and Ireland (1995–2013).

studies (Table 3). The predictor and observation data was partitioned into three defined groups using k-means distance measures.

RESULTS

The impact of EEHV on the population

During the period 1995–2013, a total of 105 Asian elephants (30 male, 73 female, and two of unreported sex) lived within the captive popula-

tion of the United Kingdom and Ireland. All were housed in and sometimes transferred between 13 zoologic institutions (which include zoos, wildlife parks, and safari parks). During this 18-yr period, 37 elephants (20 male, 15 female, and two of unreported sex) were born. Twenty (54.0%) of the Asian elephants born in captivity died (11 males [55.0%], seven females [35.0%], and two of unreported sex [10.0%]). EEHV was the cause

Table 3. A k-means cluster analysis of the fatal elephant endotheliotropic herpesvirus (EEHV) cases that have occurred within the captive Asian elephant (*Elephas maximus*) population of the United Kingdom and Ireland between 1995 and 2013. The numbered groupings indicate the three clusters formulated from a k-means cluster analysis, based on predictors and observations.^a

Case no.	EEHV strain	Contact type	Predictors				Observations						
			Stressors	Other cases present	Predictor grouping	Tongue discoloration	Facial edema	Lethargy	Appetite suppression	Gasping	Hemorrhagic conjunctiva	Observation grouping	
1A	1B	FC	Difficulty from birth	No	1	Strongly present	Present	Present	Present	Present	Present	Present	1
1B	1A	FC	Difficulty from birth	No	2	Strongly present	Absent	Present	Absent	Absent	Absent	Absent	2
1C	1A	FC	Early injury (<3 wk)	Yes (1B)	2	Strongly present	Absent	Absent	Absent	Present	Absent	Absent	2
2A	1A	PC	Early injury (<3 wk)	No	2	Strongly present	Absent	Present	Present	—	Absent	Absent	2
2B	1A	PC	Late showing ulcers	No	1	Strongly present	Absent	Present	Present	Present	Present	Present	2
2C	1A	PC	Late injury	Yes (1C)	1	Present	Present	Present	Present	Absent	Absent	Absent	1
3A	5	PC	Late showing ulcers	No	1	Present	Present	Present	Present	Present	Present	Absent	1
6A	—	—	Late injury	No	3	—	Present	Present	Present	Present	—	Absent	3

^a FC indicates free contact (keepers have barrier-free contact with the elephants of a captive herd); PC, protected contact (keepers work with the elephants of a captive herd through a secure barrier).

(as confirmed by diagnostic testing and/or consistent pathologic findings) of eight (40.0%) of these deaths, stillbirths were responsible for another eight deaths (40.0%), two (10.0%) were due to infanticide, and two (10.0%) were of unknown causes.

Overall, EEHV HD has been responsible for the fatalities of 21.6% of all Asian elephants born in captivity in the United Kingdom and Ireland between 1995 and 2013. However, there has never been a confirmed EEHV fatality in an elephant less than 1 yr of age (L. Howard, pers. comm.). Therefore, EEHV is the single greatest cause of death (29.6% of fatalities) of Asian elephants born in the United Kingdom and Ireland between 1995 and 2013 that lived to be 1 yr or older.

Case studies

All case study data have been collated into Tables 1 and 2, and are summarized in Figure 1. The case studies involved five captive-born male Asian elephants and three captive-born female Asian elephants between the ages of 15.5 and 37.5 mo (Table 1). Within the cohort, EEHV1 was responsible for six fatalities (five due to EEHV1A and one due to EEHV1B), EEHV5 was responsible for one fatality, and no diagnostic testing data were available for one fatality. Observable clinical disease courses within the cohort averaged 2.5 days, with EEHV1 infection leading to disease courses of 1.5–3 days, EEHV5 infection leading to a disease course of 6 days, and the unknown EEHV species infection leading to a disease course of 1 day.

Figure 1 provides a comprehensive clinicopathologic comparison between the disease courses of all case studies presented in this report. Although each case began with similar clinical signs of lethargy and/or abnormal behavior, the disease courses proceeded very differently between cases infected with different EEHV species (EEHV1 versus EEHV5) (Table 2). Polymerase chain reaction (PCR) testing was used to confirm the EEHV species responsible in all cases except case 6A. Certain data points in the records available for case 6A were missing, including the EEHV species responsible for the fatal infection; therefore, case 6A can be commented on but cannot be extensively compared to the other case studies. Quantitative PCR was not carried out on any of the cases.

Six of the eight case studies (cases 1A, 1B, 1C, 2B, 2C, and 3A) received antiviral treatment with famciclovir. Some also received additional antiviral treatment with ganciclovir (case 1C) or

veterinary antiviral interferon (cases 1A and 3A). Treatment and/or supportive therapy (including analgesics, i.v. and rectal fluid therapy, treatment of edema with diuretics [e.g., furosemide], intranasal oxygen therapy, antibacterials, antibiotics, and a pericardiocentesis in the case of 3A) were not successful in altering the fatal outcome of these cases.

The results of the k-means cluster analysis are shown in Table 3. Although predictors (contact management type, stressors, and the presence of concurrent cases) do not appear to predict the EEHV species involved or differences among the collections, the observations (clinical signs of tongue discoloration, facial edema, lethargy, appetite suppression, gasping, and hemorrhagic conjunctiva) do appear to differentiate between EEHV species. For example, Table 3 shows that one k-means grouping based on observation data (observation grouping 2) collates four-fifths of the EEHV1A cases together, and is mainly indicated by a strong tongue discoloration, without facial edema or hemorrhagic conjunctiva. Case 2C is not included in this grouping, despite being EEHV1A, because of the presence of facial edema.



DISCUSSION

The results show that EEHV HD was responsible for 29.6% of fatalities in Asian elephants born in captivity in the United Kingdom and Ireland between 1995 and 2013 that lived to be 1 yr or older; this makes EEHV the single greatest cause of fatality in young Asian elephants born during this time. In this discussion it is suggested that 1) stressors in captive groups may lead to an increased risk of exposure and susceptibility of young calves to viral loads that could lead to possibly fatal primary infections; 2) asymptotically shedding adults within a collection could account for the presence of a particular EEHV species affecting the juveniles they are housed with; 3) variation might exist between the disease courses of different EEHV species; and 4) in the confirmed fatal EEHV cases identified in this study, administration of famciclovir was likely instituted too late (following the onset of visible clinical signs) to have an effect on the outcome.

Figure 1 provides a comprehensive summary of the findings in the case studies. It is used as a framework to discuss the effect of stressors and the manifestation of EEHV in clinical signs and postmortem results in order to make objective recommendations for future management and treatment of EEHV.

Possible stressors

Stressors received by the elephants in early life, prior to the appearance of visible clinical signs associated with EEHV, are thought to increase the risk of fatal EEHV HD.¹⁴ Indeed, factors that cause acute or prolonged stress in young calves may have increased the risk of EEHV HD in the case studies described in this report. Five out of eight (1A, 1B, 1C, 2A, and 6A) of the fatal cases presented here received injuries or complications prior to, and independent of, clinical signs (Table 1); the disease tended to progress quicker in these cases (1–2.5 days) than in those without early injuries or complications (1.5–6 days) (Table 2). Three cases presented EEHV-like symptoms previously without succumbing to fatal disease (1C, 2B, and 2C) (Table 1). A cluster analysis, incorporating early stressors, the presence of other cases in the herd, and management type, could not associate these predictors with specific EEHV species (Table 3).

Early stressors are variable and hard to quantify; specific examples of stressors affecting these calves include suckling issues, an umbilical hernia, accidental injury, a fractured tusk, and changes in group demographics due to translocations. It is important to keep in mind that some stressors may have affected the entire group of elephants, leading to a general increase in adult shedding and thus an increase in exposure of the calves to higher levels of the virus. Although early stressors are prevalent in fatal EEHV cases, further work is needed to determine if stressors such as those mentioned above truly lead to an increase in the risk of developing fatal EEHV HD. Comparing the husbandry practices between collections with and without EEHV HD fatalities might improve understanding of possible stressors; however, this would require data from a larger sample size, and was beyond the scope of this initial study.

Clinical signs and treatments

The clinical signs and postmortem results presented in each EEHV1 case in this report are very similar to those of previous EEHV1 cases reported in North America and Europe.^{10,12,13} The EEHV1 cases proceeded with similar clinical signs, and no major differences were found in clinical presentations between the EEHV1B case and the EEHV1A cases. Comparisons between the case studies did reveal a major difference in the length of the observed clinical disease course between the EEHV1 cases and the case affected

by EEHV5 (Table 2). This may have been due to the pericardiocentesis procedure performed on case 3A, prolonging cardiovascular function. Unfortunately the small sample size in this report prevents further determination if there is a true difference in the disease course of different EEHV species, but previous research in North America did have similar findings.¹

Evidence from PCR diagnostic testing suggests that a genetically identical EEHV1A strain was responsible for two fatalities at collection 2 (cases 2A and 2B). Cases 1B and 1C died within 15 days of one another, and PCR diagnostic testing has revealed that some adult cows housed at collection 1 were confirmed or suspected asymptomatic EEHV shedders (Table 1).⁵ Therefore, it is theorized that an identical or closely related strain different from that at collection 2 was similarly responsible for the fatal cases at collection 1. Evidence from North America has shown that a fatal EEHV1A case was caused by an identical strain to that detected in an asymptotically shedding adult within the same group.¹⁷ Regarding the case studies in this report, an identical strain found between elephants housed at the same collection could similarly indicate that the infection was transmitted from a latently infected adult in the group.

Although successful treatment with famciclovir has been noted in EEHV cases occurring in North America and Europe,¹²⁻¹⁴ it was not successful in altering the outcome of the cases presented in this report. Antiviral treatment was given to cases 1B, 1C, and 2C (using the recommended dosage of famciclovir [8–15 mg/kg orally or rectally every 8 hr]² for cases 1B and 1C); however, little difference existed between the subsequent disease course and outcome of these cases and cases that did not receive any antiviral treatment. An improvement after one dose of famciclovir treatment was noted in case 2C, but further famciclovir doses were refused and clinical signs progressed. In these cases, antiviral treatment was started only after the onset of visible clinical signs. Administration of antiviral and supportive therapy before the onset of clinical illness, when subclinical viremia is detected, is more likely to result in successful treatment of an elephant ill from EEHV HD (L. Howard, pers. comm.). Supportive therapy and other treatment modalities (including rectal fluid therapy, i.v. fluid therapy and conspecific plasma administration, treatment of edema with diuretics, analgesics, antibiotics, intranasal oxygen therapy, antibacterials, and, in the case of 3A, a pericardiocentesis),

were used in all cases except case 6A, but did not alter the outcome of any case. To date, there are no confirmed survivors of EEHV HD within the captive Asian elephant population of the United Kingdom and Ireland.

Although clinical signs show associations with EEHV species in a cluster analysis (Table 3), the swiftness of disease progression following clinical presentation impacts the success of treatment programs. An evaluation of viral loads in clinical and subclinical Asian elephants found that EEHV DNA in the blood is detectable in elephants up to 28 days before the onset of clinical signs of EEHV HD.¹⁶ Therefore, the best way to identify subclinical viremia and begin treatment before clinical signs are evident, is to carry out regular PCR analysis on blood samples of young Asian elephants (between the ages of 1 and 8 yr).

Postmortem results

Although clinical signs manifest in observations such as tongue discoloration, facial edema, lethargy, appetite suppression, gasping, and hemorrhagic conjunctiva (Table 2), postmortem results show that EEHV manifests throughout the body (Figure 1). Among a range of other signs, EEHV can be seen as hemorrhaging of the subcutaneous and muscle tissues, stomach, intestines, pancreas, liver, lungs, cardiovascular system, spleen, lymph nodes, brain, and spinal cord, as well as the presence of edema in muscles, liver, lungs, brain, and spinal cord. Postmortem results show a strong agreement between the EEHV species involved and the cases, although all cases from collection 1 (1A, 1B, and 1C) did not reveal intranuclear viral inclusion bodies in the heart, liver, or small intestine. That EEHV can have such a large effect throughout the body in all cases in only 1–6 days following clinical presentation indicates the importance of identifying the disease subclinically.

Recommendations for a consistent EEHV monitoring strategy

Analysis of studbook data and zoo records provided evidence that juvenile mortality is an issue within the captive Asian elephant population of the United Kingdom and Ireland and EEHV has an impact on this. These findings are indicative of a need for an EEHV monitoring program in order to detect subclinical viremia quickly so that treatment may be started as early as possible and have a higher success rate.

The k-means cluster analysis used in this report showed that clustering of clinical observations fit quite well with EEHV species. These clinical signs (including observations of tongue discoloration, oral ulcers, facial edema, lethargy, appetite suppression, gasping, hemorrhagic conjunctiva, and elevated body temperatures; Table 3) are easy to observe and can be consistently reported if normal ranges and observations for each individual elephant are known. Certain parameters may be more difficult or impossible to obtain if elephants are not trained for examination, so there may be a need for alternative measures if the management system used in a collection is not conducive to these observations. However, the presence of clinical signs should not be used to diagnose EEHV HD, as EEHV viremia develops days to weeks before clinical signs are observed.¹⁶ In addition, EEHV is prevalent throughout the body from the first observation of visible clinical signs, and treatment is negatively impacted when administered late, following the appearance of clinical signs (Table 2). Therefore, weekly whole blood collection for PCR diagnostic testing in Asian elephant calves of at-risk age (1–8 yr old) is of great importance for monitoring and detecting low level viremia before visible clinical signs develop. Early detection would mean an early start to famciclovir treatment, as well as other supportive therapies, including fluid administration (rectal or i.v.), plasma administration (i.v.), nutritional support, and antibiotic therapy for secondary infections; this would provide a much higher probability of success in the treatment of EEHV HD.

It is clear that comparing EEHV HD cases between captive Asian elephant populations located in different countries is necessary to learn more about EEHV HD epidemiology, how treatment protocols compare in their success or failure, and how the distinct EEHV species differ from one another in clinical presentation and pathologic effects. This case study review is the first of its kind in the United Kingdom and Ireland and will hopefully have an impact not only on the captive population within these countries, but also on the EEHV field as a whole.

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