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Review of a Newly Recognized Disease of Elephants Caused by Endotheliotropic Herpesviruses

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There are two newly recognized herpesviruses that cause a fatal disease syndrome in elephants. They are known as the elephant endotheliotropic herpesviruses, of which one is fatal for Asian elephants (*Elephas maximus*) and the other for African elephants (*Loxodonta africana*) [Richman et al., Science 283:1171–1176, 1999a]. The disease syndrome affects predominantly young elephants and has been described in North America [Richman et al., J Wildl Dis 36:1–12, 2000], Europe [Richman et al., Verhandlangsbericht des 39 International Symposium uben Erkrankungen der Zoo und Wildtiere, Wien, 39:17–21, 1999b; Ossent et al., Vet Pathol 27:131–133, 1990], and Israel [Richman et al., Verhandlangsbericht des 39 International Symposium uben Erkrankungen der Zoo und Wildtiere, Wien, 39:17–21, 1999b]. The predominant clinical signs for both species include lethargy, edematous swellings of the head, neck, and thoracic limbs, oral ulceration, cyanosis of the tongue, and death of most elephants in 1–7 days [Richman et al., J Wildl Dis 36:1–12, 2000]. Three affected young Asian elephants recovered after a 3–4-week course of therapy with the anti-herpesvirus drug famciclovir [Richman et al., J Wildl Dis 36:1–12, 2000; Schmitt and Hardy, J Elephant Managers Assoc 9:103–4, 1998]. Additional reported herpesvirus-associated lesions in otherwise healthy elephants include localized skin papillomas in African elephants [Richman et al., Science 283:1171–1176, 1999a; Jacobson et al., J Am Vet Med Assoc 189:1075–8, 1986], proliferative vulval lymphoid patches in African elephants [Richman et al., Science 283:1171–1176, 1999a; Munson et al., J Zoo Wildl Med 26:353–8, 1995], and pulmonary nodules in African elephants [Richman et al., J Wildl Dis 36:1–12, 2000; McCulley et al., Onderstepoort J Vet Res 38:225–236, 1971]. Recent findings suggest that these localized herpesvirus-associated lesions in healthy African elephants may be one source of the herpesvirus that causes disseminated disease and death in the Asian species

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[Richman et al., Science 283:1171–1176, 1999a] and the African species [Richman et al., J Wildl Dis 36:1–12, 2000]. These findings have implications for management practices in facilities keeping both African and Asian elephants and in protecting natural elephant habitats from virulent forms of the virus. Zoo Biol 19:383–392, 2000.

Key words: *Elephas maximus*; famciclovir; *Loxodonta africana*; endotheliotropic virus

INTRODUCTION

Since 1983, there have been at least 18 confirmed deaths or serious illnesses in captive Asian and African elephants located in North America, Europe, and the Middle East associated with newly recognized endotheliotropic herpesviruses. The actual number of cases is likely greater because research breakthroughs [Ossent et al., 1990; Richman et al., 1999a,b], have only recently enabled diagnosticians to identify this newly recognized disease syndrome. Pre-mortem diagnosis of this disseminated herpesvirus disease has been accomplished in four Asian elephants, three of which were treated with a 3–4-week course of an anti-herpesvirus drug, famciclovir, and recovered. In this report, we review a new, fatal endotheliotropic herpesvirus disease of elephants that has been responsible for a significant proportion of perinatal deaths and that may interfere with the successful propagation of elephants for the future.

MATERIALS AND METHODS

Clinical and/or postmortem samples were obtained from 12 elephants (10 Asian, 2 African) from North American zoos, 5 Asian elephants from European zoos, and 1 Asian elephant from an Israeli zoo with endotheliotropic herpesvirus infection.

Clinical and Pathological Evaluations, Recent Cases (1995–1998)

Clinical evaluations usually included physical examinations, collection of one or more blood samples (EDTA and heparinized blood) for hemograms, blood bacterial and viral cultures, and serum for serological testing, vitamin E levels (Dr. E. Dierenfeld, Wildlife Conservation Society, New York, NY), and to assay famciclovir serum levels in one Asian elephant. Three Asian elephants (two from the United States, one from the Netherlands) were treated with an anti-herpesvirus drug, famciclovir (Famvir, SmithKline Beecham, Philadelphia, PA) administered orally or rectally at 500 mg/70 kg, three times a day for 3–4 weeks [Schmitt and Hardy, 1998].

Complete necropsies were performed on elephants that died, and representative samples were taken from all organs and fixed in buffered 10% formalin. All organs and tissues were embedded in paraffin, sectioned at 5-μm thickness, stained with hematoxylin and eosin, and examined by light microscopy. For several of the elephants, additional sections of selected formalin-fixed heart, liver, tongue, and intestinal tract were post-fixed in 2.5% gluteraldehyde and 2% osmium tetroxide, dehydrated in a series of graded alcohols, and embedded in epoxy resin. Semithin sections were stained with toluidine blue, and ultrathin sections were stained with uranyl acetate and lead citrate for electron microscopy.

Retrospective Pathological Studies

A search for pathology material from potential cases of this herpesvirus disease was carried out by sending a survey to all participating zoos via the American Zoo

and Aquarium Association (AZA, Silver Spring, MD) Species Survival Plan (Portland, OR and Indianapolis, IN) and by reviewing the Asian and African elephant studbook mortality records (held by the AZA). There were no reports of herpesvirus infections associated with elephant deaths from the survey or recorded in the studbooks from early in the century in North America. Microscopic slides and tissues (frozen and/or fixed) were obtained from more than 20 elephants whose clinical and pathological descriptions were suggestive of disseminated herpesvirus disease. Additionally, archival paraffin blocks of lung tissue reported to have herpesvirus inclusion bodies from healthy wild African elephants [McCully et al., 1971] (received from Dr. N. Kriek, Onderstepoort Veterinary College, Pretoria, Republic of South Africa) and preserved samples of skin papillomas from otherwise asymptomatic, wild-born African elephants that were imported to Florida in the early 1980s [Jacobson et al., 1986] were obtained. We also received a skin papilloma from a wild bull elephant from the Kruger National Park in South Africa (received from Dr. R. Bengis, Department of Agriculture, Skukuza, Republic of South Africa) as well as biopsy specimens of vulval lymphoid patches [Munson et al., 1995] from wild African elephants from Zimbabwe. The fixed tissues were processed for light and electron microscopy; all slides received and any new slides prepared were reviewed, and all major organs and tissues were examined by light microscopy.

Polymerase Chain Reaction Assay and Sequencing

Initially, using nested polymerase chain reaction (PCR) (performed by Dr. R. Garber, Pathogenesis Inc., Seattle, WA) with consensus redundant oligonucleotide primers that target portions of the terminase and DNA polymerase genes [VanDevanter et al., 1996] of all known herpesvirus genomes, we confirmed the presence of herpesviruses that are different from any other known herpesvirus species. Later, direct single round PCR [Richman et al., 1999a] was also performed on DNAs extracted from affected tissues of 10 deceased elephants and peripheral blood from four ill elephants with clinical signs of disseminated endothelial disease, and appropriate negative controls. Additionally, DNA isolated from lung tissue from three healthy, wild African elephants containing lymphoid nodules, three African elephants with cutaneous papillomas (two captive in the United States, one wild elephant from Kruger National Park, South Africa), and three African elephants with hyperplastic vulval lymphoid patches were processed for PCR as described previously [Richman et al., 1999a]. The PCR products were visualized on a 1.5% agarose gel stained with ethidium bromide.

RESULTS

Clinical Findings

Clinical evaluations of the recent cases of endotheliotropic herpesvirus disease revealed that the most common clinical signs were lethargy, anorexia, mild colic, and edema of the head, proboscis, neck, and thoracic limbs. The index case from North America (Kumari, National Zoological Park) had a diffusely cyanotic tongue and oral ulcers on the day that she died (day 5). In several of these elephants, cyanosis began at the tip of the tongue and moved caudally several days later. Pertinent laboratory tests included hemograms early in the course of the disease in three Asian elephants that showed mainly lymphopenia and thrombocytopenia in two of the el-

ephants, one of which was also anemic. In the three elephants that were successfully treated with famciclovir, lingual cyanosis and head and neck edema resolved during a period of 1 week [Schmitt and Hardy, 1998; D.L. Schmitt et al., 1999, personal communication; Dr. W. Schaftenaar, Rotterdam Zoo, Netherlands).

Pathological Findings

The necropsied elephants and 7 of the 20 elephants reviewed retrospectively that died between 1983 and 1993 all had similar lesions attributed to the endotheliotropic herpesviruses. Six of the retrospective cases were Asian elephants from zoos in New York, Oklahoma, Illinois, and Missouri in the United States, and in Ontario, Canada (two); one was an African elephant from a zoo in Texas, United States.

Gross findings usually included the following: pericardial effusion with extensive petechial to ecchymotic hemorrhages involving the epi- and endocardial heart surfaces and throughout the myocardium, diffusely scattered petechiae within all of the visceral and parietal peritoneal serous membranes, cyanosis of the tongue, hepatomegaly, and variably oral, laryngeal, and large intestinal ulcers [Richman et al., 2000].

Light and Electron Microscopic Findings

The microscopic findings consisted of extensive microhemorrhages throughout the heart and tongue associated with edema, and mild infiltrates of lymphocytes, monocytes, and neutrophils between myofibers. There was multi-focal hepatic sinusoidal expansion with mild sub-acute inflammation, and mild hepatocellular vacuolar degenerative changes. The capillary endothelial cells in the myocardium, tongue muscle, and within the hepatic sinusoids of the liver contained amphophilic to basophilic intra-nuclear viral inclusion bodies that were in close association with the microhemorrhages (Fig. 1). Ultrastructural microscopic studies of the endothelial inclusion bodies revealed 80–92-nm nucleocapsids morphologically consistent with herpesviruses.

Histologically, the cutaneous papillomas in African elephants were composed of hyperplastic epithelial cells with acanthosis and amphophilic intranuclear inclusion bodies in cells of the stratum spinosum. By electron microscopy, the inclusion bodies were composed of viral particles morphologically consistent with herpesviruses, and mature enveloped herpesvirions filled the intercellular spaces [Jacobson et al., 1986].

Hyperemic nodules from the distal urogenital canal of both African and Asian elephants are composed of reactive lymphoid follicles and have been attributed to nonspecific antigenic stimulation; viruses were not detected by light or electron microscopy [Munson et al., 1995, L.K. Richman, unpublished data]. The pulmonary nodules from archival African elephant tissues were composed of multiple large lymphoid follicles that surrounded epithelial cells that contained intra-nuclear inclusion bodies [McCully et al., 1971]. These epithelial cells often formed syncytia.

Clinical Laboratory Findings

In the index case, aerobic and anaerobic bacterial cultures of heart blood, pericardial and peritoneal fluid, cerebrospinal fluid, axillary lymph node, and liver were negative, and bacterial cultures of multiple segments of the gastrointestinal tract yielded no enteric pathogens. Co-cultivation of liver and heart were performed as

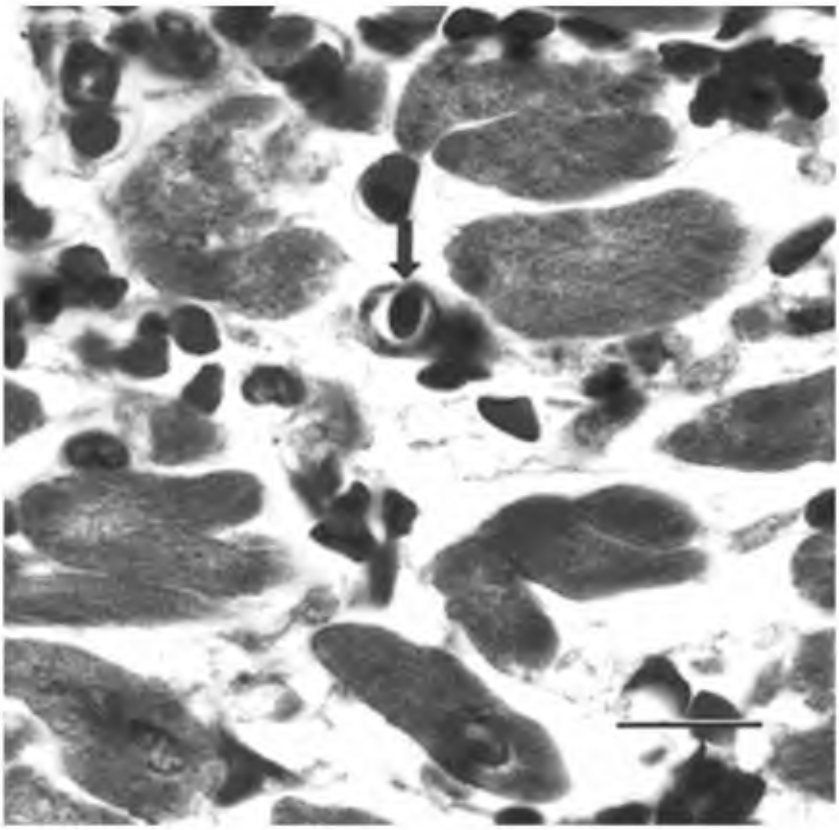

Fig. 1. Photomicrograph of heart from an Asian elephant (*Elephas maximus*) that died from endotheliotropic herpesvirus disease, demonstrating diffuse hemorrhage and an intra-nuclear inclusion body within a capillary endothelial cell (arrow). (H&E, scale bar = 25μm.)

previously described [Richman et al., 1999a] and were also negative for any of the known human or animal herpesviruses or any other viruses. In addition, serology and cultures of heart tissue were negative for encephalomyocarditis virus; fluorescent antibody test and paired sera were negative for *Leptospira interrogans* antigens and antibodies. The blood alpha tocopherol level was 0.62 μg/mL (reference range is 0.2 μg/mL to 0.9 μg/mL for Asian elephants) at the time of her illness.

PCR and Viral Sequencing

DNA from tissues of all of the deceased Asian elephants tested and in the blood of the three survivors during the course of their illness encoded herpesvirus terminase sequences that had only minor variability at the nucleotide level. Initially, no terminase PCR products were obtained from African elephants with endothelial disease and conversely, DNA polymerase-directed PCR generated products from the African cases only, but not from the Asian elephants with endothelial disease. Once new primers were constructed, PCR products were obtained from the DNA polymerase gene of four Asian elephant cases that were nearly identical to each other at the nucleotide level [Richman et al., 1999a]. However, sequence comparison of the herpesvirus

DNA polymerase regions from the two elephant species showed only a 76% protein identity between the viruses detected in Asian and African elephant cases with 65% identity at the nucleotide level [Richman et al., 1999a]. This indicates that two different species of herpesviruses are present in the elephants studied here. Similarly, PCR products from the terminase gene region were also obtained from an African elephant once a second set of specific primers was constructed. Sequence comparison of the terminase gene region from the two elephant species showed 80% identity at the nucleotide level, although in this case the changes were all synonymous and the encoded proteins showed 100% amino acid identity [Richman et al., 1999a]. Control samples that proved negative in this study included heart and/or liver tissue from eight Asian and five African elephants that died of conditions unrelated to endothelial disease. Additionally, DNAs extracted from peripheral blood of 27 asymptomatic Asian and 13 African elephants, including herdmates from facilities where herpesvirus deaths had occurred, were negative for herpesvirus using PCR primer sets specific for both viruses.

From the African elephant cutaneous papillomas and vulval lymphoid patches, we obtained some surprising results; PCR sequencing of DNA extracted from these two lesions proved to encode protein sequences identical to those obtained from the Asian elephants with endotheliotropic herpesvirus disease. Control samples that proved negative included pustular skin lesions from two African and three Asian elephants without evidence of inclusion bodies, and suppurative or non-inflammatory vulval lesions from one Asian and two African elephants. Conversely, DNA extracted from pulmonary tissue of wild African elephants with morphological evidence of herpesvirus contained viral sequences in the DNA polymerase gene region that had 100% protein identity with the herpesvirus that was fatal for the two African elephants in our study group. To date, skin, vulval, and pulmonary lesions from Asian elephants that are grossly and histologically similar to the lesions found in African elephants have not yielded herpesvirus sequences.

DISCUSSION

Before the identification of the highly fatal herpesvirus disease in captive elephants [Richman et al., 1999a; Ossent et al., 1990], the herpesviruses from skin papillomas [Jacobson et al., 1986] and pulmonary nodules [McCully et al., 1971] of African elephants were recognized, but no references had been made to herpesvirus in Asian elephants. At the time, these localized lesions with herpetic inclusion bodies in the African elephants were considered incidental with no systemic illnesses associated with them, and no documentation of herpesvirus isolation. Subsequent PCR sequencing of DNA extracted from African elephant skin papillomas showed identical sequences in the terminase gene region as the virus lethal for Asian elephants [Richman et al., 1999a]. Similarly, a nearly identical viral DNA sequence was identified by PCR in biopsies of lymphoid patches from the distal vaginal tract (vestibulum) of a wild African elephant that did not contain viral inclusion bodies histologically [Richman et al., 1999a].

The finding of intranuclear inclusion bodies within only endothelial cells of elephants that have died with the characteristic disease provides histological evidence of the viral tropism for these vascular cells. In fact, these endothelial inclusion bodies found in all the elephants that died of the herpesvirus infection are histologi-

cal hallmarks that may be pathognomonic for this disease. The endotheliotropic disease associated with the elephant herpesviruses, in conjunction with prominent intranuclear inclusion bodies, suggest that these viruses have some of the characteristics of beta herpesviruses, such as the cytomegaloviruses (CMV). Indeed, the proteins encoded by the PCR amplified DNA obtained from each of the Asian and African elephants that were affected with the disease are clearly those of herpesviruses, but they are distinct from any of the currently known herpesviruses. By comparing amino acid sequences that are characteristic for each herpesvirus subfamily, together with the results of a phylogenetic tree analysis [Richman et al., 1999a], the terminase protein of the elephant herpesviruses shows slightly greater similarity to beta herpesviruses than to alpha or gamma herpesviruses, but it is clearly not that of a CMV. Similarly, the elephant virus DNA polymerase proteins do not fit into any of the herpesvirus subgroups [Richman et al., 1999a]. These findings, together with the unique pathogenesis, suggest that the causative agents of elephant endothelial disease are either outliers of mammalian beta herpesviruses or may belong in a new subfamily.

The acute nature and high fatality rate in clinically affected elephants of this herpesvirus disease is unusual for infections caused by herpesviruses in humans and other animals. One of the major factors considered important in the epidemiology of the elephant herpesviruses is the premise of the herpesvirus being innocuous in one elephant species and highly pathogenic for the other. This relationship is known for a number of herpesviruses including herpesvirus B, latent in *Macaca* spp., and potentially fatal in humans, and malignant catarrhal fever herpesviruses carried by sheep or wildebeest and producing overt disease in cattle and acelaphine antelopes [Schuller et al., 1990]. The evidence of cross-species transmission of a herpesvirus from benign lesions in African elephants to susceptible Asian elephants was the discovery of nearly identical herpesvirus sequences in these two species [Richman et al., 1999a]. This suggested that otherwise healthy African elephants are a source of the herpesvirus that is highly pathogenic for Asian elephants.

Additionally, the second related herpesvirus identified in pulmonary nodules obtained from healthy wild African elephants has 100% protein identity (in the DNA polymerase gene region) with the previously reported virus that was lethal for the two African elephants with endotheliotropic disease [Richman et al., 2000]. Although currently limited by data obtained from a small portion of the viral genomes, and given the obvious need for additional viral sequence data, the results obtained to date may indicate that African elephants can harbor two novel herpesviruses: the one that caused fatal endotheliotropic disease in Asian elephants and the other that caused the same disease picture in African elephants. Currently, intensive work is under way to further elucidate the epidemiology of these two herpesviruses that are either carried latently, or as chronic low-level infections in African elephants. The status of herpesviruses in wild Asian elephants is currently unknown, because it has not yet been possible for us to obtain elephant tissue samples from Asian countries.

In addition to the Asian elephant from Switzerland [Ossent et al., 1990], the virus of which we determined is homologous (in the terminase gene region) with our other Asian elephant cases from North America, we have confirmed by PCR and/or morphological techniques similar herpesvirus cases in five Asian elephants from Germany, Holland and Israel [Richman et al., 1999b]. Additionally, herpesvirus sequences identical to the virus that causes lethal disease in Asian elephants have been found in heart and placenta of three Asian elephant stillborn fetuses from Germany [Richman

et al., 1999b; J. Fickel, unpublished]. Any association of the herpesvirus with the high elephant stillbirth and abortion rate in captivity remains to be determined.

In the last 3 years, four Asian elephants were suspected to have this disease based on clinical signs; suspicions were confirmed by PCR sequencing on DNA extracted from peripheral blood early in the disease. Three of these elephants were treated with famciclovir for 3–4 weeks (the fourth elephant died suddenly after diagnosis was confirmed). After several days of treatment, past the time when untreated elephants would normally die, the appetites returned, lingual cyanosis slowly regressed, and edema was no longer visible in the medicated elephants [Schmitt and Hardy, 1998]. This beneficial effect was attributed to the antiherpesviral action of the famciclovir, which has been used successfully in people to treat herpes simplex (HSV-1 and HSV-2) and shingles.

We suggest that these elephant herpesviruses have contributed to a significant proportion of captive-born elephant mortalities and that an accurate accounting of this disease has been limited by the unavailability of case material before the 1980s. Although our findings to date implicate the African elephant as a potential reservoir of herpesviruses that can cause disease in the two elephant species, direct proof of transmission has not been established between contact and exposed animals. It might be important to minimize the comingling or socializing of Asian and African elephants at the same facility or between facilities, because this may increase the risk of the transmission of herpesviruses, particularly in breeding operations, or where new elephants are being introduced.

CONCLUSIONS

1. There are currently two recognized herpesviruses that cause a fatal disease syndrome in elephants. They are known as the elephant endotheliotropic herpesviruses, of which one is fatal for Asian elephants (*Elephas maximus*) and the other for African elephants (*Loxodonta africana*).

2. Healthy African elephants with external herpetic lesions have yielded herpesvirus sequences identical to that found in Asian elephants with endothelial disease, which suggests that the Asian elephant deaths were caused by cross-species infection with a herpesvirus that is naturally latent in, but normally not lethal to African elephants.

3. Many healthy free-ranging African elephants have pulmonary lymphoid nodules that yield herpesvirus sequences identical to that found in African elephants with endothelial disease, suggesting that African elephants may also harbor the virus that causes disseminated endothelial disease in African elephants.

4. Three Asian elephants with endothelial disease have been treated with the antiviral drug, famciclovir, and survived. Early diagnosis by clinical signs and PCR on peripheral blood, followed by oral or rectal administration of famciclovir, are crucial to prevent further losses from this fatal disease.

5. Considerably more epidemiological work is required to clarify the potential carrier status of the Asian elephant for either of these viruses. Some of these factors will depend on cultivating the viruses, or on the development of serological or PCR tests so that elephants previously exposed to these viruses can be identified. Additional sequence data from both viruses are needed to determine the relatedness of the elephant viruses with other herpesviruses.

6. Some of the aspects of this study that are important to elephant managers include the possibility that comingling or socializing of Asian and African elephants at the same facility or between facilities may increase the risk of transmission of herpesviruses. Although we currently have no direct proof that cross-species transmission occurs, and much more epidemiological work needs to be done, it may be advisable to minimize comingling of Asian and African elephants, particularly in breeding operations, or where new elephants are being introduced.

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