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CLINICAL AND PATHOLOGICAL FINDINGS OF A NEWLY RECOGNIZED DISEASE OF ELEPHANTS CAUSED BY ENDOTHELIOTROPIC HERPESVIRUSES

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ABSTRACT: The unique clinical and pathological findings in nine Asian (*Elephas maximus*) and two African (*Loxodonta africana*) elephants from North American Zoos with a highly fatal disease caused by novel endotheliotropic herpesviruses are described. Identification of the viruses by molecular techniques and some epidemiological aspects of the disease were previously reported. Consensus primer polymerase chain reaction (PCR) combined with sequencing yielded molecular evidence that confirmed the presence of two novel but related herpesviruses associated with the disease, one in Asian elephants and the second in African elephants. Disease onset was acute, with lethargy, edema of the head and thoracic limbs, oral ulceration and cyanosis of the tongue followed by death of most animals in 1 to 7 days. Pertinent laboratory findings in two of three clinically evaluated animals included lymphocytopenia and thrombocytopenia. Two affected young Asian elephants recovered after a 3 to 4 wk course of therapy with the anti-herpesvirus drug famciclovir. Necropsy findings in the fatal cases included pericardial effusion and extensive petechial hemorrhages in the heart and throughout the peritoneal cavity, hepatomegaly, cyanosis of the tongue, intestinal hemorrhage, and ulceration. Histologically, there were extensive microhemorrhages and edema throughout the myocardium and mild, subacute myocarditis. Similar hemorrhagic lesions with inflammation were evident in the tongue, liver, and large intestine. Lesions in these target organs were accompanied by amphophilic to basophilic intranuclear viral inclusion bodies in capillary endothelial cells. Transmission electron microscopy of the endothelial inclusion bodies revealed 80 to 92 nm diameter viral capsids consistent with herpesvirus morphology. The short course of the herpesvirus infections, with sudden deaths in all but the two surviving elephants, was ascribed to acute cardiac failure attributed to herpesvirus-induced capillary injury with extensive myocardial hemorrhage and edema.

Key words: Elephant, *Elephas maximus*, endotheliotropic viruses, famciclovir, herpesvirus, *Loxodonta africana*, new disease, penciclovir.

INTRODUCTION

Elephants are exceptionally charismatic megavertebrates; the Asian (*Elephas maximus*) and African (*Loxodonta africana*) species are listed respectively as endangered and threatened by The World Conservation Union (IUCN). To increase elephant numbers, a great deal of effort is underway by zoo organizations to breed these majestic animals naturally and by assisted insemination techniques. However, too many young elephants still die from unknown causes. Recently, we reported the virological and epidemiological findings of a highly fatal disease of elephants in North America caused by two related but distinct novel, endotheliotropic herpesviruses (Richman et al., 1999a). In that paper, sequences from two herpesvirus gene regions, terminase and DNA polymerase, showed 80% and 65% DNA homology, respectively between the viruses that are lethal for Asian and African elephants. A similar disease attributed to an endotheliotropic herpesvirus has been reported in a young Asian elephant from a circus in Switzerland (Ossent et al., 1990) and was shown to have the same viral sequences as in our cases (Richman et al.,

1999a). In our series, a total of 11 cases of endotheliotropic herpesvirus infections were identified from eight zoos in North America by polymerase chain reaction (PCR). Six of the cases were found retrospectively after identification of the index case involving a 16-mo-old Asian elephant at the National Zoological Park in 1995 (Richman et al., 1999a). The disease was fatal in six young Asian elephants representing 18% of that species born in captivity from 1983 to 1996 (Richman et al., 1999a). One adult Asian elephant and two African elephants (a calf and an adult) also died, and two young Asian elephants recently survived the disease after treatment with an anti-herpesvirus drug, famciclovir. The relationship between the Asian and African elephant endotheliotropic herpesviruses is under further investigation; however, they appear to be closely related, novel herpesviruses that produce nearly identical lesions in the two elephant species (Richman et al., 1999a). Additionally, localized African elephant skin and vulval lesions were found to contain viral sequences nearly identical to the virus that caused the highly fatal infections in Asian elephants (Richman et al., 1999a).

The purpose of this paper is to report the clinical and pathological findings of this new herpesvirus disease of elephants and to describe a unique pattern of lesions in the organs targeted which is attributed to the endotheliotropism of the virus. In addition, we have developed an early diagnostic method for this disease by a whole blood PCR test, and propose a possible source of the herpesvirus that caused the deaths of the two African elephants with endotheliotropic disease. The gross and microscopic findings together with the molecular virological data and diagnostic tests support these herpesviruses as the causative agents of this newly recognized disease syndrome.

MATERIALS AND METHODS

General

Clinical and/or postmortem samples were obtained from 11 elephants from North American zoos with endotheliotropic herpesvirus infection which included seven Asian and two African elephants that died, and two Asian elephants that survived. A detailed account of the clinical management of one of the latter elephants is published elsewhere (Schmitt and Hardy, 1998).

Clinical and pathological evaluations of recent cases (1995–98).

Signalment and disease status of elephant (E) #1 through E#4 were assessed (Table 1); clinical evaluations were dependent upon the tractability of the animals at the time of illness but 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 (E. Dierenfeld, Wildlife Conservation Society, New York City, New York, USA), and to assay famciclovir serum levels in E#3. Elephant #3 and E#4 were treated with an anti-herpesvirus drug, famciclovir (Famvir, SmithKline Beecham, Philadelphia, Pennsylvania, USA) administered orally or rectally at 500 mg/70 kg, three times a day for 3 to 4 wk (Schmitt and Hardy, 1998).

Complete necropsies were performed on E#1 and E#2 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 microns thickness, stained with hematoxylin and eosin, and examined by light microscopy. 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 (Steele et al., 1997). Semi-thin sections were stained with toluidine blue, and ultra-thin sections were stained with uranyl acetate and lead citrate for electron microscopy (Steele et al., 1997). From E#1, fresh liver and spleen and paired serum samples were submitted for fluorescent antibody tests (FAT) for leptospirosis (*Leptospira interrogans* serotypes *pomona, hardjo, icterohaemorrhagia/copenhageni, grippotyphosa, canicola, bratislava,* and *sejroe*; New York State College of Veterinary Medicine, Ithaca, New York, USA) and fresh heart for encephalomyocarditis virus (EMCV) cultures (J. Gaskin, University of Florida, Gainesville, Florida, USA). Replicate sections of heart, liver, tongue, lymph nodes, spleen, kidney, and intestinal tract were frozen at −70 C for further viral isolation attempts.

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, Maryland, USA) Species Survival Plan (SSP, Portland, Oregon, USA and Indianapolis, Indiana, USA) 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. Pathology records, microscopic slides and tissues (frozen and fixed) were obtained from over 20 elephants whose clinical and pathological descriptions had some similarities with E#1 (the index case) and the case reported in an Asian elephant in Switzerland (Ossent et al., 1990). Additionally, archival paraffin blocks of lung tissue reported to have herpesvirus inclusion bodies from healthy wild African elephants (McCully et al., 1971) (received from N. Kriek, Onderstepoort Veterinary College, Pretoria, Republic of South Africa) were obtained. The fixed tissues were processed as described for E#1 and E#2 for light and electron microscopy; all slides received and any new slides prepared were reviewed, all major organs and tissues were examined by light microscopy, and the data collated.

PCR assay and sequencing

Polymerase chain reaction was performed on DNA extracted from either fresh or frozen (−80 C) peripheral blood from two ill elephants (E#3 and E#4) with clinical signs of endotheliotropic herpesvirus disease, and on DNA isolated from lung tissue from three healthy, wild African elephants containing lymphoid nodules reported to include herpesvirus inclusions (McCully et al., 1971) and processed for PCR as described previously (Richman et al., 1999a). Specific single primer sets for the elephant herpesvirus terminase and DNA polymerase gene regions were constructed from the sequence initially obtained from cases E#1 and E#2: Terminase primers for Asian elephants (5′GTACGTCCTTTCTAGCTCAC 3′ and 5′GTGTCGGCTAAATGTTCTTG 3′); terminase primers for African elephants (5′AATGTGATATCCTACGTATG 3′ and 5′ GTACTATATCTTATCATGTC 3′); and DNA polymerase primers for both elephant species (5′ GTGTCTGGCTATAGCAGAGT 3′ and 5′CACATCGATACGGAATCTCT 3′).

Polymerase chain reaction was performed with these primers in a 50 μl reaction volume containing PCR SuperMix (Gibco/BRL-Life Technologies, Gaithersburg, Maryland, USA), 0.3% (v/v) glycerol, and 20 pmol of each primer. Polymerase chain reaction was completed using the following protocol: Denaturation at 94 C for 1 min, annealing at 50 C for 1 min, and extension at 72 C for 2 min for a total of 36 cycles. The final extension was performed at 72 C for 7 min. The PCR product was visualized on a 1.5% agarose gel stained with ethidium bromide.

Serological testing

Selected serological tests were performed via the National Animal Disease Laboratories (NADL; Ames, Iowa, USA) on sera from three adult elephants (two Asian, one African) from the National Zoological Park (Washington DC, USA) as well as banked frozen sera from herdmates from two other zoos (located in Bronx, New York, USA and Springfield, Missouri, USA). These were the sites where previous deaths from the endotheliotropic herpesvirus had occurred. Serologic tests for antibodies to the following were performed: bluetongue virus by enzyme-linked immunosorbent assay, epizootic hemorrhagic disease virus of deer by agar gel immunodiffusion (AGID), bovine herpesvirus 1 by serum neutralization (SN), bovine herpesvirus 2 by SN, bovine herpesvirus 4 by immunoperoxidase (IPT), malignant catarrhal fever virus by IPT, equine herpesviruses by SN, equine viral arteritis virus by SN, encephalomyocarditis virus by SN, equine infectious anemia virus by AGID, equine adenovirus by IPT, pseudorabies virus by SN and porcine reproductive and respiratory syndrome virus by IPT.

High performance liquid chromatography (HPLC) analysis

Famciclovir (Famvir, SmithKline Beecham, Philadelphia, Pennsylvania, USA) is a pro-drug that is rapidly and completely converted to penciclovir by liver esterases (Vere Hodge et al., 1989). Penciclovir (SmithKline Beecham, Philadelphia, Pennsylvania, USA) is the compound that circulates in the blood after administration of famciclovir. We used, with minor modifications, a previously described method for the determination of penciclovir concentrations in serum (Fowles and Pierce, 1989) from elephant #3. The calibrators (range 10–10,000 ng/mL), quality assurance samples, and E#3 serum samples were spiked with acyclovir (Sigma, St. Louis, Missouri, USA) (50 μL of 100 μg/mL) which was used as the internal standard. Trichloroacetic acid (0.5 mL) was added to precipitate the proteins. The samples were centrifuged at 1,200 rpm in an Eppendorf 235C centrifuge (Fisher Scientific, Pittsburgh, Pennsylvania, USA) for 2 min and the supernatants were transferred to a new tube and then dried in a Speed Vac (Savant Instrument

TABLE 1. Signalment and disease status of four elephants with endotheliotropic herpesvirus disease.

ID	Species	Sex	Age	Location	Onset	Conrse	Status
Elephant #1	Asian	F	16 mo	Washington, DC	Apr-95	5 days	Died
Elephant #2	African	M	11 mo	California	Aug-96	3 days	Died
Elephant #3	Asian	F	17 mo	Missouri	Nov-97	10 days	Survived[a]
Elephant #4	Asian	M	16 mo	Florida	Sep-98	12 days	Survived[a]

[a] Animals were treated with famciclovir, beginning 3 to 5 days after onset.

Inc., Holbrook, New York, USA). The samples were resuspended in 300 μL of 0.001 M NaH_2PO_4. Aliquots of 100 μL were injected using an autoinjector (Waters Associates, Inc., Milford, Massachusetts, USA) onto a C-18 reverse phase HPLC column (Waters Associates, Inc., Milford, Massachusetts, USA) and then were eluted with 0.01 M NaH_2PO_4 (pH 7): methanol (9:1) at a flow rate of 1 mL/min using Waters HPLC pump. The signal was detected using Waters spectrophotometric detector at UV absorption of 254 nm. Data acquisition was done using Waters Millennium Chromatography Manager. Samples were obtained from E#3 several times each day during the treatment period and were compared to banked serum samples from the same elephant before treatment commenced.

RESULTS

Clinical findings

From the clinical evaluations of E#1–E#4 (Table 1), the onset of the disease appeared to be sudden with a course of 1 to 7 days. The initial clinical signs were lethargy, anorexia, mild colic, edema of the head (especially the face and proboscis) neck and thoracic limbs (Fig. 1). E#1 had a diffusely cyanotic tongue and oral ulcers on the day that she died (day 5). Cyanosis of the tongue in E#2 was not observed, but in E#3 cyanosis began at the tip of the tongue and moved caudally on the third and fourth day. E#4 had swelling and cyanosis of the tongue noted 4 days after the onset of illness. Pertinent laboratory tests included hemograms early in the course of the disease in the three Asian elephants which showed mainly lymphopenia and thrombocytopenia in two of the elephants, one of which also was anemic (Table 2). E#1 and E#2 died on the fifth and third day, respectively after the onset of the disease. In E#3, the lingual cyanosis and head and neck edema resolved over the next week, approximately 9 days after beginning treatment with famciclovir. The elephant gained strength and resumed eating. Semiquantitative blood virus levels measured daily from day 3 after disease onset decreased steadily to a non-detectable level by 8 wk after the onset of illness. Serum levels of penciclovir rose to 863 ng/ml after the initial dose and fluctuated between 0 (8 hr post-treatment) and 4,365 ng/ml (1 hr post-treatment) over the 3 wk course of oral famciclovir administration.

FIGURE 1. Asian elephant #3 has subcutanous edema of the head, neck, and thoracic limbs attributed to endotheliotropic herpesvirus disease.

Pathological findings (recent and retrospective cases).

The necropsied elephants E#1 and E#2 and seven of the 20 elephants reviewed retrospectively that died between 1983 and 1993 all had similar lesions attributed

TABLE 2. Blood parameters during the acute onset of endotheliotropic herpesvirus disease in three elephants.

	WBC	RBC	Platelets	Neutrophils	Lymphocytes	Monocytes
Mean Female[a]	(13.5 × 10^3/μl)	(3.1 × 10^3/μl)	(601 × 10^3/μl)	(4.7 × 10^3/μl)	(6.6 × 10^3/μl)	(2.4 × 10^3/μl)
#1 Female	4.6	3.89	decreased	3.1	0.90	0.40
#3 Female	19.5	2.55	166	9.55	3.5	1.6
Mean Male[a]	(16.5 × 10^3/μl)	(3.2 × 10^3/μl)	(518 × 10^3/μl)	(6.13 × 10^3/μl)	(8.2 × 10^3/μl)	(1.4 × 10^3/μl)
#4 Male	15.8	2.31	adequate	5.06	10.43	0.16

[a] From Mikota et al. (1994).

FIGURE 2. Left ventricular endocardial surface of the heart from Asian elephant #1 showing subendocardial ecchymotic hemorrhages due to endotheliotropic herpesvirus disease.

to the endotheliotropic herpesviruses. Six of the retrospective cases were Asian elephants from zoos in New York, Oklahoma, Illinois, and Missouri in the USA, and in Ontario, Canada (2); one was an African elephant from a zoo in Texas (USA).

Gross findings typically included pericardial effusion with extensive petechial to ecchymotic hemorrhages involving the epi- and endocardial heart surfaces (Fig. 2) and throughout the myocardium. In addition, diffusely scattered petechiae were within all of the visceral and parietal peritoneal serous membranes and variably, there was cyanosis of the tongue (Fig. 3). There was also hepatomegaly, and variably oral, laryngeal and large intestinal ulcers.

Light and electron microscopic findings

Pertinent microscopic findings consisted of extensive microhemorrhages throughout the heart (Fig. 4) and tongue associated with edema, and mild infiltrates of lymphocytes, monocytes and a few neutrophils between myofibers. There was mild to moderate hepatic sinusoidal expansion with multifocal subacute inflammation, and mild hepatocellular vacuolar degenerative changes. Ulcers of the oral and la-

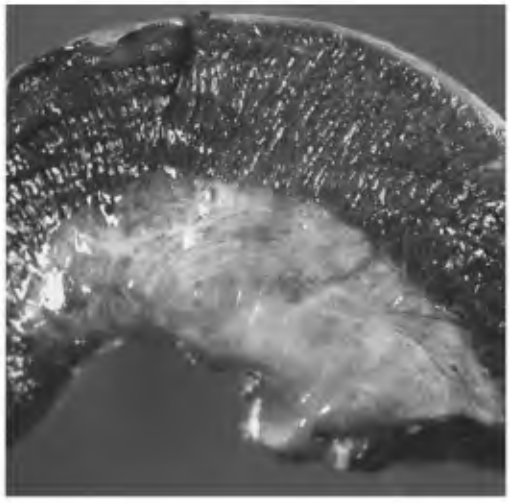

FIGURE 3. Cross section of the tongue from Asian elephant #1 demonstrating diffuse swelling and cyanosis attributed to endotheliotropic herpesvirns disease.

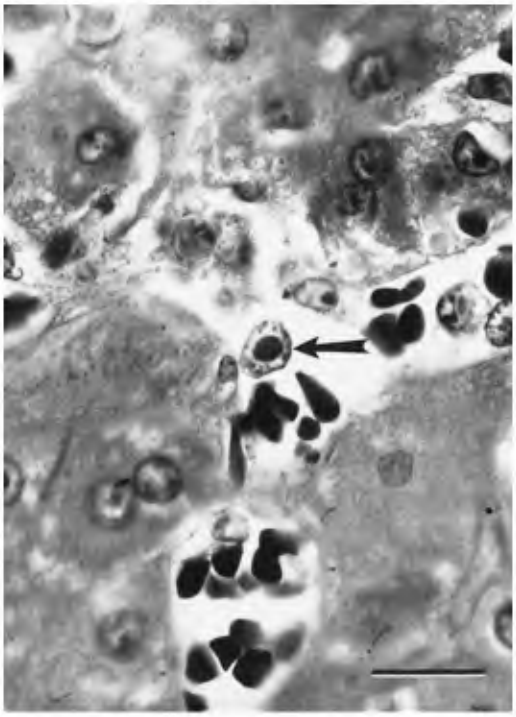

FIGURE 5. Photomicrograph of liver from Asian elephant #1 that died with endotheliotropic herpesvirus disease, showing an intranuclear viral inclusion body within a sinusoidal endothelial cell (arrow). Bar = 25 μm.

FIGURE 4. Photomicrograph of heart from Asian elephant #1, demonstrating extensive hemorrhage and separation of myocardial fibers attribnted to endotheliotropic herpesvirus. Bar = 150 μm.

ryngeal mucous membranes in E#1 were acute, with necrotic surface cells still intact in some areas. The endothelial cells of capillaries in the myocardium (9/9 animals) and tongue muscle (from 6/6 animals from which tongue was available), and within the hepatic sinusoids of the liver (9/9 animals) contained amphophilic to basophilic intranuclear viral inclusion bodies (Fig. 5). The endothelial cells with the viral inclusion bodies were in close association with the microhemorrhages in the heart and tongue. The inclusion bodies were less often seen in capillary endothelial cells in the lamina propria and smooth muscle layers of the intestinal tract, but were not evident in any of the ulcers or in blood vessels larger than capillaries. Ultrastructural microscopic studies of the endothelial inclusion bodies in all nine cases revealed 80–92 nm particles morphologically consistent with herpesviruses (Figs. 6, 7). His-

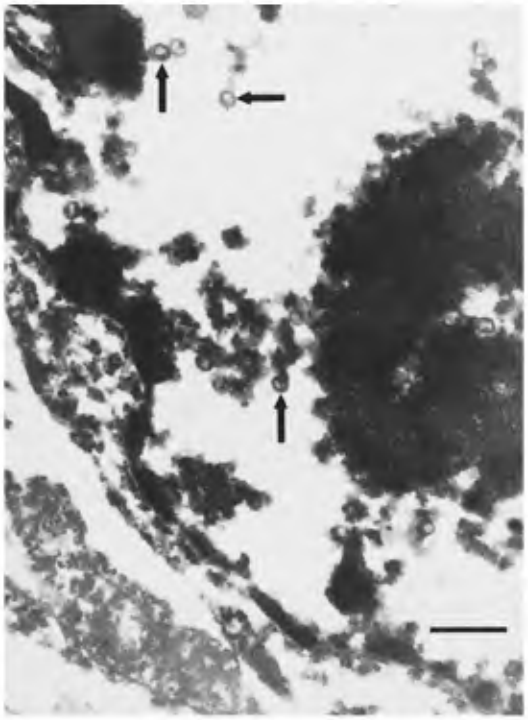

FIGURE 6. Transmission electron micrograph (TEM) of heart from African elephant #2 that died with endotheliotropic herpesvirus disease, demonstrating an intranuclear inclusion body within a capillary endothelial cell. Herpesvirus particles (arrows) are seen emerging from the inclnsion body. Bar = 500 nm.

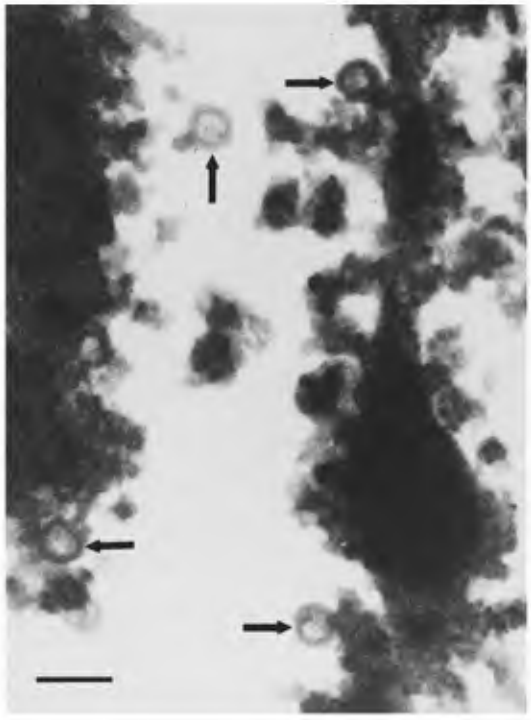

FIGURE 7. Higher magnification TEM of the intrannclear inclusion body from Figure 6, showing greater detail of the emerging herpesvirus nncleocapsids (arrows). Bar = 200 nm.

tologically, pulmonary nodules from archival African elephant tissues were composed of multiple large lymphoid follicles (Fig. 8) which surrounded epithelial cells that contained intranuclear inclusion bodies (Fig. 9). These epithelial cells often formed syncytia.

Clinical laboratory findings

In E#1, 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 previously described (Richman et al., 1999a) and also were 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; FAT and paired sera were negative for *Leptospira interrogans* antigens and antibodies. 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

Polymerase chain reaction, followed by sequencing of DNA extracted from peripheral blood from E#3 and E#4 contained nearly identical endotheliotropic herpesvirus sequences in the terminase gene region (up to 3 base pairs difference from some of the deceased Asian elephants, but all are silent mutations) as the target tissues from the deceased Asian elephants. DNA extracted from pulmonary tissue of wild African elephants with morphological evidence of herpesvirus con-

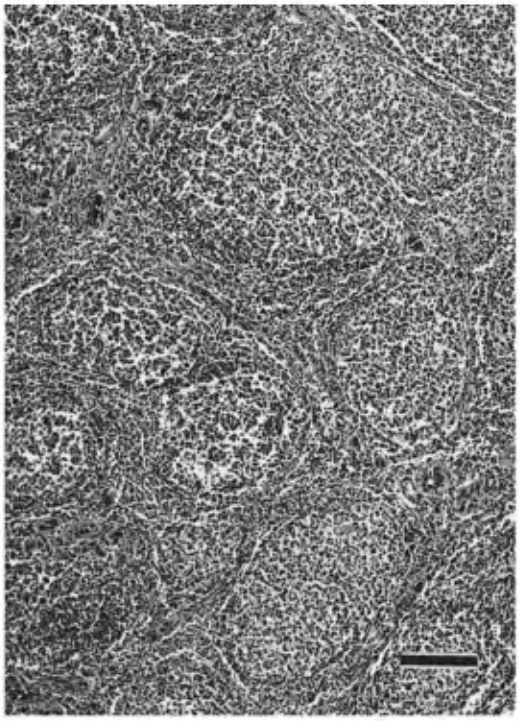

FIGURE 8. Pulmonary lymphoid nodnles from a healthy, free ranging adult African elephant. Follicular lymphoid hyperplasia snrrounds epithelial cells which contain intranuclear herpesviral inclnsion bodies, shown in Fignre 9. Bar = 250 μm.

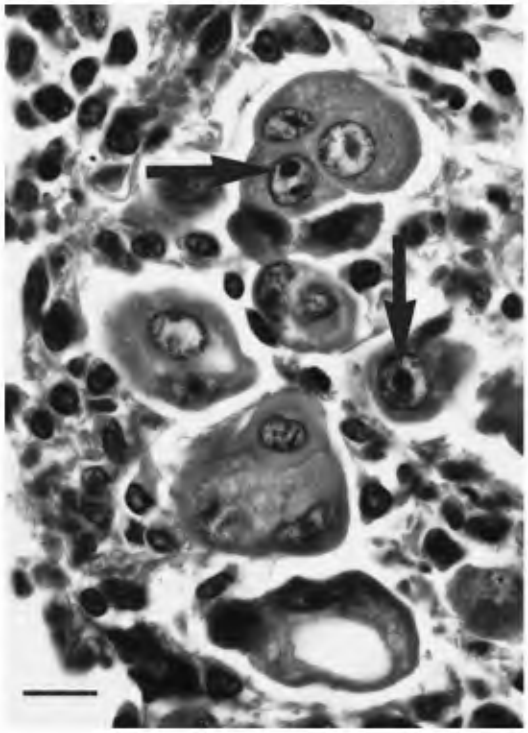

FIGURE 9. Higher magnification of the lung nodule from Fignre 8, demonstrating intranuclear herpesviral inclusion bodies (arrows) within epithelial syncytial cells. Herpesviral protein seqnences from the DNA polymerase gene region obtained from these African elephant pulmonary nodules are identical to those found in deceased African elephants with endotheliotropic herpesvirus disease. Bar = 25 μm.

tained 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.

Serological studies

One herdmate of E#1 had low serum antibody titer to bovine herpesvirus-4, and several others tested had low titers to malignant catarrhal fever virus (alcelaphine herpesvirus 1) and to equine adenovirus. Serosurveys from remaining viruses listed were all negative. Herd mates from the Swiss case (Metzler et al., 1990) also had low titers considered cross reactive to bovine herpesviruses but the significance of these serologic findings is currently unknown since there was no consistent pattern, nor were herpesviruses isolated from any of the cases.

HPLC for penciclovir

Serum levels of penciclovir from E#3 varied from 97 ng/ml to 4,365 ng/ml, depending on the time interval between treatment and blood collection. Lower levels of serum penciclovir were present in the early morning, 8 hr after the last dose of famciclovir was administered. Higher values of serum penciclovir correlated with treatment administration 1 to 3 hr prior to blood collection (data not shown). These concentrations are comparable to penciclovir concentrations in humans after administration of a 500 mg dose of famciclovir (Vere Hodge et al., 1989; Boike et al., 1994).

DISCUSSION

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 indicating probable cross-species transmission of a herpesvirus from benign lesions in otherwise asymptomatic African elephants to susceptible Asian elephants was the finding 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.

Another important factor considered in the pathogenicity and the unusual nature of the lesions observed in these elephant herpesvirus infections is the endotheliotropism of these viruses. Most animal herpesviruses are epitheliotropic or have a predilection for nervous tissue. The target organs for many of the disseminated herpesvirus infections in mammals are usually the liver, adrenal glands and brain, in which the herpesvirus forms characteristic intranuclear inclusion bodies and produces foci of necrosis in the parenchymal cells. The finding of intranuclear inclusion bodies in the endothelial cells of capillaries in the elephant lesions provides histomorphological evidence of their tropism for these vascular cells. In fact, these endothelial inclusion bodies found in all nine elephants that died of the herpesvirus infection are histological hallmarks and may be pathognomonic for this disease syndrome.

It has been reported (Metzler et al., 1990) that elephants that had contact with the Swiss case had serological evidence of exposure to bovine herpesvirus 2 (BHV2). Because of this report, we tested convalescent serum samples from Asian elephant E#3 for exposure to or cross-reactivity with BHV1, BHV2 and BHV4 (data not shown). All convalescent serum samples from E#3 were negative by ELISA for BHV1, BHV2 and BHV4 (R. Eberle, pers. comm.) and negative by immunofluorescence for BHV1, BHV2, BHV4 and other ungulate herpesviruses including equine herpesviruses 1 and 4 (M. Kennedy, pers. comm.). This supports our molecular findings, and demonstrates that the elephant herpesviruses are unique in immunogenicity such that cross reactivity with other herpesviruses is not detected. Additionally, the vitamin E (alpha-tocopherol) level in E#1 was within normal range for Asian elephants. In the earlier cases, at the time of their deaths, hypovitaminosis E was considered in the differential diagnosis of several of the elephants in our study group due to ecchymotic hemorrhages that were seen at necropsy.

The endotheliotropism of the elephant herpesviruses also may explain the organ tropism of these viruses for the heart, liver and tongue, the capillary endothelial cells of which harbor the inclusion bodies. The reason for the specific tropism for endothelial cells mainly in these targeted organs is not clear, but may reflect some difference in metabolic properties or surface receptor characteristics between endothelial cells in different organs. Other instances of endotheliotropism for herpesviruses have been reported with varicella-zoster virus (VZV) in children with fatal disseminated infections, and more recently with human herpesvirus-8 (HHV8) and its association with Kaposi's sarcoma, which are thought to be derived from endothelial-like spindle cells (Moore and Chang, 1995).

Based on these morphologic findings, the proposed pathogenesis is as follows: once the elephant becomes viremic (possibly via circulating lymphocytes after exposure to the virus), ensuing viral replication in the heart leads to endothelial cell damage resulting in capillary leakage and severe intramyocardial hemorrhage and edema. The magnitude of this damage, as reflected by the microscopic lesions, leads to cardiac failure either by disruption of the conducting system, the mass effect of the intracardiac swelling, or myocardial ischemia and localized metabolic disturbances. Cardiac insufficiency may also contribute to glossal cyanosis. The regression of lingual cyanosis as noted in E#3 treated with famciclovir reflects a similar process of capillary injury and leakage within the tongue, followed by resolution as recovery progressed. The severe hemorrhage and congestion was manifested as the blue discoloration noted clinically in E#3 and involving the entire tongue of elephant E#1 in the terminal stages of the herpesvirus infection.

In support of this pathogenesis, E#3 and E#4 were found to be viremic by PCR early in the disease, had all the classic clinical signs, and were treated with famciclovir for 3 to 4 wk. After several days of treatment, at a time when elephants would normally die, appetite returned, lingual cyanosis slowly regressed and edema was no longer visible. In E#3, temporal semiquantitative PCR showed decreasing detectable blood viral load that coincided with serum famciclovir at therapeutic levels (data not shown) which strongly suggests a beneficial effect of the antiherpesvirus drug. The recovery of the second young elephant (E#4) that was treated with famciclovir further supports the efficacy of this drug in the treatment of these elephant herpesvirus infections.

Prior to the identification and description of this highly fatal herpesvirus disease in captive elephants (Ossent et al., 1990; Richman et al., 1999a), there existed only several reports of herpesviruses occurring in skin papillomas and pulmonary nodules of African elephants by light and electron microscopy, but no references to herpesvirus in Asian elephants. At the time, these lesions with herpetic inclusion bodies in the African elephants were considered incidental or localized findings with no systemic illnesses associated with them, and no documentation of herpesvirus isolation (McCully et al., 1971; Jacobson et al., 1986). The agent observed in the African elephant cutaneous papillomas is homologous with the virus lethal for Asian elephants in the terminase gene region (Richman et al., 1999a). In that study, the same virus was also identified by PCR in biopsies of the lymphoid patches from the distal vaginal tract (vestibulum) of wild African elephants which did not contain viral inclusion bodies histologically. This herpesvirus is now considered to be a novel, indigenous herpesvirus of African elephants, possibly latent in lymphocytes of the vaginal lymphoid follicle-containing patches. These lymphoid patches periodically become hyperplastic and occasionally ulcerate (Munson et al., 1995) which then may afford transmission of the virus to another host. The additional findings in our report here indicate that there is a second related herpesvirus identified in pulmonary nodules obtained from healthy wild African elephants that 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. 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, one that can cause fatal endotheliotropic disease in Asian elephants and the other in African elephants. Currently, intensive work is underway to further elucidate the epidemiology of these two herpesviruses which are either carried latently, or as

chronic low-level infections in African elephants.

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 nine Asian elephant cases from North America, we have confirmed by PCR and/or morphological techniques similar herpesvirus cases in five Asian elephants from Germany, Netherlands, and Israel (Richman et al., 1999b). The implications of the herpesvirus in wild African elephants are unknown but no herpesvirus-associated illnesses have been observed or reported through the diagnostic laboratory in Onderstepoort, Republic of South Africa. The existence of any elephant herpesviruses in Asian elephants in the wild is currently unknown.

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. 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 by the development of serological tests so that elephants previously exposed to these viruses can be identified. It might be important to minimize the co-mingling or socializing of Asian and African elephants at the same facility or between facilities as this may increase the risk of the transmission of herpesviruses particularly in breeding operations, or where new elephants are being introduced.

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LITERATURE CITED

BOIKE, S. C., M. PUE, P. R. AUDET, M. I. FREED, A. FAIRLESS, B. E. ILSON, AND N. J. D. K. ZARIFFA. 1994. Pharmacokinetics of famciclovir in subjects with chronic hepatic disease. Journal of Clinical Pharmacology 34: 1199–1207.

FOWLES, S. E., AND D. M. PIERCE. 1989. High performance liquid chromatographic method for the determination of 9-(4-hydroxy-3-hydroxymethylbut-1-yl) gnanine (BRL-39123) in human plasma and urine. Analyst 114: 1373–1375.

JACOBSON, E. R., J. P. SUNDBERG, J. M. GASKIN, G. V. KOLLIAS, AND M. K. O'BANION. 1986. Cutaneous papillomas associated with a herpesvirus-like infection in a herd of captive African elephants. Journal of the American Veterinary Medical Association 189: 1075–1078.

MCCULLY, R. M., P. A. BASSON, J. G. PIENAAR, B. J. ERASMUS, AND E. YOUNG. 1971. Herpes nodules in the lnng of the African elephant (*Loxodonta africana*). Onderstepoort Jonrnal of Veterinary Research 38: 225–236.

METZLER, A. E., P. OSSENT, F. GUSCETTI, A. RUBEL, AND E. M. LANG. 1990. Serological evidence of herpesvirus infection in captive Asian elephants (*Elephas maximus*). Journal of Wildlife Diseases 26: 41–49.

MIKOTA, S. K., E. L. SARGENT, AND G. S. RANELAK. 1994. Medical management of the elephant. In-

dira Publishing House, West Bloomfield, Michigan, pp. 54–58.

MOORE, P. S., AND Y. CHANG. 1995. Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and without HIV infection. New England Journal of Medicine 332: 1181–1185.

MUNSON, L., W. KARESH, S. SHIN, J. BALKE, P. CALLE, R. CAMBRE, M. CRANFIELD, S. CITINO, AND R. JUNCE. 1995. Lymphoid follicular vulvitis in African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants. Journal of Zoo and Wildlife Medicine 26: 353–358.

OSSENT, P., F. GUSCETTI, A. E. METZLER, E. M. LANG, A. RUBEL, AND B. HAUSER. 1990. Acute and fatal herpesvirus infection in a young Asian elephant (*Elephas maximus*). Veterinary Pathology 27: 131–133.

RICHMAN, L. K., R. J. MONTALI, R. L. GARBER, M. A. KENNEDY, J. LEHNHARDT, T. HILDEBRANDT, D. SCHMITT, D. HARDY, D. J. ALCENDOR, AND G. S. HAYWARD. 1999a. Novel endotheliotropic herpesviruses fatal for Asian and African elephants. Science 283: 1171–1176.

———, ———, T. HILDEBRANDT, J. FICKEL, D. SCHMITT, AND G. S. HAYWARD. 1999b. Status of a new, fatal herpesvirus disease in elephants in North America and Europe. Verhandlangsbericht des 39 International Symposium uben Erkrankungen der Zoo und Wildtiere, Wien 39: 17–21.

SCHMITT, D. L., AND D. A. HARDY. 1998. Use of famciclovir for the treatment of herpesvirus in an Asian elephant. Journal of the Elephant Managers Association 9: 103–104.

SCHULLER, W., S. CERNY-REITERER, AND R. SILBER. 1990. Evidence that the sheep associated form of malignant catarrhal fever is caused by a herpesvirus. Zentralbl Veterinarmed 37: 442–447.

STEELE, K. E., F. Y. SCHULMAN, H. MENA, AND E. O. STRIMPLE. 1997. Rhabdoid tumor in the brain of a dog. Veterinary Pathology 34: 359–363.

VERE HODGE, R. A., D. SUTTON, M. R. BOYD, M. R. HARNDEN, AND R. L. JARVEST. 1989. Selection of an oral prodrug (BRL 42810; famciclovir) for the antiherpesvirus agent BRL 39123 [9-(4-hydroxy-3-hydroxymethylbut-1-yl) guanine; penciclovir]. Antimicrobial Agents and Chemotherapy 33: 1765–1773.

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