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Systemic equid alphaherpesvirus 9 in a Grant's zebra

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Abstract. A 2-y-old female Grant's zebra (*Equus quagga* [*burchellii*] *boehmi*) was presented with a clinical history of depression, anorexia, and weakness of 1-wk duration. Postmortem examination identified ulcers on the tongue and palate; a large abscess adjacent to the larynx; left lung consolidation; mild swelling, darkening, and congestion of the liver with accentuation of the lobular pattern; and edema and congestion of the distal small and large intestines. Histologic examination identified necrotizing bronchopneumonia, necrotizing hepatitis, nephritis, and enterocolitis. Eosinophilic intranuclear inclusions were detected in syncytial cells and degenerate bronchial epithelium in the lungs and in some hepatocytes associated with necrotic foci. Bacterial cultures of the lung, liver, and laryngeal abscess failed to detect any significant pathogen. Lung and liver tested positive for equine herpesvirus with neuropathogenic marker by real-time PCR. Subsequently, equine herpesvirus was isolated in tissue culture, and the entire viral DNA polymerase gene (ORF30) was sequenced. The zebra lung isolate had a very close nucleotide and amino acid sequence identity to equid alphaherpesvirus 9 (EHV-9; 99.6% and 99.8%, respectively) in contrast to the neuropathogenic T953 strain of EHV-1 (94.7% and 96.6%, respectively). Although zebras are considered the natural host for EHV-9, we document an unusual acute systemic, fatal EHV-9 infection in a 2-y-old Grant's zebra.

Key words: Equid alphaherpesvirus 9; equine herpesviruses; EHV-9; Grant's zebra; zebra herpesvirus.

Herpesviruses are ubiquitous in nature and they have been isolated from many species of animals. At least 9 herpesviruses infect equids (horses, donkeys, zebras, and onagers) and they belong to the subfamilies *Alphaherpesvirinae* and *Gammaherpesvirinae* in the family *Herpesviridae*.[14] Equine herpesviruses (EHV) 1–5 are endemic in the domestic horse (*Equus caballus*) population.[9] EHV-1, -3, and -4 are alphaherpesviruses; EHV-2 and -5 are gammaherpesviruses. Donkeys are infected with 6 asinine herpesviruses, which include asinine herpesvirus (AHV) 1, 2, and 3 (EHV-6, -7, and -8, respectively). AHV-1 and AHV-3 are alphaherpesviruses, whereas AHV-2 is a gammaherpesvirus.[6] Species *Equid alphaherpesvirus 9* (EHV-9) is the newest member in the subfamily *Alphaherpesvirinae.* EHV-9 has been shown to be a highly neurotropic herpesvirus that was originally isolated from the encephalitic brain of a Thomson's gazelle (*Eudorcas thomsonii*).[16] EHV-9 is most closely related to the neuropathogenic EHV-1.[21] The natural host of EHV-9 appears to be the zebra (*Equus grevyi*) and the onager (*Equus hemionus onager*) but it can cross the species barrier and infect a number of zoo and wild animals such as Thomson's gazelle, giraffe, polar bear, llama, and alpaca.[10,12,15–17,20,21] Infection in captive polar bears (*Ursus maritimus*) has caused serious disease including encephalitis.[10,21] Experimental infection of cattle, goats, and swine with EHV-9 has also resulted in encephalitis.[11,18,19,23] EHV-9 infection was detected by seroconversion and PCR of the trigeminal ganglia in a wild Burchell's zebra from Tanzania that did not demonstrate disease.[5,6,8] Thus, it has been suggested that EHV-9 is naturally adapted to the zebra and onager given the absence of clinical disease.[21] We document herein an unusual acute systemic, fatal EHV-9 infection in a 2-y-old Grant's zebra.

A 2-y-old female Grant's zebra (*Equus quagga* [*burchellii*] *boehmi*) had acute onset of anorexia, depression, and weakness of 1-wk duration. No neurologic signs were reported. This animal was 1 of 4 Grant's zebras housed in a dry-lot with no contact with horses. The zebra was in a separate pen within 25 m of a burro (*Equus africanus asinus*), but had no nose-to-nose contact with the burro. At the time of physical examination by the referring veterinarian, the zebra was hypothermic, had marked leukopenia (1.2×10^9/L, reference interval (RI): 4.6–11.5 × 109/L; IDEXX, Sacramento, CA) with absolute neutropenia (0.95×10^9/L, RI: 2.26–8.58 × 10^9/L) and lymphopenia (0.18×10^9/L, RI: 1.5–7.7 × 10^9/L), was hypoglycemic (1.44 and 1.28 mmol/L, RI: 4.16–6.38

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mmol/L), and had a purulent, fetid, bloody nasal discharge. The animal was treated supportively with fluids, B-complex vitamins, vitamin E–selenium, potassium penicillin, and gentamicin. The animal died on day 2 of treatment and was presented for postmortem examination.

At autopsy, the animal was in fair-to-good nutritional state with adequate perirenal and pericardial fat stores. The animal was small for its age, but in relatively good body condition. The sublingual oral mucosa had a 1-cm linear ulcer. Multiple ulcers up to 5.0 mm were noted on the hard and soft palate. The oropharyngeal region had a single large, 2-cm diameter abscess that involved the perilaryngeal connective tissues and adjacent oropharynx and had a large core of thick purulent exudate. The left lung had a focally extensive area of consolidation and reddening of the cranial portion of the caudal lobe in the region of the cardiac notch. The remaining portions of right and left lungs were expanded, with multiple small red (up to 3 mm diameter) nodules present throughout. The liver was mildly swollen, dark, and bloody on cut sections with slight accentuation of the lobular pattern. The wall of the ileum was thickened, wet, red, and edematous. The wall of the cecum and large intestines was thickened with mild serosal edema; the mucosa was diffusely reddened and congested. All other tissues appeared grossly normal.

The lesional lung was cultured for aerobic bacteria and *Mycoplasma*. The liver and oropharyngeal abscess were also cultured using standard aerobic culture. Large intestinal content was cultured for *Salmonella*. Fresh lung, liver, kidney, and spleen were collected and frozen for additional testing. Bacterial cultures of the consolidated regions of the left lung identified small numbers of *Enterobacter cloacae* and mixed flora. The oropharyngeal abscess culture yielded mixed bacteria, including a *Proteus* sp. and some coliforms. The liver cultures had no bacterial growth at 48 h. Cultures of the large intestines failed to detect *Salmonella* sp.

Representative sections of the brain, heart, lungs, liver, kidney, spleen, adrenal gland, stomach, small intestines, cecum, large intestines, and oral ulcers (palate) were fixed in 10% neutral-buffered formalin, trimmed and processed routinely, sectioned at 5 μm, and stained with hematoxylin and eosin. Histologic examination of the lungs identified necrotizing bronchopneumonia characterized by necrosis and loss of bronchiolar epithelium with luminal sloughed necrotic cells. Numerous multinucleate syncytial cells were present in affected bronchioles and bronchi (Fig. 1). Occasional pale eosinophilic intranuclear inclusions (Cowdry type A bodies) were present in affected degenerate epithelium and syncytial cells. The bordering alveoli contained variable numbers of neutrophils, plus cellular debris, fibrin, and edema fluid. The liver had random areas of lytic necrosis with abundant cellular debris and neutrophils (Fig. 2). Eosinophilic intranuclear inclusions were occasionally present in some of the bordering degenerate hepatocytes. The kidney had a focally extensive area of tubular necrosis, and lymphocytic infiltrates in the adjacent renal interstitium. The mucosa of the ileum, cecum, and large intestines had multiple foci of necrosis. Remaining intestinal crypts were dilated, denuded, and filled with debris, variable numbers of neutrophils, and sloughed epithelium. Remaining glandular epithelium was often flattened. The lamina propria also had variable numbers of neutrophils. The affected mucosa and submucosa were edematous and contained fibrin. The oropharyngeal tissues had both partial and complete loss of the surface epithelium with some foci having necrosis of the associated submucosal connective tissue. Affected areas contained variable numbers of neutrophils, some edema, fibrin, and bacteria. A few scattered small submucosal blood vessels also had fibrin thrombi. All other tissues examined were within normal limits.

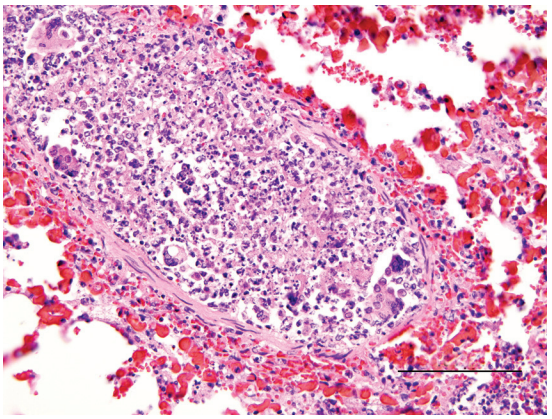

Figure 1. Necrotic epithelium, abundant cellular debris, and scattered multinucleate syncytial cells are present in the bronchial lumen. Adjacent alveoli contain fibrin and necrotic debris. H&E. Bar = 100 μm.

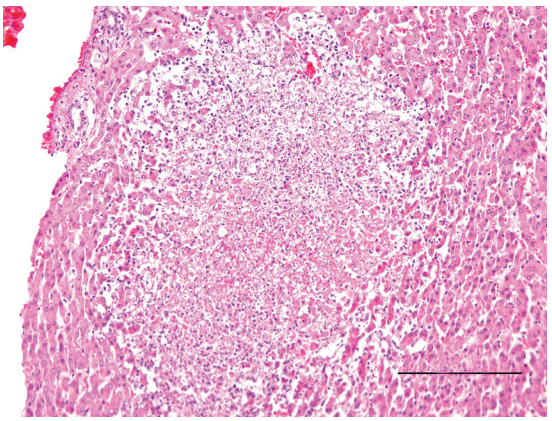

Figure 2. The hepatic parenchyma has areas of lytic necrosis with abundant cellular debris, a few inflammatory cells, and degenerate hepatocytes at the periphery. H&E. Bar = 200 μm.

Immunohistochemical staining for EHV-1 (Polyvalent antibodies, Gluck Equine Research Center, College of Agriculture, Food and Environment, Department of Veterinary Science, University of Kentucky, Lexington, KY) was performed

on the affected lung and identified positive immunolabeling of the affected bronchial epithelium and syncytial cells. Fresh and frozen lung and liver tissues were submitted for EHV-1 real-time (rt)PCR. DNA was extracted from tissue samples using a commercial kit following the recommendations of the manufacturer (High Pure PCR template preparation kit, Roche Diagnostics, Indianapolis, IN). Real-time PCR was performed as described previously,[3] with the exception that the samples were tested in single-plex assays[3] rather than in a multiplex format. Both tissues tested positive for EHV-1 containing the neuropathogenic marker (G_{2254}) only. Partial sequence analysis was performed on the rtPCR amplicon to verify the presence of the neuropathogenic marker.[4] Virus isolation was successfully attempted in equine endothelial cells from lung tissue (10% suspension) according to a standard laboratory protocol at the Maxwell H. Gluck Equine Research Center (Lexington, KY).[22] Subsequently, the entire open reading frame (ORF)30 encoding the viral DNA polymerase was sequenced using a standard laboratory protocol. Comparative nucleotide and amino acid sequence analysis of ORF30 (GenBank accession KC924786) showed that our zebra lung isolate was 99.6% and 99.8% identical to the EHV-9 sequence in GenBank (NC_011644.1). In contrast, the zebra lung isolate had only 94.7% and 96.6% nucleotide and amino acid sequence identity, respectively, to the neuropathogenic T953 strain of EHV-1 (KM593996). These data indicate that the viral isolate from the Grant's zebra associated with the systemic infection was EHV-9.

Systemic herpesviral infections involving natural or adaptive host species are observed most often in young or otherwise immunocompromised individuals or aborted fetuses. Devastating systemic herpesviral infections occur when herpesviruses infect susceptible non-adapted host species. In aberrant hosts with documented clinical EHV-9 infections, central nervous system (CNS) lesions are often identified. Experimental intranasal aerosolization of EHV-9 in horses resulted in fever for a few days but no other clinical signs.[24] Horses were clinically normal at 14 d post-experimentation. Histopathology on the horses at 14 d post-exposure only identified mild lesions in the brain (perivascular mononuclear infiltrates and mild gliosis primarily in the olfactory and limbic system).[24] In our case, histologic lesions were consistent with systemic herpesviral infection affecting the lungs, liver, kidney, and intestines. However, there was no involvement of the CNS, suggesting that EHV-9 may not be neurotropic in the zebra. Testing for EHV-9 on herd mates of the affected zebras was not performed; consequently, herpesvirus status and shedding of virus by herd mates was unknown. The young zebra in our report may represent a primary infection following exposure to an infected herd mate or may represent recrudescence of a latent infection. Aside from its small stature, no lesions were detected to indicate immunosuppression or underlying disease in our case. No other zebras in the herd became ill after the initial infection in this zebra.

Serologic surveys of wild zebras in Africa have demonstrated EHV-9 in many of the species and subspecies of zebra.[5,7] In most studies, no association with clinical disease has been found. This suggests that the zebra may represent the host equid species for this virus. In previous cases of systemic herpesviral infection in zebras and an onager, EHV-1 was initially identified or suspected as the agent responsible for the clinical features observed. However, subsequent sequencing of the virus from several of these zebra cases confirmed EHV-9 rather than EHV-1 infection. Previous results may be attributed to the fact that EHV-9 is closely related to EHV-1, and antibodies and type-specific PCR targeting EHV-1 will cross-react with EHV-9.[20] It is possible these cases may have been identified as EHV-9 had more specific methods (e.g., EHV-9–specific PCR) or subsequent genetic analysis of viral isolates been utilized.[1,2,13,22]

Declaration of conflicting interests

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