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Hemorrhagic Disease in Deer in Arizona

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ABSTRACT: Two mule deer (*Odocoileus hemionus*) and one white-tailed deer (*Odocoileus virginianus*) in Arizona (USA) were submitted for necropsy. Gross and microscopic lesions compatible with hemorrhagic disease (HD) were observed in all three deer. Epizootic hemorrhagic disease virus type 2 (EHDV-2) was isolated from two of the deer. To our knowledge, this is the first documentation of HD in deer in Arizona. Two of the mortalities were attributed to EHDV-2 infection.

Key words: EHDV-2, epizootic hemorrhagic disease, mule deer, *Odocoileus hemionus*, *Odocoileus virginianus*, orbivirus, white-tailed deer.

Epizootic hemorrhagic disease (EHD) has long been recognized as a common disease of white-tailed deer (*Odocoileus virginianus*) in the southeastern United States (Nettles and Stallknecht, 1992) with reported mortality during epizootics ranging between 60 and 90 percent (Prestwood et al., 1974; Roughton, 1975; Hoff and Trainer, 1981). White-tailed deer are reported to be the most sensitive wild species to the EHD viruses (Nettles and Stallknecht, 1992; Nettles et al., 1992b). Mule deer (*Odocoileus hemionus*), though often affected less severely than white-tailed deer (Thorne et al., 1982), are also susceptible to disease caused by EHD viruses (Barker et al., 1992).

The first EHD virus was isolated in 1955 from white-tailed deer in New Jersey (Shope et al., 1960). By 1990, 32 states in the United States had reported hemorrhagic disease (HD) in deer due to either the EHD viruses or to the antigenically similar bluetongue (BT) viruses (Gibbs and Greiner, 1989; Nettles and Stallknecht, 1992). During a 1971 epizootic across the southeastern United States, both EHD and BT viruses were isolated from a single animal (Prestwood et al., 1974; Thomas et al., 1974). Both viruses can be active concurrently in the same deer population (Hoff and Trainer, 1981) and in the same vector population (Barker et al., 1992). The midge (*Culicoides variipennis*) is the primary vector for both EHD and BT viruses in the United States (Foster et al., 1977; Jones et al., 1977; Nettles et al., 1992a).

The absence of reports of HD in wild ungulates in the southwest United States is notable because serologic studies have revealed high prevalence for antibody to BT virus in both wild and domestic ungulates in these areas (Trainer and Jochim, 1969; Metcalf et al., 1981; Nettles et al., 1992a). Several reviews of HD in the United States have not mentioned disease in deer from Arizona though disease has been reported in various wild ungulate species in neighboring states (Trainer and Jochim, 1969; Hoff and Trainer, 1978; Nettles et al., 1992a, b; Nettles and Stallknecht, 1992; Pearson et al., 1992b).

This report documents the first three cases of HD in deer diagnosed by the Arizona Veterinary Diagnostic Laboratory (AZVDL; Tucson, Arizona, USA). The first two cases occurred in 1993 and were temporally and geographically related; the third occurred in 1996 and was geographically unrelated to the first two.

On 14 July 1993, a mule deer doe was found dead at the Buenos Aires National Wildlife Refuge (BANWR; 31°34′N, 111°30′W, elevation 1065 m) in southern Arizona. The refuge is bordered on the south by the Republic of Mexico. The deer was found 1.6 km north and 1.6 km west of the BANWR main entrance in desert

grassland-type habitat. The doe was estimated to have died 6 hr prior to being found and was transported to the AZVDL for necropsy (AZVDL Case #93-2016).

At necropsy corneas of both eyes were cloudy. Several circular areas of faint white discoloration suggestive of mucosal erosions were present on the lateral aspect of the tongue and the buccal mucosa of the lips. Locally extensive ecchymotic hemorrhages covered much of the serosal surfaces of the rumen and intestine. Patches of red discoloration were visible on the ruminal mucosa. Extensive hemorrhage with hematoma formation was present along the sciatic nerves of both hind legs. The renal cortices were pale.

Microscopically there was generally mild lymphoplasmacytic perivasculitis and vasculitis in the brain, the tongue, and occasionally in the lung. Partial atelectasis was evident in the lung. Hemorrhage was prominent in lymph nodes, adrenal cortex, and in the muscularis, serosa, and serosal fat of the rumen. Ruminal papillae were hemorrhagic or necrotic and sloughing; adjacent viable tissue often contained intense infiltrates of neutrophils. Necrosis of myofibers accompanied the hemorrhagic lesions in hindleg musculature. Severe necrosis and hemorrhage of the renal and adrenal cortices was observed. Pulmonary microvasculature was congested along with multifocal alveolar edema. Acute uveitis and keratitis were present in both eyes. Hypopyon occurred in one eye.

Spleen tissue was sent to the National Veterinary Services Laboratories (NVSL; Ames, Iowa, USA) where spleen-derived inoculum was injected intravenously into embryonating chicken eggs (ECE) and onto baby hamster kidney (BHK-21) cell cultures using standard methodology as described by Pearson et al. (1992a). Virus isolation attempts were negative for BT and EHD viruses.

The second case, a white-tailed deer doe, occurred 18 days later on 1 August 1993. The doe was found behind the Santa Margarita Ranch corrals by ranch personnel (31°41′N, 111°35′W, elevation 1199 m). The ranch lies 14.5 km northwest of the BANWR on similar desert grassland-type habitat. The doe was observed for approximately 1.5 hr. She had difficulty standing and walking and appeared to be blind. Generalized hair loss was evident. The doe was captured manually by ranch hands. She began thrashing and died during transport to the AZVDL (AZVDL Case #93–2252).

At necropsy, the cornea of one eye was cloudy. There was prominent thinning of the hair over the dorsal and lateral aspects of the body along with several areas of moderate edema and bruising of the underlying subcutis. The lungs were dark red. A small amount of ingesta was present in the rumen, and very few fecal pellets were present in the colon, suggesting that she had been anorectic for some time. The uterus contained a late-term female fetus. Placental separation from the maternal caruncles was evident.

Microscopically there was generally mild lymphoplasmacytic perivasculitis and vasculitis in the brain, spinal cord, and lung. Pulmonary microvasculature was congested, and there was spotty alveolar hemorrhage and multifocal alveolar edema along with partial atelectasis. Mild hemorrhage was evident in one section of skeletal muscle, and spotty hemorrhages were occasionally found in the lamina propria of some villous tips of intestinal mucosa. There was multifocal necrosis of chorionic epithelium and intravascular thrombosis in a section of uterine caruncle. Acute keratitis with erosion of the corneal epithelium was present in one eye and was accompanied by an acute uveitis and hypopyon. The NVSL isolated epizootic hemorrhagic disease virus type 2 (EHDV-2) from spleen using the methodology described previously (Pearson et al., 1992a).

The third case, a lactating mule deer doe, was found on 22 October 1996 by Arizona Game and Fish Department (AGFD) personnel in Hualapai Mountain County Park, Mohave County, Arizona,

(35.0931°N, 113.8748°W, elevation 1981 m). The doe was found dead approximately 0.64 km from a private lake which was fed by a slow-moving stream in chaparral and ponderosa pine. She was observed having several seizures 24 hr prior to death. The animal was transported to the AZVDL for necropsy (AZVDL Case #96–3346).

Gross lesions included a few mucosal erosions on the tip of the tongue. Edema was observed in muscle and subcutis at the thoracic inlet, in bone marrow, coronary grooves of the heart, lesser omentum, and adventitial tissues around the esophagus and trachea. The parotid salivary glands were red-tan and firm.

Microscopically there was mild lymphoplasmacytic vasculitis in the brain, meninges, and submucosa of the tongue and abomasum. Small foci of interstitial hemorrhage with multifocal coagulative necrosis of myofibers was present in the heart. Multifocal interstitial hemorrhage with focal acinar necrosis occurred in parotid salivary glands. Epizootic hemorrhagic disease virus type 2 was isolated from spleen at the NVSL using the previously described methods (Pearson et al., 1992a).

The lesions of HD described in this report are generally compatible with those previously reported (Karstad et al., 1961; Karstad and Trainer, 1967; Hoff and Trainer, 1981; Barker et al., 1992). Though finding typical lesions is considered adequate for a diagnosis of HD, virus isolation is necessary to establish an etiologic diagnosis of either EHD or BT (Hoff and Trainer, 1981). Failure to isolate BT or EHD viruses in cases of HD is not unusual (Nettles and Stallknecht, 1992; Fischer et al., 1995).

Woods et al. (1996) reported the occurrence of a new disease in California deer which has been named adenovirus hemorrhagic disease (AHD). Lesions are similar to those of EHD, but AHD is characterized (and differentiated from EHD) by the formation of intranuclear inclusions in endothelial cells. Archived sections of lung tissue from all three cases were reviewed retrospectively and compared with a reference slide from a deer with AHD. There was no evidence of adenovirus infection in our cases.

Hemorrhagic disease is not recognized as a significant problem among wild cervids in Arizona. This may be due to enzootic stability in which a near-perfect host-virus relationship exists with high levels of herd immunity and low incidence of clinical disease (Stallknecht et al., 1996). In the fall of 1993, serum samples from both mule deer and white-tailed deer in Unit 36 (which encompasses the BANWR) were collected at a hunter check station. Fifteen (75%) of 20 had serum neutralizing antibody to EHDV-2, indicating substantial activity of the virus among deer in the area (AZVDL Case #93-3154). Additionally, testing indicated 55% had antibody to BTV-17, 50% to BTV-11, 35% to BTV-13, and 5% to EHDV-1. None of the samples tested had antibody to BTV types 2 or 10. Serologic testing was performed at the NVSL according to methods described by Pearson et al. (1992a).

Prestwood et al. (1974) suggested that high host population densities may contribute to the occurrence of disease. Helicopter surveys by AGFD Region V personnel reported higher population densities of mule deer in Unit 36 (which encompasses the BANWR) in 1992, as compared to the preceding 3 yr, after which the population began declining (Fink and Heffelfinger, 1999).

Disease is also associated with "blooms" of *Culicoides* which may cause animals to be exposed to high levels of virus (Nettles and Stallknecht, 1992; Tabachnick, 1996). *Culicoides* larvae need moisture for development; even as little as that held in the damp decaying parts of cacti in the desert will suffice (Blanton and Wirth, 1979). Several man-made water catchments, including stock ponds and a watering trough, were also present in the area of the first two dead deer. Rainfall data for 1993 were analyzed in the area Sasabe 6 NNE

(BANWR); it was found to be higher for the summer "monsoon" months of June, July, and August when compared with previous years and may have contributed to a higher vector population.

Other factors that may also contribute to the lack of reported disease may relate to innate host resistance, fawn protection through maternal antibody transfer, poor detection of affected animals, and vector seasonality (Stallknecht et al., 1996).

To our knowledge, this is the first report of HD in deer in Arizona and the first confirmation of EHDV-2 in Arizona. Studies of *Culicoides* spp. in Arizona, including vector capacity and competence as defined by Tabachnick (1996) and additional serosurveys of various wild ungulates in Arizona should be considered for the future.

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