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Hemorrhagic Disease in Bighorn Sheep in Arizona

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ABSTRACT: Two bighorn sheep from Arizona (USA) were submitted for necropsy. One was a Rocky Mountain bighorn (*Ovis canadensis canadensis*) and the other was a desert bighorn (*Ovis canadensis mexicana*). Both had lesions consistent with those of hemorrhagic disease (HD). Epizootic hemorrhagic disease virus (EHDV) type-2 and bluetongue virus (BTV) type-17, respectively, were isolated from the sheep tissues. To our knowledge, HD caused by either EHDV or BTV infection has not been documented previously in Arizona bighorn sheep.

Key words: Arizona bighorn sheep, bluetongue, desert bighorn sheep, EHD, epizootic hemorrhagic disease, orbivirus, *Ovis canadensis canadensis, Ovis canadensis mexicana,* Rocky Mountain bighorn sheep.

Epizootic hemorrhagic disease (EHD) and bluetongue (BT) viruses are antigenically closely related orbiviruses (Gibbs and Greiner, 1988) that have been reported to cause hemorrhagic disease (HD) in wild ungulates (Sohn and Yuill, 1991). Ungulate species vary in their degree of susceptibility to each virus (Sohn and Yuill, 1991; Barker et al., 1992). The most sensitive wild species to both EHD and BT viruses is reported to be white-tailed deer (WTD; *Odocoileus virginianus*) (Nettles and Stallknecht, 1992; Nettles et al., 1992b). Mule deer (*Odocoileus hemionus hemionus*) are also susceptible to disease caused by EHD and BT viruses (Barker et al., 1992). Disease due to bluetongue virus infection (BTV) has been documented in pronghorn antelope (*Antilocapra americana*) (Thorne et al., 1988) and several species of zoo ungulates (Hoff et al., 1973). Bluetongue virus was first documented as causing disease in a desert bighorn sheep (DBHS; *Ovis canadensis*) in Texas (USA; Robinson et al., 1967).

Previous reports of epizootic hemorrhagic disease virus (EHDV) infection in bighorn sheep (BHS) include the isolation of EHDV type-1 from a sick, captive peninsular bighorn lamb (*Ovis canadensis cremnobates*) from the Santa Rosa herd in California (USA) in 1983 (Jessup, 1985). In addition, EHDV type-2 was isolated from a BHS in British Columbia, Canada in association with an outbreak of EHD in other ungulates (Dulac et al., 1988). Lesions and clinical signs in the affected animals were not specifically described in either report.

Several excellent comprehensive reviews of HD (Trainer and Jochim, 1969; Hoff and Trainer, 1981; Nettles et al., 1992a; Nettles et al., 1992b; Nettles and Stallknecht, 1992; Pearson et al., 1992b) do not mention any occurrence of HD in BHS in Arizona, however a high prevalence of antibody to BTV has been reported in cattle in Arizona, indicating that viral activity exists in the state (Metcalf et al., 1981).

This report documents two cases of HD in Arizona BHS seen by diagnosticians at the Arizona Veterinary Diagnostic Laboratory (AZVDL; Tucson, Arizona, USA). In both cases, virus was isolated; the mortalities were attributed to EHDV infection in the first case and to BTV infection in the second case.

On 6 October 1995, a dead male Rocky Mountain bighorn lamb (*Ovis canadensis canadensis*) was found during a helicopter survey of the Blue River and Clear Creek

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area north of Clifton, Arizona in pinyon pine and juniper-type habitat (33°20′N, 109°11′W, elevation 5500′). The lamb was estimated to have been dead 1 to 2 hrs, and the age was estimated to be 3 mo. A herd of Rocky Mountain bighorn sheep (RMBHS) was observed within 200 m of the dead lamb. The RMBHS were part of a group of 28 sheep that were translocated from the Gunnison (Colorado, USA) area to the Clifton area on 24 January 1995 as part of an attempt to reintroduce RMBHS to habitat that they had previously occupied in Arizona (Lee, 1996).

A field necropsy examination was performed by one of the authors (Cagle). Externally, there was greenish discharge from the nose and blood around the anus. Small dark spots suggestive of hemorrhages were noted on the conjunctival membranes of the eyes. Internally, hemorrhages were present on the serosal surfaces of the rumen and intestine.

Tissue samples were collected at necropsy and submitted to the AZVDL for microscopic evaluation (AZVDL Case #95-2762). In a section of heart there was a focus of myocardial necrosis. Hemorrhages were present in the epicardium, epicardial fat, myocardium, and tunica muscularis and submucosa of the rumen. In the lung, there was diffuse congestion of microvasculature. Alveolar edema was evident in one section of lung. The lesions were felt to be consistent with those reported for HD (Barker et al., 1992).

A spleen sample was sent to the National Veterinary Services Laboratories (NVSL; Ames, Iowa, USA) where inoculum was injected into embryonating chicken eggs (ECE) by the intravenous route and inoculated onto baby hamster kidney (BHK-21) cell cultures using standard methodology described by Pearson et al. (1992a). Epizootic hemorrhagic disease virus type-2 (EHDV-2) was isolated.

The source of the EHDV-2 infection in the RMBHS was unknown. Mule deer and cattle were present in portions of the habitat and domestic sheep were grazed approximately 16 km away. The serologic status of the deer, domestic sheep, and cattle was not determined.

Midges of the genus *Culicoides* are the primary vector for EHD and BT viruses (Mullens and Dada, 1992; Jones et al., 1996). The presence of *Culicoides* spp. within the habitat was not determined. Perennial riparian areas (Clear Creek and Blue River), where breeding of midges might occur, were within 5 to 6 km of the location where the RMBHS lamb was found.

Additional studies to establish the serologic status of resident ungulates within the habitat, as well as the presence of competent *Culicoides* spp. vectors are felt to be warranted, particularly if additional transplants of RMBHS are contemplated.

The second case, a captive 10-yr-old female desert bighorn sheep (DBHS) (*O. canadensis mexicana*), was received for necropsy examination at the AZVDL on 29 September 1998 (AZVDL Case #98-3818). The ewe had lived since birth at the Arizona-Sonora Desert Museum (ASDM; 32°14′39″N, 111°10′07″W, elevation 2821′) which houses a collection of living flora and fauna indigenous to the Sonoran Desert and is located 19 km west of Tucson, (Arizona). The animal was first examined at the ASDM five days prior to death for a swollen head, face, and lips. Treatment included diphenhydramine, prednisone, and antibiotics. The following day, most of the head swelling had resolved. The next 2 days, the animal was lethargic but eating. On the fourth day, the animal's condition deteriorated and death occurred the following day.

Necropsy findings included extensive ventral subcutaneous edema from the jaw to the pelvis. The thorax and pericardial sac contained excess clear yellow fluid. There were multifocal epicardial hemorrhages and a hemorrhage in a papillary muscle of the left ventricle. There were scattered gray plaques on the surface of the gall bladder mucosa. The uterine lu-

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men contained approximately 1 l of tan waxy material.

Microscopically, there were scattered perivascular hemorrhages in sections of the cerebral cortex, midbrain, medulla, and thalamus of the brain. In addition, there was a mild encephalitis characterized by focal gliosis and a focal perivascular cuff of lymphocytes in the cerebral cortex. Infiltrates of lymphocytes were present in the adrenal medulla. There was multifocal lymphocytic perivasculitis in the endocardium, epicardium, and myocardium of the heart with focally extensive myocardial necrosis and hemorrhage in a papillary muscle. Collectively, the lesions were felt to be compatible with those of HD (Barker et al., 1992).

Spleen tissue was sent to the NVSL where polymerase chain reaction (PCR) testing performed on the sample was positive for BT viral RNA and negative for EHD viral RNA. The specimen was also inoculated intravenously into ECE and onto BHK-21 cell cultures. Bluetongue virus was isolated and identified as serotype-17 by PCR testing. Standard methods for testing performed at the NVSL have been described (Pearson et al., 1992a; Katz et al., 1993; Wilson, 1994; Eaton, 1996; Johnson et al., 2000).

Culicoides variipennis sonorensis is the primary North American vector of the BT viruses and has recently been elevated to species rank *C. sonorensis* (Holbrook et al., 2000); the presence of competent *C. sonorensis* is thought to determine BT distribution in the USA (Tabachnick, 1996). The arid Sonoran Desert habitat surrounding the ASDM would not normally be expected to harbor breeding sites for *Culicoides* spp. since the larvae need moisture to develop; however, as little as that held in damp decaying parts of cacti in the desert will suffice (Blanton and Wirth, 1979). Precipitation records at the ASDM indicated that the summer "monsoon" rainfall (July–September) for 1998 was higher than in the previous 3 yr. Subsequently, a survey by two of the authors (Noon and Wesche) revealed approximately 15 potential breeding sites for *Culicoides* at the ASDM. These included pooled run-off from washing down animal pens, fountains, and artificial ponds in exhibits throughout the Museum.

On three occasions in October 1998, 10 to 14 days following the DBHS ewe's death, insects were collected using CDC miniature light-traps (Model 512, John W. Hock Company, Gainesville, Florida, USA) by one of the authors (Mead) around suspected breeding sites at the ASDM. Samples were transported to the Medical Entomology Laboratory (University of Arizona, Tucson, Arizona) on dry ice, then sorted and pooled by species (84 individuals, 13 pools total) based on morphologic characteristics. Four species of *Culicoides* were found, with the predominant species being *C. sonorensis.* Pools were individually macerated in Hank's balanced salt solution (HBSS) and clarified by low speed centrifugation (10 min at 3000 RPM; Beckman, Model GS-6R, Fullerton, California). Pools were screened according to methods described by Smith et al. (1996) for HD viruses by inoculating resultant supernatant fluids on BHK-21 and cattle pulmonary artery endothelial (CPAE) cells (American Type Cell Culture Collection, Rockville, Maryland, USA) and incubating at 37 C in a 5% CO_2 atmosphere. Cells were observed daily for cytopathic effects. None were observed. After 14 days all samples were determined to be negative.

Hemorrhagic disease is not currently recognized as a significant problem in Arizona ungulates. This may be due to enzootic stability, where a near-perfect host-virus relationship exists among indigenous populations and a high level of herd immunity occurs with a low incidence of clinical disease (Stallknecht et al., 1996). Grazing of domestic ungulates does not occur adjacent to the ASDM, however an indigenous mule deer population exists in the surrounding Sonoran Desert habitat. In the course of a previous study in 1995, five

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mule deer were captured at distances between 3 to 6 km from the ASDM (P. Krausman, pers. comm.). Blood samples drawn for routine disease surveillance purposes (AZVDL Case #95-3192) were all positive for serum neutralizing (SN) antibody to BTV-17, the same agent that apparently caused HD in the female DBHS at the ASDM. All five mule deer tested also were found to have SN antibody to BTV-11, EHDV-1, and EHDV-2. Four of the five had antibody to BTV-13. Serologic testing was done at the NVSL using methodology described by Pearson et al. (1992a). Clinical disease was not observed in the deer population. However, the lack of disease in indigenous deer populations does not mean that HD could not present a disease risk to other ungulate species (Stallknecht et al., 1996). Transplanted animals from nonenzootic areas or captive animals may be susceptible to disease caused by these viruses.

We believe these two cases of HD are significant for several reasons: there are very few reports of HD in bighorn sheep species; there is good evidence that HD viruses are enzootic in arid environments and do not normally cause disease in these areas; and these viruses are a factor to be considered in wildlife management plans (Hoff and Trainer, 1981), especially those involving translocation or maintenance of susceptible species in captivity. To the best of our knowledge, this is the first documentation of HD caused by either BTV or EHDV infection in BHS in Arizona. This is also the first reported isolation of BTV from a captive DBHS in Arizona, and of EHDV-2 from a lamb which was the offspring of transplanted RMBHS from Colorado. Studies to further define the ecology of these viruses in the various ungulate habitats of Arizona should be considered for the future, including vector capacity and competence of *Culicoides* spp. as defined by Tabachnick (1996), as well as additional serosurvey studies on resident ungulates of the arid southwest.

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