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## Malignant Catarrhal Fever Associated with Ovine Herpesvirus-2 in Free-ranging Mule Deer in Colorado

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**ABSTRACT:** Malignant catarrhal fever (MCF) was diagnosed in four free-ranging mule deer (*Odocoileus hemionus*) in January and February of 2003. Diagnosis was based on typical histologic lesions of lymphocytic vasculitis and PCR identification of ovine herpesvirus-2 (OHV-2) viral genetic sequences in formalin-fixed tissues. The animals were from the Uncompahgre Plateau of southwestern Colorado. Deer from these herds occasionally resided in close proximity to domestic sheep (*Ovis aries*), the reservoir host of OHV-2, in agricultural valleys adjacent to their winter range. These cases indicate that fatal OHV-2 associated MCF can occur in free-ranging mule deer exposed to domestic sheep that overlap their range.

**Key words:** Colorado, free-ranging, malignant catarrhal fever, mule deer, *Odocoileus hemionus*, ovine herpesvirus-2.

Malignant catarrhal fever (MCF) is an infectious disease that affects a variety of wild and domestic ruminant species. Clinical signs of MCF in domestic cattle (*Bos taurus*) include fever, lacrimation, corneal opacity, anorexia, salivation, diarrhea, melena, and hematuria, often accompanied by mucopurulent ocular and nasal discharge (Heuschele and Reid, 2001). On postmortem, ulcerations of the upper respiratory and gastrointestinal mucosae and enlarged lymph nodes are observed, and histologic lesions typically include lymphocytic vasculitis, lymphoid proliferation, and epithelial necrosis in the mucosae of the gastrointestinal tract, urinary bladder, and respiratory tract (Heuschele and Reid, 2001). The disease has been recognized in cattle for many years and more recently has been recognized in other animal species. Typical clinical presentation and postmortem findings can vary among affected species, as

has been reported in American bison (*Bison bison*) (Schultheiss et al., 2000).

Two major forms of MCF are recognized, a wildebeest-associated form caused by alcelaphine herpesvirus 1 (AHV-1) and a sheep-associated form caused by ovine herpesvirus 2 (OHV-2). These gamma herpesviruses can reside in peripheral blood leukocytes of their respective host animal species without causing clinical disease, but can spread to susceptible animal species through direct contact and cause clinical MCF. In clinical MCF, the viruses infect lymphocytes and epithelial cells. The sheep-associated form of MCF occurs in North America. Antibodies to OHV-2 or viral DNA of OHV-2 can be found in almost all adult domestic sheep, and domestic sheep are considered the major reservoir of the agent of MCF in North America (Li et al., 1995). Recently, other viruses associated with MCF have been identified in a variety of animal species, including caprine herpesvirus-2 (CpHV-2) (Keel et al., 2003) and other rhadinoviruses similar to MCF virus of white-tailed deer (Li et al., 2003a).

Diagnosis of MCF in domestic animals and wildlife is based on histologic identification of typical lymphocytic vasculitis that can be found in multiple tissues and in North America is further supported by PCR demonstration of OHV-2 or CpHV-2 viral sequences in tissues. In the present study, we offer evidence for OHV-2 infection in four free-ranging mule deer from Colorado with histologic lesions of MCF.

Cases of MCF have been described in many captive wildlife species in the United States and other countries (Ta-

TABLE 1. Captive wildlife species in which malignant catarrhal fever has been reported in the United States and other countries.

Common name	Scientific name	Location	Citation
Axis deer	<i>Axis axis</i>	Arizona, USA	Li et al., 1999
Mule deer	<i>Odocoileus hemionus</i>	Arizona, USA	Li et al., 1999
Reeve's muntjac	<i>Muntiacus reevesi</i>	Arizona, USA	Li et al., 1999
Reindeer	<i>Rangifer tarandus</i>	Arizona, USA	Li et al., 1999
Barbary red deer	<i>Cervus elaphus barbarus</i>	California, USA	Klieforth et al., 2002
Bison	<i>Bison bison</i>	Colorado, Utah, USA	Schultheiss et al., 2000
Shira's moose	<i>Alces alces shirasi</i> Nelson	Wyoming, USA	Williams et al., 1984
Sika deer	<i>Cervus nippon</i>	Arizona, USA	Keel et al., 2003
White-tailed deer	<i>Odocoileus virginianus</i>	Minnesota and Texas, USA	Li et al., 2003
White-tailed deer	<i>Odocoileus virginianus</i>	Missouri, USA	Kleiboeker et al., 2002
White-tailed deer	<i>Odocoileus virginianus</i>	Arizona, USA	Li et al., 1999
White-tailed deer	<i>Odocoileus virginianus</i>	New Jersey, USA	Brown and Bloss, 1992
Brown brocket deer	<i>Mazama gouazoubira</i>	Brazil	Driemeier et al., 2002
Père David's deer	<i>Elaphurus davidianus</i>	England	Flach et al., 2002; Reid et al., 1987
Red deer	<i>Cervus elaphus</i>	New Zealand	Audige et al., 2001
Roan antelope	<i>Hippotragus equinus</i>	England	Gulland et al., 1989
Rusa deer	<i>Cervus timorensis</i>	Australia	Tomkins et al., 1997
Swamp deer	<i>Cervus duvauceli</i>	England	Flach et al., 2002

ble 1). Contact with domestic sheep was found in cases involving captive bison, Shira's moose, brown brocket deer, and rusa deer. Cases of MCF in white-tailed deer and Sika deer have been associated with CpHV-2, and contact between goats and affected deer has been documented (Li et al., 2003b).

In contrast to captive species, the only previous case of MCF reported in free-ranging animals in North America was in black-tailed deer (*Odocoileus hemionus columbianus*) in California (Jessup, 1985), but this report does not conclusively identify MCF as the disease. The gross findings described, such as hyperemia of the oronasal mucosa, conjunctivitis, mucopurulent nasal discharge, ulcers of the tongue and oral mucosa, coronitis, pulmonary edema, and histopathologic lesions, such as fibrinoid necrosis of arteries and lymphocytic perivascular cuffing, are compatible with but not exclusively found in MCF cases. Similar gross and histologic lesions have been described for other viral infections of deer, including epizootic hemorrhagic disease of deer virus (EHDV), bluetongue virus (BTV), adeno-

virus hemorrhagic disease virus (AHDV), and bovine viral diarrhea virus (BVDV). Moreover, the depletion and necrosis of lymphoid tissues described by Jessup (1985) are not characteristic of MCF cases. The author was unable to establish an etiology using animal inoculation and virus isolation in cell culture. The cell types in which virus isolation was attempted do not support replication of OHV-2, and other viruses including non-cytopathic BVDV, BTV, or EHDV were not detected by these methods. The molecular biological techniques to detect the DNA genome of OHV-2 were not available at that time.

Four cases of MCF in free-ranging mule deer occurred in January and February of 2003 on the Uncompahgre Plateau of southwestern Colorado. Deer were radio collared in two separate winter range study areas (38°26'00"N, 108°01'00"W and 38°21'00"N, 107°48'30"W) as part of an ongoing mule deer study evaluating habitat effects on survival (Bishop et al., 2003). Both areas comprise lower-elevation pinyon-juniper habitat adjacent to agricultural valleys. One winter range study area is

a sheep grazing allotment. Sheep were not present on the allotment during the winter months; however, deer were occasionally in close proximity to sheep in the valley. Residual forage in the agricultural fields is used by livestock operators for winter feeding. Deer feed in these same fields, particularly from late October through December and again during mid-March through April. The approximate center of the summer range was 38°12'00"N, 107°56'00"W, in the southern Uncompahgre Plateau and San Juan Mountains. Most of the radio-collared deer in the area have summer range located on private lands. The majority of these private lands are used for sheep and cattle grazing in the summer, and the remaining lands have been converted to variable-density housing developments. The amount of contact between deer and sheep varied spatially and temporally, being mostly dependent on the movement of sheep between different summer pastures. Contact between deer and sheep was greatest when sheep were first released into a new pasture, particularly during fawning in June when adult does were often sedentary because of newborn fawns. There was minimal contact between deer and sheep on winter range because of differential timing of land use. Although one winter study area contained a sheep grazing allotment, deer and sheep were not present on the allotment at the same time. However, deer were occasionally in close proximity to sheep in the adjacent agricultural valley. It is estimated that approximately 25% of the deer share summer range with sheep, based upon the extent of overlap between land used for sheep grazing and the summer home ranges of the radio-collared mule deer sample.

The affected animals were three adult (>2 yr old) females and one fawn of approximately 7 mo of age. The first animal was an adult doe that was observed to be thin, weak, and have diarrhea. The second adult doe and the fawn were found dead. The fourth animal was a moribund

adult doe. Various formalin-fixed tissues, including lung from all four animals, were submitted to the Colorado State University Veterinary Diagnostic Laboratory (Fort Collins, Colorado, USA) for histologic examination and ancillary tests. The first three animals had histologic lesions consistent with MCF. Lungs had mild to moderate lymphocytic vasculitis, capillary congestion, and accumulation of low-protein edema fluid in alveolar spaces. Heart sections submitted from the two does had lymphocytic vasculitis and acute necrosis of adjacent myofibers. Kidney from the fawn had lymphocytic vasculitis, which was most prominent at the corticomedullary junction but also present in scattered areas of the cortex. The bladder wall of the fawn had lymphocytic vasculitis but the lesions had not progressed to hemorrhage. Liver from the fawn had no significant changes. The lung from the fourth animal, an adult doe, had severe congestion but did not have vasculitis. The severe congestion could reflect peracute vascular injury due to OHV-2 infection and would have contributed to death. Kidney from this doe had nephrosis with many oxalate crystals in renal tubules, possibly due to consumption of oxalate containing plants; thus, renal failure would also have been a contributing factor in her death. Fibrinoid vascular necrosis, a prominent lesion in cattle with MCF, was not observed in any tissues from the four mule deer. The absence of fibrinoid vascular necrosis has also been noted in bison affected with MCF (Schultheiss et al., 2000).

In all four mule deer, genetic sequences of OHV-2 were detected in lung tissue by polymerase chain reaction (PCR) technique. Lung tissue was fixed in 10% buffered formalin for approximately 24 hr, routinely processed, and embedded in paraffin. Unstained histologic lung sections from paraffinized blocks were deparaffinized by immersing the slides into xylene substitute histologic clearing agent (Sigma-Aldrich Inc., St. Louis, Missouri, USA) for 10 min at room

temperature, followed by sequential 5-min incubations at room temperature in 100% ethanol, 90% ethanol, and deionized, distilled water. Extraction of DNA was performed using the QIAamp DNA Mini Kit (Qiagen Inc., Valencia, California, USA) following the tissue protocol provided by the manufacturer. The PCR assay for detection of OHV-2 DNA was performed with the use of a nested process with primer sets targeting OHV-2 genetic sequences gene as described by Li (Li et al., 1995). The amplification reactions were performed in a total volume of 25  $\mu$ l consisting of High Fidelity PCR Master Mix (Roche Diagnostics, Indianapolis, Indiana, USA) and 2  $\mu$ M each of the forward and reverse primers. Five microliters of DNA extract were added to the flank PCR reactions. Five microliters from the completed flank reaction were used as template for the nested PCR reaction. Amplification was performed on a Techne Genius thermal cycler (Techne Inc., Cambridge, England) under conditions of one cycle of 5 min at 94 C followed by 35 cycles of 94 C for 1 min, 60 C for 1 min, and 72 C for 1 min and subsequently a final extension of 5 min at 72 C. Amplification of the targeted OHV-2 polymerase gene results in a 423-bp (base pair) product in the flank reaction and a 238-bp product in the nested reaction. These reaction products were detected in all four deer samples by 1.5% agarose gel electrophoresis to which 15  $\mu$ l of the reaction product was applied. Therefore, OHV-2 DNA was detected in lung tissue from all four deer tested.

Deparaffinized tissues were also subjected to RNA extraction with the RNeasy Mini Kit (Qiagen Inc., Valencia, California, USA) and tested by RT-PCR for the presence of RNA of bluetongue virus (Wilson and Chase, 1993), EHDV (Wilson, 1994), and bovine viral diarrhea virus (Ridpath et al., 1994). The RNA from these viruses was not detected in any of the lung tissue samples.

In attempt to identify more cases of

OHV-2 infection in clinically normal mule deer in the herd, the peripheral blood leukocytes (PBLs) from an additional 32 animals from the herd were tested for genetic sequences of OHV-2 by PCR and all were negative. This finding is not unexpected. It is possible that all OHV-2-infected deer succumb to MCF rather than developing a detectable carrier state. Alternatively, the percentage of deer infected with OHV-2 may be very low, and the sample size of 32 may have been too small to identify deer infected with OHV-2. Also, it is possible that infected deer could have a latent viral infection with virus not present in PBLs and therefore undetectable in these living animals. Finally, PCR assays are subject to false-negative results due to inhibition of the enzymatic reaction by undefined substances present in tissues (Dr. Tim Crawford, pers. comm.) and viral DNA in the surveyed deer may have escaped detection because of this type of non-specific interference with the PCR assay. These four cases show that OHV-2 associated MCF occurs in free-ranging mule deer, resulting in mortality. Diagnosis of MCF was based on typical histologic lesions and demonstration by PCR of OHV-2 viral DNA in these deer. Infection of these mule deer with OHV-2 presumably occurred via exposure to domestic sheep with which they share range.

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