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Malignant catarrhal fever-like disease in sheep after intranasal inoculation with ovine herpesvirus-2

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Abstract. A malignant catarrhal fever (MCF)–like disease was induced experimentally in 3 sheep after aerosol inoculation with ovine herpesvirus-2 (OvHV-2). Each of 3 OvHV-2–negative sheep was nebulized with 2 ml of nasal secretions containing approximately $3.07 \times 10^5$ OvHV-2 DNA copies from a sheep experiencing an intensive viral-shedding episode. Ovine herpesvirus-2 DNA became detectable by polymerase chain reaction in the peripheral blood leukocytes of all 3 sheep within 3 days, and all 3 seroconverted between 6 and 8 days postinfection (PI). The sheep developed clinical signs, with copious mucopurulent nasal discharge and fever around 14 days PI. One of the 3 clinically affected sheep was euthanized at 18 days PI. Major lesions at necropsy were multifocal linear erosions and ulcers in mucosa of the cheeks, tongue, pharynx, and proximal esophagus and mild disseminated pneumonia. Microscopically, there was extensive moderate superficial histiocytic–lymphocytic rhinitis with epithelial dissociation and degeneration. Moderate multifocal histiocytic bronchointerstitial pneumonia was associated with loss of terminal bronchiolar epithelium. Lymphocytic vasculitis was present only in the lung. The remaining 2 sheep recovered clinically, approximately 25 days PI. The study revealed that clinical signs and lesions resembling MCF can develop when uninfected sheep are exposed to a high dose of aerosolized OvHV-2.

Key words: Experimental MCF disease; ovine herpesvirus-2; sheep.

Malignant catarrhal fever (MCF) is a fatal disease syndrome primarily of ruminant species such as cattle, bison, and deer.2,3,14,16 The disease is caused by one of several rhabdoviruses belonging to the gammaherpesvirus subfamily. Two major epidemiological forms of MCF exist, each defined by the reservoir ruminant species from which the causative virus arises. One is commonly known as the African form or “wildebeest-associated” MCF (WA-MCF), caused by a herpesvirus carried by wildebeest and designated alsophage herpesvirus 1 (AlHV-1).14 The other is referred to as “sheep-associated” MCF (SA-MCF). Virtually, all cases of MCF in cattle and bison in the United States are caused by the SA-MCF virus.11,12 designated ovine herpesvirus-2 (OvHV-2). The virus is ubiquitous, and infection in sheep is subclinical.7 The virus has never been successfully propagated in vitro.

Acute MCF in cattle caused by either OvHV-2 or AlHV-1 is indistinguishable, clinically and pathologically.14 Severe nasal discharge and high fever are characteristic.12 Other clinical signs include inflammation, erosions, and mucopurulent exudation affecting upper respiratory, ocular, and oral mucosa; swollen lymph nodes; lameness; and neurological signs.10 The principal microscopic lesions are inflammation and necrosis of alimentary, urinary, and upper respiratory epithelium; generalized lymphoid proliferation and necrosis; and vasculitis, which is characterized by fibrinoid-necrotizing vasculitis with intramural and perivascular aggregates of large lymphoblastoid cells.9,12 Spontaneous cases of an MCF-like syndrome in sheep have been reported on the basis of histopathological lesions,15 but none have ever been confirmed by other laboratory tests, such as in situ polymerase chain reaction (PCR).17 This report describes an MCF-like syndrome that was induced in domestic sheep in the course of studying the experimental transmission using high doses of OvHV-2.

Nasal swabs were collected daily from a group of 15, 6-month-old, OvHV-2–infected sheep. The status of the infection of these sheep was confirmed by OvHV-2–specific PCR.6 DNA from the nasal secretions was purified by using FastDNA kit,a and OvHV-2 DNA copy numbers were determined by real-time PCR.5,6 Within 6 hours of initial sampling, 8 additional nasal swabs were collected from any sheep whose initial sample contained $\geq 10^6$ OvHV-2 DNA copies/2 µg DNA. The swabs were agitated in cold phosphate-buffered saline and the supernatant collected after clarification by low-speed centrifugation at $129 \times g$ for 5 minutes. The clarified supernatant was kept on ice for aerosol transmission. The viral DNA copy number in the inoculum was determined by real-time PCR. In this experiment, the 2 ml of inoculum contained $3.7 \times 10^5$ OvHV-2 DNA copies. Each of 3 uninfected, 9-month-old sheep (Nos. 1–3) from an OvHV-2–free flock that was repeatedly confirmed negative by PCR7 and competitive enzyme-linked immunosorbent assay (cELISA)b was inoculated with 2 ml of fresh inoculum. For aerosol inoculation, a liter-sized plastic bottle with the bottom removed was placed around each sheep’s muzzle, and the inoculum was delivered for a 5-minute period, using a commercial nebulizer,b which generates a mist.
Figure 1. A representative sheep (No. 1) in the aerosol transmission experiment. The OvHV-2 DNA copy numbers/2 μg total DNA in nasal secretions by real-time PCR (solid circles); OvHV-2 DNA copy numbers/2 μg total DNA in PBLs by real-time PCR (open circles); the solid arrows represent the times of peak fever; and the open arrow represents the time when the animal became antibody-positive by cELISA.

Figure 2. Head, OvHV-2–inoculated sheep. Note profuse mucopurulent nasal discharge (arrow). There is mild lachrymation.

Figure 3. Larynx and fauces, OvHV-2–inoculated sheep. Note multifocal ulcerative pharyngitis (arrowhead), diffuse mucosal edema, and suppurative exudate (asterisk).

Figure 4. Ovine herpesvirus 2–inoculated sheep. There is diffuse edema of lung with multifocal red discoloration and consolidation (arrowheads).

of fine particles smaller than 5 μm diameter. Blood and nasal secretion samples were collected daily, and clinical signs and temperature were recorded on a daily basis. MCF viral antibody in plasma was monitored by cELISA.6 Ovine herpesvirus-2 DNA in peripheral blood leukocytes (PBLs) and in nasal secretions was detected and measured by seminested PCR7 and real-time PCR.3,4

Ovine herpesvirus-2 DNA became detectable in PBL and nasal secretions of all 3 sheep, within 3 days postinfection (PI). Viral DNA levels in the nasal secretions reached peaks ranging from $2.4 \times 10^5$ to $2.5 \times 10^6$ copies/2 μg DNA, at 3–4 days PI and declined rapidly during the subsequent 2 days (Fig. 1). At 6 days PI, the 3 sheep developed fever, ranging from 41.2 C to 41.6 C, which lasted 2–4 days, without other obvious signs. MCF viral antibody became detectable in all animals between 6 and 8 days PI. The levels of OvHV-2 DNA in the PBLs of all 3 animals increased about 100-fold at 11 days PI compared with the viral DNA level at 8 days PI. By 14 days PI, all 3 sheep displayed distinct clinical signs. All animals had a profuse malodorous mucopurulent nasal discharge (Fig. 2) and fever up to 40.8 C, which lasted 4–8 days. The sheep continued to eat and drink without loss of appetite. Sheep No. 2 was euthanized and necropsied at 18 days PI. The 2 remaining sheep (Nos. 1 and 3) recovered from the clinical disease at approximately 25 days PI and remained clinically healthy until termination of observations at 60 days PI.
The principal macroscopic changes in the euthanized sheep were in the respiratory tract, oral cavity, and esophagus. There were multifocal erosions and ulcers in the mucosa of the cheeks, tongue, and pharynx (Fig. 3). There were multiple, 10–20 mm shallow linear erosions and ulcers in the mucosa of the proximal esophagus. No other gross changes were found elsewhere in the digestive tract. In addition to profuse malodorous mucopurulent nasal discharge, the trachea contained abundant foamy fluid and the lungs were moderately, diffusely edematous. There were multiple, 2–3-mm lobular areas of consolidation and red discoloration in all lobes (Fig. 4).

Microscopically, there was locally extensive moderate superficial histiocytic-lymphocytic rhinitis, with epithelial dissociation and degeneration (Fig. 5). Similar changes were present in respiratory seromucous glands and in tracheal mucosa. There was moderate multifocal histiocytic bronchiolar-interstitial pneumonia, with loss of terminal bronchiolar epithelium (Fig. 6a, 6b). Alveoli contained macrophages admixed with lymphocytes and proteinaceous fluid (Fig. 6b inset). Partial to complete loss of terminal bronchiolar epithelium was a consistent finding. Peribronchiolar lymphoid aggregates were moderately enlarged, with predominance of large lymphoblastoid cells. Acute segmental lymphocytic arteritis of medium caliber arteries was present in several areas (Fig. 8). Vasculitis was present only in the lung. Ulcerative lesions in the oral cavity corresponded to the areas of moderate to severe histological stomatitis, with widespread basilar and suprabasilar epithelial degeneration and cell death (Fig. 7). Mild lymphoplasmacytic sialoadenitis and dacryoadenitis were also present. There was no evidence of encephalitis. Serum neutralization tests for bovine respiratory syncytial virus, parainfluenza virus 3, and border disease pestivirus were performed on samples collected at 18 days PI (sheep No. 2) and 32 days PI (sheep Nos. 1 and 3). Only sheep No. 1 had a low titer (1:8) against parainfluenza virus 3 and sheep No. 3 had low titer (1:8) against bovine respiratory syncytial virus, suggesting earlier exposure to these agents. However, neither bovine respiratory syncytial virus antigen nor parainfluenza virus 3 antigen was found in lung tissues of sheep No. 2 by immunohistochemistry. The serum neutralization tests and immunohistochemical assays were performed by the Washington State Animal Disease Diagnostic Laboratory, Pullman, Washington.

The data from the study show that an MCF-like disease developed in sheep after experimental aerosol inoculation of nasal secretions containing a high dose of OvHV-2. Because no direct controls could be used in the experiment, the authors cannot know with absolute certainty whether the disease was caused by the inoculated OvHV-2 or whether it might be attributable to intercurrent disease. The clinical syndrome that occurred was distinctive, consistent among the 3 sheep, and had features similar to those of acute MCF in cattle and bison (see below). Seroconversion and detection of OvHV-2 in leukocytes shortly after exposure showed that the sheep were infected recently with OvHV-2 and that infection preceded the onset of clinical disease. The spectrum of lesions (rhinitis, tracheitis, bronchiointerstitial pneumonia, stomatitis, and pharyngitis) was distinctive and to the knowledge of the authors does not occur in combination in other diseases that are in sheep endemic in the United States. Aerosolization using lower concentrations of virus than used in this study ($2.7 \times 10^7$ viral DNA copies in bison vs. $3.7 \times 10^9$ in this study) has successfully induced MCF in bison.
Figure 7. Oral mucosa, OvHV-2–inoculated sheep. Multifocal degeneration and cell death of oral keratinocytes, some in small groups (arrowheads) (HE).

Figure 8. Lung, sheep. Mural lymphocytic arteritis in medium-caliber vessel. Note dense periarterial infiltration of large lymphoid cells (HE).

This is the first report of an MCF-like disease in sheep, experimentally induced with MCF virus derived from sheep. In an earlier study from Scotland, 5 out of 19 fetal lambs inoculated with lymph node cells from clinically affected red deer or rabbits were born full term, and 4 out of 5 live-born lambs subsequently developed clinical signs suggestive of MCF and died or were killed 10–175 days after birth. Lesions consisted of lymphoproliferation, arteritis, and interstitial infiltration of lymphocytes in multiple organs. MCF-like disease in sheep has been suspected on the basis of the presence of disseminated fibrinoid vasculitis but could not be confirmed because of the nonspecific nature of the lesion, which also occurs in other diseases of sheep. Whether MCF occurs naturally in sheep is unclear. Confirmation of naturally occurring MCF in sheep is difficult because of technical limitations. Virtually all sheep raised under natural conditions are infected with OvHV-2. Whether MCF occurs naturally in sheep is unclear. Confirmation of naturally occurring MCF in sheep is difficult because of technical limitations. Virtually all sheep raised under natural conditions are infected with OvHV-2. Demonstrating OvHV-2 colocalized with lesions by in situ PCR in the diseased sheep may help confirm OvHV-2–induced disease but is a laborious and technically demanding procedure.

Ovine herpesvirus-2 is well adapted in sheep and does not induce disease under most conditions. The 3 sheep that were aerosolized with a high dose of OvHV-2 developed clinical signs, but the 2 sheep allowed to survive recovered from the disease at approximately 25 days PI, and the third was euthanized at 18 days PI for pathological examination. In subsequent aerosol transmission studies using lower doses of nasal inocula ranging from $10^4$ to $10^6$ viral DNA copies/inoculum developed a short-duration (<48 hours) fever (40°C), without other clinical signs (Taus NM, Traul DL, Oaks JL, Crawford TB, Lewis GC, Li H, unpublished data). This suggests that induction of clinical MCF in normal sheep requires a very high dose of the virus, which is unlikely to be reached during natural transmission. Therefore, naturally occurring clinical MCF in sheep is rare and may occur only in those individuals with genetic or acquired immunological deficiency.

Clinical signs and lesions in these affected sheep mimicked some but not all the changes that occur in susceptible ruminant species, such as cattle and bison. Similarities included the presence of disseminated ulcerative stomatitis, pharyngitis and esophagitis, and marked rhinitis. Vasculitis involving medium caliber arteries is a useful diagnostic feature of MCF and was present although its distribution was limited to lung. Severe disseminated arteritis–phlebitis is one of the diagnostic stigmata of acute MCF in domestic cattle. The restriction of lymphocytic arteritis to the lungs in this sheep is an interesting but unexplained phenomenon. Lymphoproliferation, another helpful diagnostic feature of MCF, was restricted to bronchiolar lymphoid aggregates and was not evident in lymph nodes. A feature that is not seen in classical MCF in cattle, deer, or bison was bronchiointerstitial pneumonia with destruction of terminal bronchiolar epithelium. These differences could be because of viral host cell tropism difference related to long-term viral host adaptation.

The patterns of viral DNA present in the nasal secretions of the aerosol-inoculated sheep were similar to the shedding pattern of sheep naturally infected with OvHV-2. However, high levels of viral DNA in the nasal secretions of naturally infected sheep typically occur a few months later, after initial infection. In dose-dependent aerosol transmission experiments, high levels of OvHV-2 DNA in nasal secretions were also observed early in some of the sheep with high doses of inoculum. The question of whether OvHV-2 initially targets lymphocytes or other undefined permissive cells in the mucosa of the respiratory tract is unresolved. Most lambs acquire OvHV-2 at approximately 10 weeks of age under natural flock conditions. The most intensive viral shedding originates from adolescent lambs (6–9 months of age) several months after initial infection. To explain the early appearance of high viral DNA levels in the nasal secretions of these aerosolized sheep, the authors speculate that a large number of lymphocytes were infected in the submucosal tis-
sues of the respiratory tract by the high dose of viral inoculum. These latently infected lymphocytes progressed to lytic infection, to provide infectious virus to undefined permissive cells in the mucosa of the respiratory tract, resulting in high production of the virus.

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