Lymphocytic enteritis and systemic vasculitis in sheep

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Abstract
Lymphocytic enteritis and systemic lymphocytic vasculitis may be a new or previously unrecognized syndrome in sheep suffering from chronic wasting. Seven cases in three separate flocks were seen over an eight-year period at Veterinary Laboratory Services in Brighton, Ontario. Most of the animals were between five and twelve months of age and exhibited weight loss and inappetence, with or without diarrhea. All were Suffolks or crossbred Suffolks. In one of the flocks, there were also several sheep with lymphoma.

Postmortem examination of carcasses and ancillary testing did not reveal an etiology; however, the intestinal and vascular lesions resembled those described in certain viral diseases, such as malignant catarrhal fever and Border disease, and immune-mediated diseases, such as polyarteritis nodosa.

Submission for necropsy of sheep that exhibit signs of chronic wasting is encouraged, to help establish the etiology of the disease and its significance to the industry.

Résumé
Entérite lymphocytaire et vasculite systémique chez le mouton
Le complexe entérite lymphocytaire et vasculite systémique est un syndrome récemment identifié chez les moutons qui présentent un dépérissement chronique. Les Laboratoires de Services Vétérinaires de Brighton, en Ontario ont diagnostiqués sept (7) cas provenant de trois (3) élevages différents sur une période de 8 ans. La majorité des animaux étaient âgés entre 5 et 12 mois et présentaient une perte d’appétit et de poids avec ou sans diarrée. Les moutons étaient de race Suffolks ou Croisé Suffolks. Dans l’une des bergeries, plusieurs moutons présentaient un lymphome. L’examen post-mortem et les épreuves effectuées n’ont pu permettre d’identifier l’étiologie. Toutefois, les lésions intestinales et vasculaires ressemblaient à celles décrites lors de pathologies d’origine virale, telles que la fièvre catarrhale maligne et la maladie de Border, et lors de maladies à médiation immunitaire comme la polyarthritis nodulaire.

Les auteurs recommandent qu’une autopsie soit effectuée sur les moutons présentant des signes débilitants chroniques afin de préciser l’étiologie de la maladie et de déterminer son impact dans l’industrie.

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a 5% CO₃ incubator. All other plates were incubated aerobically at 37°C.

Fecal flotations were performed on samples from three of the seven animals, using a supersaturated sodium nitrate solution and standard technique.

Plasma protein levels were determined in three of the animals using a refractometer (Atago Co. Ltd., Tokyo, Japan).

Serological screening for antibody to bovine viral diarrhea virus, using a serum neutralization assay, and bovine leukemia virus and maedi/visna virus, using an agar gel immunodiffusion (AGID) technique, was carried out on single serum samples collected from one animal in flock A with enteritis and vasculitis, the single animal from flock B, and seven animals in flock C, exclusive of the one submitted for necropsy.

Cultures of lamb kidney and goat synovial cells were inoculated with 10% suspensions of spleen, mesenteric lymph nodes, and brain from the two affected animals from flocks B and C.

Results

Two of the animals with weight loss were emaciated; most of the others were thin. Hydration status ranged from normal to moderate dehydration. In most carcasses, the mesenteric lymph nodes were enlarged. In all, the large and small intestines were dilated and filled with green to brown watery liquid. In two animals, the mucosa of the entire or the distal half of the small intestine had a ridged or corrugated appearance. In two other animals, there were small intestinal mucosal ulcers and erosions or plaques, 0.5–1 mm x 1–3 mm in size. In two animals, the heart and kidneys contained pale areas centered around blood vessels. In one carcass, thrombi were found in mesenteric and testicular blood vessels.

In the 10-month-old animal with lymphoma, adequate fat stores were present. The lungs, liver, and kidneys were diffusely infiltrated with white, solid, discrete masses, most of which were nodular with an occasional umbilicate form present. Similar masses were present throughout the thoracic fat, the pericardial sac, and attached to the ribs and sternum. The ewe with lymphoma was in fair bodily condition. She had several neck masses and enlarged retropharyngeal and prescapular nodes. The cut surfaces were firm to soft and white and had a lobular pattern. Several other masses ranging up to 25 cm in diameter were present along the left side of the neck and at the angle of the left mandible. The mass removed from the yearling at slaughter had a gross appearance similar to the above.

On histological examination, lymphocytic enteritis was observed in all seven animals. Some of these animals had lesions involving the entire length of the small intestine. The lamina propria was filled with diffuse infiltrates of lymphocytes that were mainly small and mature in appearance. Usually small numbers of plasma cells and polymorphonuclear leukocytes were also present. There was compression and, frequently, loss of crypts, and in all cases, mild cryptitis. Villous atrophy was always present and, in some cases, was quite marked. On occasion, there was focal ulceration of the epithelium. In most cases, the lymphocytic infiltrates were mild to moderate and transmural, with extension into the mesentery.

Bacteria were not seen in sections of intestine stained by the Ziehl-Neelsen method.

Systemic vasculitis involving small to medium-sized arteries and, in some cases, veins was observed in four of the seven animals submitted. On occasion, it was not possible to identify the type of vessel affected due to the severity of the reaction. Intestine, heart, and kidney were the organs most frequently affected; but in some cases, vasculitis was also found in adrenal gland, mesentery, liver, thyroid gland, and testicle. The acute vascular lesions consisted of segmental to circumferential fibrinoid necrosis of the wall with light to moderate transmural infiltration of lymphocytes and, occasionally, small numbers of polymorphonuclear leukocytes and histiocytes. The infiltrates varied from segmental to circumferential in distribution. More chronic changes were characterized by mild to marked proliferation of myointimal cells with cytoplasmic vacuolation (Figure 1), which, in some instances, reduced the lumen of affected blood vessels. Variable degrees of lymphocytic perivascular cuffing, occasionally combined with plasma cells and histiocytes, were also observed. Thrombi were seen in some of the vessels, but thrombosis was not a consistent finding. Fungi were not seen in sections stained with periodic acid-Schiff or Grocott-Gomori's methenamine silver method.

Other lesions included light to moderate periportal lymphocytic infiltrates in the liver and light lymphocytic perivascular cuffing in the lungs of most animals. In some animals, the kidneys contained multifocal, cortical, interstitial lymphocytic infiltrates.

In the cases of lymphoma, the tumor masses consisted of diffuse sheets of lymphocytes that obliterated normal architecture. The lymphocytes were large with oval and often convoluted or indented nuclei. Nucleoli were frequently apparent and chromatin was clumped. Mitoses were obvious and averaged three per 400 X field.

Clinically significant bacteria were not recovered from the cultured organs.

Fecal flotations were negative; however, a few Moniezia spp. were observed in the intestine of one lamb at necropsy.

Plasma protein values of 36 g/L, 30 g/L and 48 g/L were determined (reference range 60–75 g/L).

The results of the AGID test were negative for bovine leukemia virus and maedi/visna virus in all nine animals tested. The animals from flocks A and C had titers

Figure 1. Histological section of kidney showing an artery with myointimal cell proliferation. (Bar = 100 μm).
of <1:8 or <1:128 to bovine viral diarrhea virus; there was insufficient serum from the lamb from flock B for testing.

Discussion

All of the sheep examined had lymphocytic enteritis; four of the seven also had nonsuppurative systemic vasculitis involving primarily small and medium-sized arteries. The occurrence of both entities in one animal suggests a single etiology. The occurrence in multiple animals over a four-year period in at least one of the flocks suggests an infectious etiology.

Vasculitis has infrequently been reported in sheep (1-4) and, as in any species, can be of infectious or noninfectious origin. Of the viral causes of vasculitis in animals (6), the herpesvirus of MCF is not known to produce disease in sheep. In cattle, MCF is described as lymphoproliferative, and vasculitis is present. The vasculitis is morphologically similar to that present in these sheep, as are the lymphoid infiltrates in kidney and liver. Intestinal mucosal lymphoid infiltrates in MCF can be focal or diffuse and could resemble the intestinal lesions in these sheep, although ulceration and necrosis, seen in MCF, were not features in all of the animals. Lesions such as lymphoproliferation within lymphoid tissue, focal necrosis of lymph nodes, and vasculitis within the brain, as well as characteristic naso-ocular lesions of MCF, were not found.

Vasculitis produced by members of the Togaviridae [bovine viral diarrhea (BVD) virus, Border disease (BD) virus, equine viral arteritis virus] is also morphologically similar to what was observed in this investigation (5). Periarteritis has been described in sheep experimentally infected with BD virus (1,2), as have lymphoproliferative lesions in several organs, including kidney, liver, and intestine, in diarrheic sheep (6,7), reminiscent of the lesions in these sheep and in cattle with MCF. Interestingly, the latter workers noted the absence of vascular lesions, but further investigation into the possible role of BD virus is warranted. Serologically, there were low titers against BVD virus in all but one animal surveyed. However, serology may not be diagnostically helpful in all cases, as naturally infected animals can have low or nonexistent titers (8).

A group of related viruses also producing lesions of a lymphoproliferative nature is the subfamily Lentiviridae to which the antigenically-related viruses causing maedi/v Nelson (M/V), ovine progressive pneumonia (OPP), and caprine arthritis-encephalitis (CAE) belong (9-14). The lymphocytic infiltrates described in mammary gland, brain, lung, and joints are reminiscent of the type of lesions seen in some of the tissues in these sheep. While neither lymphocytic enteritis nor vasculitis are features of lentiviral diseases in sheep, vasculitis of a morphologically similar nature has been found in some sheep with OPP (15) and goats with CAE (14). Although serological results were negative in the animals tested for antibodies to M/V, the possible role of lentiviruses warrants further investigation.

The arbovirus causing bluetongue (BT) has not been identified in Ontario sheep, and while it produces vasculitis with thrombosis, BT is typically a microvascular disease (5) affecting capillaries, arterioles, and venules. Lesions, clinical signs, and culture results did not suggest or support a diagnosis of bacterial vasculitis.

An immune-mediated mechanism is thought to account for many types of vasculitis in man (16,17). Immune-mediated diseases with vasculitis include polyarteritis nodosa (PAN), serum sickness, and systemic lupus erythematosus. Classic PAN is a disease of the young adult, and six of the sheep in this group were one year of age or younger. Also, several similarities exist between classic PAN lesions in man and the vascular lesions in these sheep; for example, similarity in the size of vessels (small and medium arteries) and location of vessels (in order of frequency — kidney, heart, liver, and gastrointestinal tract) involved (16,18). Although the early cellular infiltrate within the vessel wall in PAN is mixed (mononuclear cells and neutrophils), it may progress to a solely mononuclear cell infiltrate and could resemble the lesions found in these sheep. Vasculitis in some infectious diseases may be immune-mediated or have an immune component; this has been suggested in MCF (5,18), CAE (12-14), and M/V (19).

Lymphocytic enteritis was present in all of the animals examined, and the similarities between the lesions seen and those described for MCF in cattle and BD in sheep have been mentioned above. Another possible cause worthy of consideration is Mycobacterium paratuberculosis, as it has been reported to cause heavy lymphocytic and other cellular infiltrates in the lamina propria of the small intestine (20). However, the absence of characteristic lesions in the large intestine and mesenteric lymph nodes and the presence of vasculitis make the involvement of M. paratuberculosis less likely.

The lymphoproliferative nature of the intestinal lesions also resembles a group of lymphoplasmatocytic enteritides described in other species (21-23). It is interesting that these conditions, namely, lymphoplasmatocytic enteritis in dogs, celiac disease and the early stages of alpha-chain disease in man, are thought to have an immunological basis, and that both celiac disease and alpha-chain disease are believed to predispose to the development of alimentary lymphoid neoplasia (22,23). Morphologically, the resemblance of the intestine of these sheep to that in horses with alimentary lymphoma is also striking (24,25). That the intestinal lesion resembles equine alimentary lymphoma is even more interesting, since three animals in flock A had lymphoma diagnosed during the same time period. In dogs and horses, submucosal or transmural lymphoplasmatocytic infiltrates may occur as a precursor to lymphoma (5).

Neoplasia in sheep is uncommon, although lymphoma is felt to be the most common neoplasm seen (26,27). In naturally occurring cases, the distribution of lymphoma tends to be multicentric with widespread involvement of lymph nodes (27-29). Nodes in the iliac, mediastinal, and cervical regions are most commonly involved (29). Lymphoma tends to be found in mature animals (26-28), but two of the three sheep that were examined were less than one year of age.

Ovine lymphoma is caused by a retrovirus which, in some cases, has been shown to be indistinguishable from bovine leukemia virus (BLV) (29). It is known that BLV is oncogenic for sheep (30-33). The sheep in
flock A had no direct contact with cattle, although cattle had been kept on the farm several years earlier. Whether or not BLV was involved in the tumor induction in the sheep in this study has not been proven. Serum was only available from one animal in the flock for serological testing and this animal did not have tumors. The AGID test for BLV was negative in this case. Screening of all animals in flock A for BLV would, in retrospect, have been a useful procedure, but no longer possible because the flock has been dispersed. The animals tested for BLV from flocks B and C, were also negative. Although no cases of lymphoma were diagnosed in either flock B or flock C, testing animals in these flocks might have been useful in the detection of BLV infection in flocks affected with lymphocytic enteritis. However, negative results may not necessarily rule out BLV or some other retroviral infection, because antibody titers to BLV take a long time to develop in both sheep and cattle (31,32).

Although diagnostics failed to provide evidence of a specific etiology, speculation can be made regarding the pathogenesis of this condition. The clinical histories and lesions are possibly best explained by a viral agent or agents, producing enteritis and vasculitis by an immune-mediated mechanism. The intestinal lesion could be a precursor to lymphoma. The significance of lymphoma in other tissues in flockmates of sheep experiencing vasculitis and enteritis is unclear. It may represent a second infection occurring independently of the vasculitis or enteritis, as the two were not observed concurrently in any one sheep.

Clearly, further investigation into possible involvement of BVD virus or BD virus, herpesviruses, BLV, and M/N virus is warranted in future cases. Serology and virus isolation should be carried out in the future on any affected and contact animals. Such tests as serum protein electrophoresis, antinuclear antibody testing, and immunofluorescent staining of tissues might help to substantiate an immunological basis for this syndrome.

Postmortem examination is also important to help determine the prevalence of the disease, as an aid in establishing its significance to the sheep industry. It is likely that many of these animals are culled due to poor bodily condition. Awareness of this disease may encourage the submission of these culled to laboratories.

References