Turkish Journal of Veterinary & Animal Sciences

Volume 35 | Number 6 Article 14

1-1-2011

Maedi-Visna virus infection in a Merino lamb with nervous signs

AHMET AKKOÇ

MERİÇ KOCATÜRK

AYLİN ALASONYALILAR DEMİRER

SEZGİN ŞENTÜRK

GIACOMO RENZONI

See next page for additional authors

Maedi-Visna virus infection in a Merino lamb with nervous signs

Authors
AHMET AKKOÇ, MERİÇ KOCATÜRK, AYLİN ALASONYALILAR DEMİRER, SEZGİN ŞENTÜRK, GIACOMO RENZONI, and SILVIA PREZIUSO

TÜBİTAK

Case Report

Turk. J. Vet. Anim. Sci.
2011; 35(6): 467-470

doi:10.3906/vet-1102-784

Maedi-Visna virus infection in a Merino lamb with nervous signs

Ahmet AKKOÇ[1], Meriç KOCATÜRK[2], Aylin ALASONYALILAR DEMİRER[1], Sezgin ŞENTÜRK[2], Giacomo RENZONI[3], Silvia PREZIUSO[3]

[1]Department of Pathology, Faculty of Veterinary Medicine, Uludağ University, Bursa - TURKEY

[2]Department of Internal Medicine, Faculty of Veterinary Medicine, Uludağ University, Bursa - TURKEY

[3]School of Veterinary Medical Sciences, University of Camerino, Matelica (MC) - ITALY

Received: 16.02.2011

Abstract: A Maedi-Visna virus (MVV) infection was diagnosed in a 10-month-old lamb from a Turkish flock. The animal suddenly showed nervous signs and died within 2 days. At necropsy, different pulmonary and nervous lesions were observed. A *Coenurus cerebralis* cyst was also present in the right-brain hemisphere. Severe nonpurulent meningoencephalitis and lymphoproliferative pneumonia were detected by histopathology. MVV proviral DNA was demonstrated by polymerase chain reaction (PCR) in formalin-fixed and paraffin-embedded samples collected from the brain and the lungs. This unusual report in a young animal confirms that not only adult sheep but also lambs can develop MVV disease. The hypothesis that concurrent nervous and pulmonary diseases increase the severity of the lesions due to MVV and reduce the incubation time should be better evaluated.

Key words: Lamb, pathology, PCR, Turkey, Maedi-Visna virus

Sinirsel bulgular gösteren bir Merinos kuzuda Maedi-Visna virüs enfeksiyonu

Özet: Bursa bölgesindeki bir koyun sürüsünde sinirsel belirtiler göstererek ölen 10 aylık bir tokluda Maedi-Visna virüs (MVV) enfeksiyonu teşhis edildi. Nekropside özellikle akciğer ve merkezi sinir sistemine ait lezyonlar gözlendi. Sağ beyin hemisferinde bir adet *Coenurus cerebralis* kistine rastlandı. Histopatolojik olarak şiddetli non-purulent meningoensefalitis ve lenfoproliferatif pnömoni teşhis edildi. PCR incelemelerinde formalin ile fikse edilmiş beyin ve akciğer doku örneklerinde MVV proviral DNA'sı teşhis edildi. Bu rapor ile hastalığın Türkiye'deki varlığı ilk kez PCR ile ortaya konuldu ve enfeksiyonun sadece yetişkinleri değil genç hayvanlarıda etkileyebileceği rapor edildi. Dolayısıyla koyunlarda, yaşa bakılmaksızın sinirsel belirtilerle seyreden hastalıkların ayırıcı tanısında MVV enfeksiyonun da göz önünde tutulmasının gerekliliği ortaya konuldu.

Anahtar sözcükler: Toklu, patoloji, PCR, Türkiye, Maedi-Visna virüs

Case history

A 10-month-old male Merino sheep from a Turkish flock of 850 sheep suddenly showed ataxia, severe tremors, lateral recumbency, and unilateral facial paralysis. Body temperature, pulse, and respiratory rates were within normal limits, but the

* E-mail: silvia.preziuso@unicam.it

mucous membranes were mildly pale. A routine complete blood count showed regenerative anemia, while the leukocyte parameters were within normal limits. Creatinine kinase was highly increased (166 IU/L). The analysis of the cerebrospinal fluid (CSF) showed a density of 1.015 g/mL with pH 7.0, protein 2+, and glucose 1+. An evaluation of the CSF cells was not performed. Bacteriology results for the CSF were negative, but no aliquots were stored for virology or polymerase chain reaction (PCR). The lamb died within 2 days after the first clinical signs. At necropsy, gross findings were only observed in the lungs and in the central nervous system (CNS). The lungs were congested and contained well-demarcated, red, consolidated areas of pneumonia in the ventral regions of the cranial and median lobes. Numerous gray foci, about 2 mm in diameter, were scattered over the pleural surfaces and on the cut sections. A *Coenurus cerebralis* cyst, 7 cm in diameter, was found in the right-brain hemisphere at the passage from the right occipital lobe and the cerebellum (Figure 1). In the CNS, the meninges appeared congested and some grayish, discolored foci were seen, especially in the cerebellum region. No evidence of discoloration of the parenchyma and no other gross changes were found at the coronal section of the entire brain. Samples from the CNS, the lungs, and the mediastinal lymph nodes were fixed in formalin and embedded in paraffin for histopathology.

Results and discussion

The histopathological examination of the samples from the cranial pulmonary lobes showed severe chronic alveolitis. Furthermore, the lungs showed lymphoid interstitial pneumonia, consisting of marked disseminated peribronchial lymphoid hyperplasia, lymphoid follicle formation, perivascular lymphocytic cuffing around the small arterioles, and moderate clusters of mononuclear cells in the alveolar septa. In the brain, there was a severe nonsuppurative encephalomyelitis consisting of perivascular cuffs and inflammatory infiltrate in the neuroparenchyma (Figure 2). Perivascular cuffing was more evident in the cerebellum and in the hippocampus. The inflammatory cells were mainly lymphocytes and a few macrophages. No clear areas of myelin vacuolation were observed. Furthermore, columnar epithelioid macrophages and/or a few giant cells were observed around the *Coenurus* cystic space. On the basis of these findings, a mixed respiratory and nervous form of Maedi-Visna (MV) was suspected. Immunohistochemistry was carried out on brain, lung, and mediastinal lymph node slides by using a commercial monoclonal antibody, as previously described (1). Despite the negative immunohistochemical results, the strongest diagnostic suspect remained Maedi-Visna virus (MVV) infection. In order to confirm the diagnostic

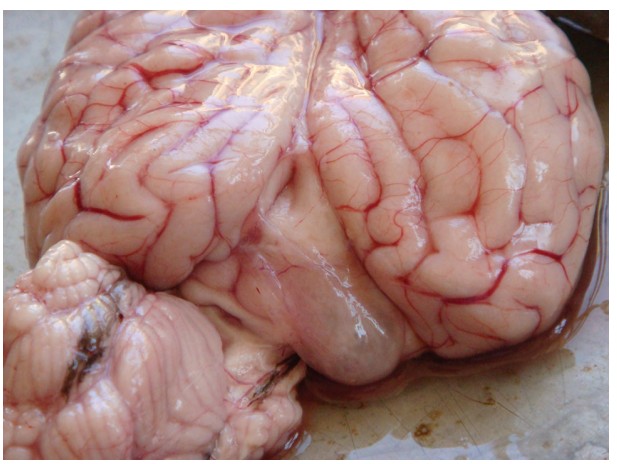

Figure 1. Brain of the 10-month-old lamb affected by MV: congestion of the meninges and a *Coenurus cerebralis* cyst in the right hemisphere.

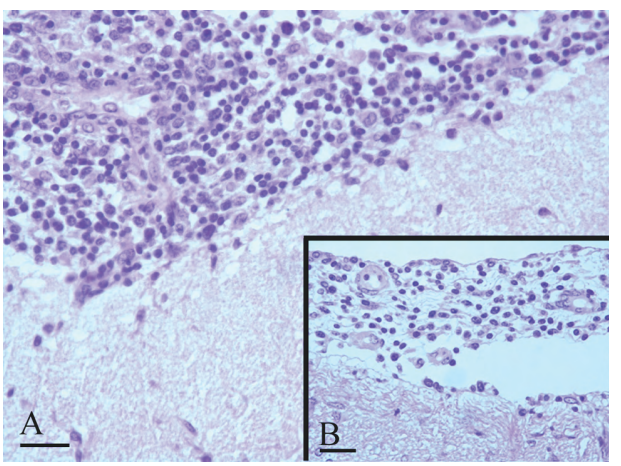

Figure 2. Severe, diffuse mononuclear cell infiltration in the cerebellum (A) and in the subependymal zone (B). (H&E, bar = 50 μm).

suspect, PCR was used to detect a conserved region of the MVV proviral genome. Because of the lack of fresh or frozen tissues, the DNA was obtained from the paraffin-embedded blocks. The DNA was extracted from 4 slides of 10 μm cut from each paraffin block, and then put into a 1.5-mL tube. After serial passages in xylene, in ethanol (100%, 95%, 70%, 50%), and in water, the DNA was extracted from the dried pellet with the QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany) in 50 μL of final volume. In order to verify the quality of the DNA obtained, a first PCR was carried out to amplify a 283-bp sequence of the ovine 28S rRNA (2). Once the expected band was obtained, the samples were tested by PCR for the amplification of a 291-bp sequence of the LTR gene of MVV (3). The finding of a clear band of the expected size in the CNS, lung, and mediastinal lymph node samples confirmed the MVV infection.

At the same farm, a history of similar clinical cases was recorded in another 4 lambs, but unfortunately the farmer did not admit the animals for veterinary examination.

This study describes, for the first time in Turkey, the clinical, pathological, and laboratory findings in a Turkish young sheep affected by MV, which is caused by the lentivirus MVV and is a global multisystemic chronic infection of adult sheep affecting the lungs, mammary glands, CNS, and/or joints with a lymphoid proliferation and mononuclear interstitial infiltration. Because of the long incubation period, the clinical signs of MV are usually observed in sheep of 2-4 years old. The pulmonary form, called Maedi, is the most frequent form described worldwide. The nervous form, called Visna, is very rarely described except in Spain, where some cases have been recently diagnosed (4). While Maedi has been previously described in Turkey (5), no cases of Visna have been reported yet. In our case, the nervous disease was associated with respiratory lesions, as also reported in other Visna cases (6,7). The nervous lesions appeared severe, and large parts of the CNS were involved. The most striking finding was the occurrence of a clinical case of Visna in a 10-month-old lamb. To our knowledge, natural cases of the nervous form of MV have mainly been diagnosed in sheep older than 2 years (4,7,8). Only 4 Visna cases have been described in young lambs, from 4 to 6 months old (9). The rapid progression of the disease and the severity of the histopathological lesions observed in our case could be due to the implication of a particularly neurotropic and virulent MVV strain. However, the lack of fresh samples and the availability of only paraffin-embedded tissues make any further characterization of the viral strain very difficult. On the other hand, it cannot be excluded that the severity of the clinical signs and of the nervous lesions was increased by the concurrent infection by *Coenurus cerebralis*. Indeed, previous reports on MVV-infected sheep with concurrent infection due to *Brucella ovis* or ovine pulmonary small strongyles have shown increased mononuclear cell infiltration and increased expression of the p28 MVV antigen in epididymal and pulmonary samples (1,10).

Another 4 suspected cases from the same flock were not admitted for a definitive diagnosis, demonstrating that in the absence of specific national and international laws, the study of some infectious diseases is practically difficult. A strong collaboration between farmers and veterinarians is very important for the study and eradication of infectious diseases. Furthermore, a strong collaboration between clinicians, pathologists, and virologists is important for the collection of the proper samples for a correct etiological diagnosis. Indeed, even if positivity was obtained in this case by PCR on the formalin-fixed and paraffin-embedded samples, the histopathological procedures usually result in DNA degradation and in a lower PCR sensitivity. Furthermore, the availability of fresh or frozen samples would allow the molecular characterization of the viral strain. Immunohistochemistry usually supports histopathological suspects but, in this case, the antigenic variability of small-ruminant lentiviruses and the availability of diagnostic antibodies reacting with a few viral strains made these techniques inadequate for a correct diagnosis.

In conclusion, MVV infection has to be considered as responsible for neurological disorders not only in adult sheep but also in lambs. During necropsy, fresh samples should be routinely stored in case the histopathological findings suggest further virological investigations. Immunohistochemistry may result in false negative reactions due to the antigenic

variability of the small-ruminant lentiviruses, while PCR protocols to detect conserved MVV genomic regions may be suitable for MV diagnosis in different geographic areas.

Acknowledgement

The authors wish to thank Dr. Gian Enrico Magi for his laboratory support in immunohistochemistry.

References

1. Preziuso, S., Taccini, E., Rossi, G., Renzoni, G., Braca, G.: Experimental Maedi Visna Virus Infection in sheep: a morphological, immunohistochemical and PCR study after three years of infection. Eur. J. Histochem., 2003; 47: 373-378.
2. Bellis, C., Ashton, K.J., Freney, L., Blair, B., Griffiths, L.R.: A molecular genetic approach for forensic animal species identification. Forensic Sci. Int., 2003; 134: 99-108.
3. Preziuso, S., Magi, G.E., Valente, C., Cuteri, V.: Detection of the Maedi Visna Virus in the popliteal lymph nodes of sheep infected by the respiratory route. Vet. Res. Commun., 2009; 33: S153-155.
4. Benavides, J., Gomez, N., Gelmetti, D., Ferreras, M.C., Garcia-Pariente, C., Fuertes, M., Garcia-Marin, J.F., Perez, V.: Diagnosis of the nervous form of Maedi-Visna infection with a high frequency in sheep in Castilla y Leon, Spain. Vet. Rec., 2006; 158: 230-235.
5. Yilmaz, H., Gurel, A., Turan, N., Bilal, T., Kuscu, B., Dawson, M.M., Morgan, K.L.: Abattoir study of maedi-visna virus infection in Turkey. Vet. Rec., 2002; 151: 358-360.
6. Georgsson, G.: Maedi-visna, pathology and pathogenesis. In: Petursson, G., Hoff-Jørgensen, R., Eds. Maedi-Visna and Related Diseases. Kluwer Academic Publishers, Boston. 1990; 19-54.
7. Biescas, E., Preziuso, S., Bulgin, M., DeMartini, J.C.: Ovine lentivirus-associated leucomyelitis in naturally infected North American sheep. J. Comp. Pathol., 2005; 132: 107-116.
8. Sigurdsson, B., Palsson, P., Bogaert, L.: Pathology of visna. Transmissible demyelinating disease in sheep in Iceland. Acta Neuropathol., 1962; 1: 343-362.
9. Benavides, J., Garcia-Pariente, C., Ferreras, M.C., Fuertes, M., Garcia-Marin, J.F., Perez, V.: Diagnosis of clinical cases of the nervous form of Maedi-Visna in 4- and 6-month-old lambs. Vet J., 2007; 174: 655-658.
10. Preziuso, S., Sanna, E., Sanna, M.P., Loddo, C., Cerri, D., Taccini, E., Mariotti, F., Braca, G., Rossi, G., Renzoni, G.: Association of Maedi Visna virus with *Brucella ovis* infection in rams. Eur. J. Histochem., 2003; 47: 151-158.