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Immunohistochemical detection of scrapie prion proteins in clinically normal sheep in Pennsylvania

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Abstract. Following diagnosis of scrapie in a clinically suspect Suffolk sheep, 7 clinically normal flockmates were purchased by the Pennsylvania Department of Agriculture to determine their scrapie status using an immunohistochemical procedure. Two of the 7 euthanized healthy sheep had positive immunohistochemical staining of the prion protein of scrapie (PrP-Sc) in their brains, nictitating membranes, and tonsils. The PrP-Sc was localized in the areas of the brain where, histopathologically, there was neurodegeneration and astrocytosis. The PrP-Sc occurred within germinal centers of the affected nictitating membranes and tonsils and was located in the cytoplasm of the dendrite-like cells, lymphoid cells, and macrophages. These results confirm that immunohistochemical examination of the nictitating membrane can be used as a screen for the presence of scrapie infection in clinically normal sheep at a capable veterinary diagnostic laboratory. In sheep with a PrP-Sc–positive nictitating membrane, the diagnosis of scrapie should be confirmed by histopathology and immunohistochemical examination of the brain following necropsy. Following full validation, immunohistochemistry assays for detection of PrP-Sc in nictitating membrane lymphoid tissues can improve the effectiveness of the scrapie control and eradication program by allowing diagnosis of the disease in sheep before the appearance of clinical signs.

Scrapie is the prototype of a heterogenous group of transmissible spongiform ecnephalopathies that occur in sheep, humans, cattle, cats, mink, and cervids and are characterized by the deposition of altered prion proteins in the central nervous system of affected individuals.[11] Scrapie in sheep has become a target of control measures and eradication programs. Crucial for the effectiveness of these measures is the detection of infected sheep. After infection, the disease has a particularly long incubation period during which the infected sheep may be able to transmit the disease to noninfected sheep.[3] Scrapie infectivity has been detected in the lymphoreticular system of sheep well before symptoms occur.[4,8,9] Detection of scrapie prion protein (PrP-Sc) in nictitating membrane or tonsil has been proposed as a diagnostic test for scrapie infection.[5,11,13] In this study at the Pennsylvania Veterinary Laboratory (PVL), an immunohistochemical procedure was applied to detection of PrP-Sc in clinically normal euthanized Suffolk sheep.

A farm had 10 sheep in a flock that had been in existence since October 1991. A 7-year-old ewe from the farm was submitted to the PVL for necropsy on April 15, 1998. The ewe had shown signs of pruritis for a few months and had recently developed neurologic signs, including staggering, stumbling, and falling. The ewe was diagnosed as having scrapie by routine histopathology conducted at PVL and immunohistochemistry (IHC) of brain tissue conducted at the National Veterinary Services Laboratory (Ames, IA). On August 11, 1998, the Pennsylvania Department of Agriculture purchased the last 7 sheep in the flock to determine their scrapie status; they were 4-year-old, clinically normal Suffolk sheep. The 7 sheep were euthanized and necropsied. Gross examination revealed no scrapie-specific lesions, and the sheep had abundant fat reserves. Representative samples of the brain, nictitating membrane, and tonsil from each of the 7 animals were collected in 10% buffered formalin for histopathologic and IHC examination. All tissues were routinely processed for paraffin embedment, sectioned at 4–5 μm, and stained with either hematoxylin and eosin (HE) or the IHC procedure.

Commercially available kits[a] and an automated immunostainer[a] were used for IHC. Tissue sections of brain stem, nictitating membrane, and tonsil were mounted on positively charged slides. These sections were heated in a flame until the wax had melted, deparaffinized with solvents, and placed in a steam bath for 10 minutes. The last step is considered heat-mediated antigen retrieval. Formalin fixation eliminates the immunoreactivity of the epitope for the anti-prion protein antibody used in this study in ovine lymphoid tissues. Heat retrieval is necessary for unmasking the epitope on PrP-Sc. The primary antibody was a monoclonal mouse anti-prion protein antibody, F89/160.1.5,[b] which recognizes a conserved epitope on the PrP-Sc of cattle, sheep, mule deer, and elk.[8] The primary antibody was used at a dilution of 1:400 for 2 hours at room temperature. The secondary antibody was biotinylated goat anti-mouse IgG[a] and was applied to tissue sections for 30 minutes at room temperature. A commercially available avidin–biotin–horseradish peroxidase complex[a] was used according to the manufacturer's instructions. The chromagen/substrate was aminoethylcarbazole. All slides were counterstained with hematoxylin. Appropriate positive and negative controls were run with every test. These were tissues from sheep diagnosed with scrapie, tissues from sheep with no exposure to scrapie, and a primary antibody of the same isotype as the primary antibody used for staining the PrP-Sc but against another disease. A commercially available rabbit anti-glial fibrillary acidic protein (GFAP) primary antibody[c] was also used in sequential sections at a dilution of 1:500 for 1 hour at room temperature to detect astrocytes.

Two of the 7 euthanized healthy ewes were diagnosed as scrapie positive, based on the presence of histologic lesions and the detection of the PrP-Sc antigens in the lesions by IHC with anti-scrapie antiserum as previously described.[8,10]

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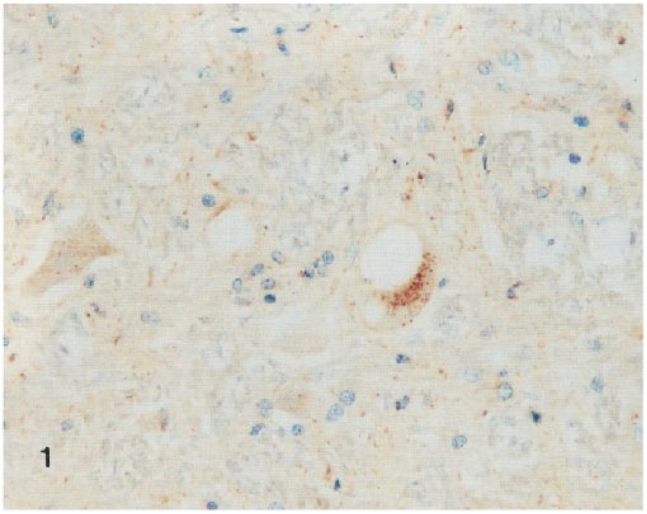

Figure 1. Immunohistochemical staining of PrP-Sc in the midbrain from a clinically normal sheep with a histopathologic diagnosis of scrapie from a flock with a history of scrapie. PrP-Sc antigen has accumulated (red) in the outer rim of vacuoles of neurons. Immunoperoxidase labeling, hematoxylin counterstain.

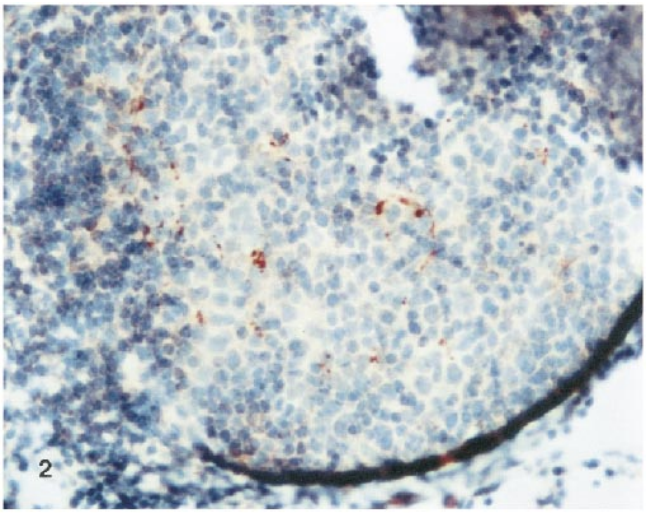

Figure 2. Immunohistochemical staining of PrP-Sc in nictitating membrane from a clinically normal sheep with a histopathologic diagnosis of scrapie from a flock with a history of scrapie. Lymphoid follicle germinal center has punctate multifocal PrP-Sc immunostaining (red). Immunoperoxidase labeling, hematoxylin counterstain.

Histologic examination revealed focal, intracytoplasmic neuronal vacuolation and mild spongiform changes in the midbrain. A mild increase in astrocyte numbers (astrocytosis) was also observed in the lesions. Neuronal vacuolation ranged from a single large vacuole to a few smaller vacuoles. These vacuoles were empty and were not stained with HE. The neuropathologic changes were mainly found in the midbrain and not in the cerebrum and cerebellum. No histopathologic changes were found in the tonsils and nictitating membranes of the 2 scrapie-affected animals.

IHC detected PrP-Sc in the brain, tonsil, and nictitating membrane of the 2 sheep with histopathologic changes of scrapie. In positive brains, the PrP-Sc antigen accumulated in the outer rim of intraneuronal vacuoles (Fig. 1) of the midbrain, whereas in negative control brains the neurons had no such staining. The immunoreactivity was comprised of densely stained granules and globules around the periphery of intraneuronal vacuoles. GFAP-immunoreactive hypertrophic astrocytes surrounded the intracytoplasmic vacuolated neurons. The nictitating membrane of the affected sheep had distinct foci of PrP-Sc staining within lymphoid cells of the germinal centers (Fig. 2). The tonsilar lymphoid tissues also had a similar deposition of granules within cells of the germinal centers. Most immunoreactivity within germinal centers was located in the cytoplasm of the dendrite-like cells, lymphoid cells, and macrophages.

Scrapie of sheep and goats was recognized in Europe at least 200 years ago, although the disease was not diagnosed in the United States until 1947.[7] The most widely accepted histologic lesion is astrocytosis and vacuolation in neuronal cells, leading to the classification of the disease as a spongiform encephalopathy. An astroglial reaction is a common histologic feature in natural[1,2,6] and in experimental[12] scrapie. Astrocytes are a target for the scrapie agent in the early pathogenesis of the disease.[6] Astrocytes, upon stimulation by PrP-Sc, are thought to respond by releasing a variety of active molecules, including nitric oxide. GFAP-immunoreactive hypertrophic astrocytes were readily identified in brain specimens from scrapie-infected hamsters, particularly in those areas where the tissue damage was the most extensive.[7] In scrapie infection of the brain, PrP-Sc was localized in areas where there was neurodegeneration and astrocytosis. PrP-Sc is thought to be toxic to neurons and trophic for astrocytes.[12]

These IHC studies support the histopathologic observations; PrP-Sc antigen was found in animals with intracytoplasmic vacuolation of neurons (spongiform changes) and astrocytosis. The IHC assay of the nictitating membrane and tonsil provides a practical method for early detection of PrP-Sc in live affected sheep before clinical signs appear.

IHC examination of the nictitating membrane can be used as a screen for the presence of scrapie infection in live sheep. In sheep with a PrP-Sc–positive nictitating membrane, the diagnosis of scrapie can be confirmed by histopathology and IHC examination of the brain following necropsy at a capable veterinary diagnostic laboratory in sheep >3 years of age. In younger sheep or those infected with the scrapie agent as adults, immunostaining of the lymphoid tissue may be positive in lymphoid tissue but negative in brain tissue for 1–2 years following infection. A large-scale validation study to determine the specificity and sensitivity of PrP-Sc detection in nictitating membrane lymphoid tissue as a live animal test for scrapie is underway. Use of this test can improve the effectiveness of the scrapie control and eradication program by allowing diagnosis of the disease in sheep before the appearance of clinical signs.

Sources and manufacturers

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b. Animal Disease Research Unit, US Department of Agriculture, Pullman, WA.
c. Sigma Chemical Co., St. Louis, MO.

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Preliminary findings on the experimental transmission of chronic wasting disease agent of mule deer to cattle

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Abstract. To determine the transmissibility of chronic wasting disease (CWD) to cattle and to provide information about clinical course, lesions, and suitability of currently used diagnostic procedures for detection of CWD in cattle, 13 calves were inoculated intracerebrally with brain suspension from mule deer naturally affected with CWD. Between 24 and 27 months postinoculation, 3 animals became recumbent and were euthanized. Gross necropsies revealed emaciation in 2 animals and a large pulmonary abscess in the third. Brains were examined for protease-resistant prion protein (PrP^{res}) by immunohistochemistry and Western blotting and for scrapie-associated fibrils (SAFs) by negative-stain electron microscopy. Microscopic lesions in the brain were subtle in 2 animals and absent in the third case. However, all 3 animals were positive for PrP^{res} by immunohistochemistry and Western blot, and SAFs were detected in 2 of the animals. An uninoculated control animal euthanized during the same period did not have PrP^{res} in its brain. These are preliminary observations from a currently in-progress experiment. Three years after the CWD challenge, the 10 remaining inoculated cattle are alive and apparently healthy. These preliminary findings demonstrate that diagnostic techniques currently used for bovine spongiform encephalopathy (BSE) surveillance would also detect CWD in cattle should it occur naturally.

Since about 1967, a scrapie-like transmissible spongiform encephalopathy (TSE), chronic wasting disease (CWD), has been identified in captive and free-ranging cervids in Colorado and Wyoming.[15,17–20] Chronic wasting disease has been documented in mule deer (*Odocoileus hemionus hemionus*), black-tailed deer (*Odocoileus hemionus columbianus*), white-tailed deer (*Odocoileus virginianus*), and Rocky Mountain elk (*Cervus elaphus nelsoni*).[9,15,17–20] The disease has been experimentally transmitted by intracerebral inoculation of brain from mule deer into a variety of wild and laboratory animal species.[19] Except for intracerebral transmission of CWD to one goat,[19] CWD has not been transmitted to other domestic animals.

The primary objectives of this study were to determine if

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