PRECLINICAL DIAGNOSIS OF OVINE SCRAPIE BY IMMUNOHISTOCHEMISTRY OF LYMPHOID TISSUE USING A PAN-SPECIFIC MONOCLONAL ANTIBODY COCKTAIL

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Abstract:

Scrapie is a naturally occurring prion-associated disease of sheep and goats. A major impediment to research and control of scrapie has been the lack of a method to predict infection prior to the onset of clinical disease in live sheep or in slaughter surveillance. The scrapie-associated prion isoform PrP-Sc is detectable in lymphoid tissues, including those of the third eyelid (nictitating membrane) and tonsil, from infected sheep months or years before development of clinical disease. Third eyelid tissue can be collected using topical anesthesia and is suitable for screening live animals over 18 months of age for scrapie. In this study, we show a high concordance between the third eyelid preclinical test for ovine scrapie and the current diagnostic standards of spongiform lesions and/or immunohistochemistry of the medulla at the level of the obex. The results of third eyelid assay agreed with the scrapie status of sheep in 251 of 258 sheep sampled, including 27 sheep that progressed to clinical disease with confirmed scrapie 3 to 20 months following biopsy. Tonsil was positive for PrP-Sc at post mortem analysis in all sheep with immunostaining of brain. Standardized protocols for high throughput automated immunostaining and for manual staining with commercially available reagents were developed. Lymphoid based testing for PrP-Sc was performed with MAb F89/160.1.5, which binds residues 142-145 of ovine PrP, and MAb F99/97.6.1, which binds residues 220-225. One or both monoclonal antibodies in this cocktail recognize PrP sequences conserved in most mammalian species in which natural TSEs have been reported. The third eyelid test on live sheep and addition of tonsil tissue to postmortem or slaughter surveys will be useful in diagnostic, surveillance and research programs.

Introduction

Scrapie is a fatal neurodegenerative disease occurring naturally in sheep and goats in many parts of the world. Scrapie is endemic in sheep in the United States and in many other countries around the world. Emerging scrapie control measures include husbandry conditions to lower the risk of transmission from infected ewes at lambing, introduction of breeding stock of lower genetic susceptibility to scrapie, and preclinical testing for scrapie.

Scrapie is classified as a transmissible spongiform encephalopathy, a diverse group of diseases including bovine spongiform encephalopathy, transmissible mink encephalopathy, and chronic wasting disease of deer and elk. These diseases are associated with accumulation of abnormally folded proteins called prions (1). Prions are produced in their normal form by all mammals (2); in the diseased animal, the prions assume an abnormal shape that renders them relatively resistant to denaturation by formalin, heat, and common chemical disinfectants (reviewed in reference 3).

Most of the reported cases of scrapie in U.S. sheep occur in 3 to 5 year old ewes, although the disease has occurred in older sheep in the US (4) and typically occurs earlier in sheep with differing genetic backgrounds or varying prion agents (5;6). Early signs may include behavioral changes (apprehension, failure to stay with the flock, reluctance to approach feed bins) followed by weight loss, incoordination, and loss of wool through rubbing or biting. In some sheep, central nervous system signs become more pronounced over the course of several weeks and include high stepping of the front legs, hopping of the back legs when moving quickly, or inability to rise. Some sheep with prion accumulation in the brain are found dead following stressful events such as shearing, vaccinations, or transport. The role of scrapie in these losses remains to be determined but unexplained losses in sheep, particularly in animals newly introduced into the flock, should be investigated by preclinical testing (described below).

The natural route of transmission is not known but exposure to an infected ewe at the time of lambing is associated with a high probability of scrapie. Infectivity has been detected in placenta and fetal membranes (7), and the prion protein has been detected in placenta using various methods (8;9). Infection may occur through the oral route when infectious materials contaminate the bedding, feed, water, or pen surfaces. The infectious agent is probably taken up by lymphoid tissues in the gut (10), disseminated to other lymphoid tissues, particularly of the head and neck (11), and eventually spread to the brain through the nervous system. Infectivity and prion proteins are typically found in the lymphoid tissues in US sheep at 10-14 months of age (11) and in the central nervous system by 2.5 to 5 years.

Genetic susceptibility

Scrapie is a transmissible disease and is not an inherited or genetic disorder (12). However, early studies with natural and experimental scrapie established that relative susceptibility is associated with the genetics of the sheep (13-15). The gene for the prion protein is polymorphic, appearing in slightly different forms among individual sheep (5; 16). Variation at codons 136 (A to V) and 171 (Q to R) are associated with relative susceptibility to at least 2 scrapie strains. Classical breeding studies (17;18), experimental inoculations (19-20), and field studies (21-24) have demonstrated that sheep with diploid genotypes 136VV or 136AV are susceptible to a scrapie strain designated strain A, and sheep with diploid genotype 136AA 171QQ are susceptible to a strain designated strain C (19). Additional strains or subgroups may be described in the future but currently, sheep with the diploid genotype 136AA 171RR are rarely reported with clinical scrapie or PrP-Sc detectable in lymphoid or nervous tissue. Field studies in France (25) indicate that heterozygous sheep (136AA 171QR) do not become silent carriers but additional studies on a possible carrier state are in progress in the US, the UK, and Europe. Diploid genotypes for various breeds have been determined and ranked by relative susceptibility to scrapie in those breeds (26). Many producers are reducing the risk of scrapie in their flocks by selecting breeding stock with the lower susceptibility genotypes. The most cost-effective approach is identification of a group of candidate rams with good breeding potential, followed by codon 136 and 171 testing to select the least susceptible sheep as founder stock.

Scrapie diagnosis

Scrapie is diagnosed by postmortem examination of the brain for the characteristic histologic changes (astrocytosis, gliosis, and vacuolation). However, these changes appear late in the clinical course and more reliable diagnosis can be made by detection of PrP-Sc in brain tissue by immunohistochemistry (27), Western immunoblot (28-33) or electron microscopy (34). These tests are useful in confirming the diagnosis of clinically affected sheep. Prions may not be detectable in the brain in sheep young younger than 30-36 months of age, however (36), and testing of the brain in younger sheep lost to other causes may not be informative. Infectivity and prion proteins accumulate in lymphoid tissues of sheep much earlier in disease (33;36-37). A test based on collection of tonsil tissue from anesthetized sheep is in use in the Netherlands for early detection of infected sheep (38). In the U.S., Dr. Steven Parish, a clinician at Washington State University, suggested an alternative site, the small patches of lymphoid tissue behind the "third eyelid"(39). This tissue is readily accessible using only anesthetic eyedrops and a good restraint technique. Collection of the biopsy takes less than a minute. A live animal test using this tissue has been described (39; 40). The abnormal prions are detected by immunohistochemistry (41) following inactivation of the normal cellular PrP with formalin and formic acid, and antigen retrieval using heat in a citrate buffer. Primary antibodies include a cocktail of two monoclonal antibodies that bind distinct epitopes on the ovine prion protein (40; 41). One or both antibodies bind all the reported ovine prion peptides and are therefore useful in testing sheep of a wide variety of genetic sources. The test has completed the first two stages of validation, as described in the OIE manual of standards for diagnostic tests (42) and has an estimated sensitivity of 88% and specificity of greater than 97%. (Table 1). The test is currently the focus of a large-scale USDA stage 3 validation study. The test methodology is also suitable for postmortem testing of lymphoid tissue, particularly tonsil tissue, retropharyngeal lymph node, Peyer's patches, ileocecal lymph node, and placenta.

Table 1: Immunohistochemistry assay of third eyelid lymphoid tissue for diagnosis of ovine scrapie

Scrapie status	Number	Eyelid positive[a]	Eyelid negative[a]
Clinical[b] suspects, confirmed scrapie[c]	42	41	1
Clinical suspects, scrapie not confirmed by IHC of brain[d]	7	1	6
No exposure to scrapie[e] (N=48) or exposed to scrapie but uninfected[f] (n=120)	168	0	168
Scrapie-exposed, clinically normal, confirmed scrapie[c] following necropsy at time of eyelid biopsy (n=14) or progression to clinical disease 3 to 20 months after biopsy (n=27)	41	36	5

[a]Results of immunohistochemistry assay performed with MAb F89/160.1.5
[b]Clinical signs included wool rubbing, weight loss and/or ataxia
[c]Scrapie infected sheep showed PrP-Sc immunostaining in medulla oblongata at the level of the obex (U.S. samples) or routine histopathology of brain (U.K. samples)
[d]Scrapie uninfected sheep showed no lesions characteristic of scrapie (U.K. samples, 5 with a negative eyelid immunostain and 1 with a positive eyelid immunostain) or no PrP-Sc detectable in brain, tonsil, retropharyngeal lymph node, or submandibular lymph node (U.S. samples, n=1).
[e]Sheep from three flocks with no reported exposure to scrapie for the last 5 years.
[f] Scrapie uninfected sheep showed no PrP-Sc in brain, tonsil, retropharyngeal lymph node, or submandibular lymph node

The risk of scrapie can be reduced through management, genetics, and preclinical testing. Purchase of healthy animals is the cornerstone of a good management program. In the US, state and federal programs, such as the USDA Voluntary Scrapie Flock Certification Program, provide producers with a source of sheep from flocks monitored for scrapie. Purchased ewes from other flocks, particularly ewes of the high susceptibility genotype, should be considered a potential risk. Good lambing records, including date and month of lambing with information on housing of ewes during this period, should be maintained and kept for 6 years. Provision of separate lambing quarters for genetically susceptible purchased ewes, with eyelid testing at least 14 months after arrival, may reduce the risk of transmission to other animals in the flock. Good sanitation, including prompt disposal of placental tissue, fetal membranes, and contaminated bedding, with cleaning of implements and surfaces with a solution of 40% household bleach should reduce the amount of infectivity in the lambing quarters. Selection of high quality rams that carry the lower susceptibility 171QR or 171RR genotype will decrease the number of susceptible lambs. Eyelid testing of live sheep may be recommended or required by regulatory agencies following possible exposure to scrapie. Postmortem testing of brain, tonsil, and ileocecal lymph node of sheep dying of unknown causes is recommended, particularly of high susceptibility sheep purchased from flocks of unknown scrapie status.

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