Reference Point

What veterinary practitioners should know about scrapie

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Many practitioners do not have time to keep up with new information pertaining to TSEs and, particularly with BSE, learn much of what they know from newscasts and newspapers, which can be dubious sources of information. Reliable peer-reviewed information is available in numerous scientific journals, but most are not readily available to practitioners. Scrapie is the longest known and most widely spread disease among the TSEs and remains the model for much research regarding these diseases. Scrapie affects animals in the United States and is a reportable disease and the subject of an active eradication program. However, to the authors' knowledge, the last scientific review of scrapie was published more than 10 years ago[1] in a journal that is not read by numerous veterinary practitioners. As a result, many sheep producers are more knowledgeable about the disease than many veterinarians. We undertook to review TSEs, update practitioners, expose certain inaccuracies regarding what is known about the disease, and answer questions that many producers expect veterinarians to know.

The TSEs are a unique group of diseases that includes scrapie and are thought by many scientists to result from accumulation of a modified cellular protein in the brain. This abnormal protein is believed to act as a template for conversion of a normal cellular protein (prion protein; PrP^{C}) to a modified protein (PrP^{SC}) in susceptible animals.[2] Susceptibility appears to be controlled genetically in some species (eg, sheep and humans [which contract Creutzfeldt-Jakob disease] and goats[3] [which contract scrapie]), whereas in bovids (which contract BSE) and deer and elk (which contract CWD), a genetic connection has not been identified.

As it accumulates, this modified cellular protein is deposited as amyloid plaque in lymphoreticular and nervous tissue, where accumulation is hypothesized to cause the signs of CNS disease associated with the various TSEs. Although some researchers do not believe that the PrP^{SC} protein itself causes the disease,[4-6] results of an experimental infectivity study[2] have established that it is generally a reliable indicator of the presence of the infective scrapie agent.

Abbreviations

TSE	Transmissible spongiform encephalopathy
BSE	Bovine spongiform encephalopathy
CWD	Chronic wasting disease
USDA APHIS VS	USDA, Animal and Plant Health Inspection Services, Veterinary Services

At present, the widely accepted theory is that, in the vicinity of the abnormal prion, PrP^{C} is induced to undergo a conformational change in which the α-helical content diminishes and the β-sheet content increases. Although the chemical composition of the molecule does not change, the structural difference changes its chemical properties.[2] For example, PrP^{C} is soluble in denaturing detergents and is digested by cellular proteases such as proteinase K.[7] The PrP^{SC} (and its infectivity) is not destroyed by detergents, but is resistant to breakdown by rendering processes used at present, heat sterilization temperatures, UV light, ionizing radiation, and many disinfecting agents and is only partially inactivated by proteinase K.

The genetics of scrapie in sheep have been described.[8] Located on chromosome 13, the PrP gene (Prnp) is composed of 3 sections called exons and another section called an open reading frame. The open reading frame is 256 amino acid codons in length. A codon is a 3-nucleotide segment of DNA and is comparable to a street address. For example, codon 136 is the 136th sequence of 3 DNA molecules that code for a given amino acid. In sheep, scrapie is linked to at least 3 polymorphisms in the PrP gene that are responsible for amino acid changes in PrP^{C} encoded by codons 136, 154, and 171.

Codon 136 codes for valine (V), alanine (A), or threonine (T); codon 154 codes for arginine (R) or histidine (H); and codon 171 codes for glutamine (Q), lysine (K), H, or R. Resistance to scrapie appears to be influenced by 136A,[9,10] 154H,[11] and 171R.[12] Genotypes 136T, 171K, and H are apparently rare worldwide, and 136V and 154H are also rare in the United States.[12] Genotypes 136V and 171Q appear to be linked because 136V is never found concurrently with 171R. These small changes in the amino acid components of this

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glyco-plasma membrane protein enable it to resist reconfiguration.

The purpose of the prion protein has only recently begun to be determined. Laboratory rodents that are devoid of prion protein have been developed, and no deleterious effects of that mutation have been detected.[a] The fact that the prion protein is found in most animal species and is highly conserved suggests that it has an important function. Nevertheless, prion-free knockout rodents appear to be clinically normal and have complete resistance to TSEs. A recent study,[b] however, revealed that irradiated mice that received repeated transfers of prion-free bone marrow stem cells became less hardy and weaker with each subsequent transplant. Stem cells from clinically normal mice containing prion protein established themselves in irradiated mice, which survived subsequent sequential transplantations and remained healthy. Prion protein appears to protect cells from stress resulting from multiple replications. Furthermore, in cell culture, it appears to protect a breast cancer cell line against tumor necrosis factor-α–mediated cell death.[13] It also appears to promote cellular survival in vivo under certain conditions and to have superoxide dismutase (antioxidant) and neuroprotective properties related to uptake of divalent copper.[13]

The prion protein is not identical among the various animal species affected by TSEs. However, there is sufficient similarity that multiple antibodies bind to prion proteins from different species. These multispecies antibodies are useful for screening tests.[14] Hence, the same tests are used for detection of BSE, scrapie, and CWD in deer and elk. So far, all diagnostic tests for TSEs function on the basis of serum antibody reactions. Because animals generally do not make antibodies against self, antibodies are mostly monoclonal in origin or are made in knockout rodents that lack prion protein. Most of these antibodies react against both normal and abnormal prions, and positive test results generally rely on the ability of the abnormal prion to withstand denaturing detergents, heat, and proteinase K digestion.[7]

Diagnostic tests commonly used for identification of scrapie are immunohistochemical testing, Western blot analysis, and ELISAs. At least 4 ELISAs are used at present in the European Union for scrapie surveillance at slaughter.[15,c] None of those assays are validated for use in the United States for diagnosis of scrapie, although several ELISA test kits have been licensed by the USDA Center for Veterinary Biologics for diagnosis of CWD in wild elk and deer.[16] The immunohistochemical test is considered to be the gold standard for testing in the United States, and it is also the confirmatory test at some labs of the World Organization for Animal Health.[17] The Western blot test is used primarily as a research tool and confirmatory test for BSE. An early report[18] on an immunocapillary electrophoresis assay indicated that it might be a valuable tool for detection of human BSE (ie, new-variant Creutzfeldt-Jakob disease) and scrapie in blood, but it is still undergoing evaluation as a diagnostic tool. Recently, other blood tests have been described that involve more sophisticated techniques such as protein misfolding cyclic amplification, aggregation-specific ELISA, and fluorescent amplification catalyzed by 17-RNA polymerase. All of these tests depend on amplification or replication of the prion many thousands of times.[19] The latter test can detect target protein at femtomolar concentrations and can also detect naturally infected mule deer with CWD and scrapie-inoculated mice with no clinical signs with 100% sensitivity and specificity.

The disease—At the time of manuscript preparation, the prevalence of scrapie in the United States was 0.1% to 0.3% (percentages that decreased from approx 0.5% before the eradication program began), as determined by slaughter surveillance.[20] Scrapie primarily affects blackface sheep breeds (eg, Suffolk, Hampshire, and crosses of those breeds), which account for approximately 96% of the overall prevalence of the disease.[20] In the United States, the disease is chiefly related to polymorphisms at codon 171 because the 136AA and 154RR genotypes are reportedly the norm among US sheep.[20,21] Ninety-one percent of brain samples with positive test results for scrapie originated from sheep with the AARRQQ genotype.[20] However, individual flocks may have high prevalences of sheep with the 136V genotype,[22,23] and genotype 154H is also occasionally detected. Given the predominance of scrapie in the Suffolk breed, many producers believe that Suffolk sheep are more susceptible to developing scrapie than other breeds, but this has not been confirmed. Although genotyping of 2,300 blackface sheep (primarily Suffolks) in a 2003 report[12] revealed that 43% of those sheep were of the 171QQ genotype and only 13% were of the 171RR genotype, other domestic sheep breeds have a higher frequency of susceptible genetics. Columbia sheep, for example, are predominantly of the 171QQ genotype.[21]

The high prevalence of scrapie in Suffolks in the United States is likely to be related to other factors, including that the disease was imported in Suffolk sheep; imported animals are expensive, and expensive sheep genetics are typically not wasted on crossbred or inferior flocks but rather remain congregated in purebred flocks; if crossbreeding is done, males are usually bred to females of different breeds and the disease is not transmitted by males; and Suffolk sheep may be used surreptitiously to confer desirable traits to sheep of Hampshire breeding, which has the next highest prevalence (5%) of the disease. Sheep of whiteface breeds (eg, Rambouillet, Southdown, and Cheviot) have also developed scrapie,[20] but most had a history of contact or likely contact with Suffolks. So far, scrapie has not been diagnosed in whiteface sheep grazed on public lands in the western United States.

The 136/171 AAQQ genotype has been associated with all instances of scrapie reported in the United States except for a few sheep with the 171QR genotype, which, to date, has been associated with the 136AV genotype.[20] The 171QR genotype is considered to confer resistance to scrapie, particularly when coupled with 136AA. In the United Kingdom, the 136/154/171 AARRQR genotype is one of the most resistant genotypes.[24] That genotype has been detected in 28% of sheep in the United Kingdom, but the prevalence of the disease there is only 0.4 cases/million head of sheep.

In the new *Uniform Methods and Rules for Scrapie Control*[25] published by the USDA, it is recognized that sheep with codon 136V are at high risk for developing scrapie even when they also carry codon 171R. The definition of genetically susceptible sheep now includes ewes with the 136/171 AVQR genotype that are epidemiologically associated with flocks in which scrapie has been diagnosed in sheep with the 136VV or 136AV genotypes.

In the United States and European Union, efforts to control and eliminate the disease by selecting sheep with at least one 171R codon and removing sheep with genotype 171QQ are in progress,[25,26] with the expectation that the remaining sheep will be resistant to scrapie infection. In the states of Texas[27] and Washington,[28] breeding animals entering the state must be of the 171RR or 171QR genotypes. Veterinarians from the USDA APHIS VS require elimination of all sheep with the 171QQ genotype from flocks affected with scrapie before lifting a flock quarantine, but allow sheep with the QR genotype to remain unless associated with codon 136AV.[25] Skeptics of this scheme worry that reliance on genetics for elimination of the disease may induce a false sense of security given that there have been several reports[29-31] of scrapie in Suffolk sheep with the 171RR genotype. However, the genotype susceptibility differences seem to be related to scrapie strain differences.

In 1988, Irish researchers using scrapie strain SSBP/1 reported[32,33] that Cheviot sheep had a short-incubation (2 to 3 years) genotype for scrapie manifestation, which was later found to be codon 136VV. Those investigators also found that sheep with the 136AV genotype had a long-incubation genotype (4 to 6 years). Sheep homozygous for the 136AA genotype were resistant or had an incubation period that was longer than the sheep's lifetime. When another scrapie strain (CH1641) was inoculated into Cheviot sheep with the 136/171 AAQQ genotype, however, those sheep were susceptible to scrapie, whereas sheep with the 136/171 AARR genotype were resistant to the CH1641 strain. Furthermore, the 171RR codon also seemed to convey partial resistance to experimentally induced BSE in sheep that underwent intracerebral inoculation.[33,d]

In flocks containing scrapie-infected sheep that carry the 171QR genotype, all or nearly all sheep with positive results of tests for scrapie also have the 136AV or 136VV genotype. Epidemiologists from the USDA APHIS VS suggest that these data strongly support the possibility that there is a second scrapie type in the United States in which codon 136V is the primary determinant of susceptibility; that strain has been referred to as a V-dependent strain.[e]

Strains of scrapie specific to the United States are unknown. However, because CH1641, the prototype for strain C, causes disease in sheep of the 171QQ genotype regardless of the 136 genotype, some researchers hypothesize that the C strain is the most prevalent US strain; it may be reasonable to suppose that SSBP/1, the prototype for scrapie strain A, would be the V-dependent strain in this country. Strain differentiation, however, requires comparison of the biochemical properties of the PrP^{SC} or strain typing by mouse bioassay and lesion-profile scoring performed by inoculating 3 conventional mouse lines.[34] The mouse bioassay is arduous and time-consuming, and its use has not been reported to the author's knowledge for US scrapie strains.

Nor98, a more recently discovered strain, was detected during surveillance testing by 1 of the 4 ELISAs[f] that are presently used in Europe as rapid screening assays.[35-37,g] The strain was originally reported in 5 unrelated Norwegian sheep with clinical signs that had the unusual genotype of 136/154/171 AAHHQQ or AAHRQQ, genotypes that are typically associated with resistance conferred by the H at codon 154. In those sheep, no PrP^{SC} was found in either lymphoid tissues or at the level of the obex, but was detected primarily in the cerebellum. Results of Western blot analysis indicated that the glycotype was different from other known scrapie strains or BSE and had a distinctive PrP^{SC} electrophoresis profile that included a low–molecular-weight protein band of 12 kDa.[35] More recently, a double leucine or leucine-phenylalanine polymorphism at a newly reported site, codon 141, has also been associated with some of these cases.[37] As of June 2004, 38 sheep with scrapie involving this strain had been identified.

More worrisome are the findings of other atypical cases of scrapie in the European Union. An obligatory active surveillance program was implemented there in 2002 that was based on large-scale testing of both slaughtered and recumbent small ruminants and that led to a considerable increase in the number of animals with a diagnosis of scrapie. The program has also revealed a number of cases of so-called discordant disease. Interestingly, these cases have also been detected with only 1[f] of the 4 screening ELISAs. In some of these discordant cases, the low–molecular-weight band associated with Nor98 was detected and some of the involved sheep were homozygous for 171R.[30,31] Moreover, by the beginning of 2005, the Veterinary Laboratories Agency of Great Britain had detected 83 atypical cases of scrapie from 110,000 tested samples. Of those atypical cases, 12 sheep had the RR genotype, which is associated with the greatest resistance to scrapie.[31] No explanation has been given for the increased sensitivity[b] of that ELISA for detection of atypical cases of scrapie, but the anti-prion antibody used in the test may play a role.

The Nor98 strain and other cases of discordant scrapie involved single sheep in flocks with no obvious contact with affected flocks, suggesting that the infectious agent may not have been transmitted by direct contact.[38] It has been hypothesized that such cases represent spontaneous prion disease, analogous to sporadic Creutzfeldt-Jakob disease in humans, and that the affected sheep were not infectious. However, the disease has been transmitted from samples of Nor98 infected brain and the brains of 3 other sheep with discordant scrapie (genotype 171RR) to transgenic mice expressing sheep prion protein.[37] It is also possible that these scrapie strains are similar to BSE in that they do not appear to be transmitted by natural means. Nevertheless, it is of concern that sheep with the 136/154/171 AARRRR genotype can no longer be assumed to be free of naturally acquired TSE. This finding challenges the foundation of selective breeding programs sponsored by the USDA APHIS VS and certain members of the European Union. In March 2007, the USDA announced that it had identified a single Wyoming ewe with Nor98 strain of scrapie; however, other discordant strains have not yet been found in the United

States. Unless the USDA APHIS VS endorses use of the ELISA test that has successfully detected the atypical scrapie strains, it is not likely that we will know what the prevalence of these odd strains in the United States really is.

Disease transmission—Interestingly, scrapie does not appear to be transmitted in utero despite the fact that the placenta and placental fluids contain PrP^{SC}. Lambs delivered via Caesarian section from infected dams and isolated from infected sheep remain disease free.[39]

Despite the wide distribution of PrP^{C} in reproductive, placental, and certain fetal tissues and fluids, PrP^{SC} has been detected only in the caruncular portion of the endometrium and cotyledonary chorioallantois (the fetal-maternal interface) of pregnant scrapie-infected ewes[40] and only if both dam and fetus are of a susceptible genotype.[41] The embryo or fetus is not exposed to scrapie while in utero in a scrapie-infected dam because there is physical separation from PrP^{SC}-containing allantoic fluid and chorioallantois by the amnion, which remains free of PrP^{SC} even when the other placental tissues are infected.[41,42] In other words, even if tissues of the maternal side of the placenta carry a susceptible prion protein, susceptible prion-containing cells from the fetal side of the placenta appear to be necessary for conversion to PrP^{SC}. Thus, an infected ewe introduced into a clean flock and bred to a ram with the 171RR genotype is unlikely to transmit scrapie. That does not necessarily hold true, however, if the ewe is bred to a ram with the 171QR genotype. In that instance, the spatial relationship between fetuses in utero can influence PrP^{SC} accumulation in ewes carrying fetuses with different genotypes. It appears that partial or incomplete anastomosis can exist in the blood supply to cotyledons of fetuses of different genotypes on the same side of the uterine horn,[42] and this can result in accumulation of PrP^{SC} in cotyledons with resistant genotypes.

The disease is naturally transmitted from infected dams during lambing via ingestion of infected placenta or allantoic fluids by flockmates and newborn lambs. Infected males are not believed to transmit the disease.

Findings from earlier research suggested that other body excretions remain free of scrapie infectivity. However, it is now known that scrapie can be transmitted to other sheep via blood transfusion,[43] and antemortem detection of scrapie prions in the blood has recently been reported.[19] Infectivity via that route is low, however, with a large volume of blood (400 to 500 mL) required to transmit disease.

Replication of scrapie prion requires the participation of cells related to the immune system (such as follicular dendritic cells and B-lymphocytes expressing tumor necrosis factor) and lymphotoxin α and β (factors present in the spleen and lymph nodes).[44] Chronic inflammatory conditions are usually accompanied by accumulation of B and T lymphocytes, follicular dendritic cells, and lymphotoxins at the site of inflammation. In 1 study[45] involving mice with chronic hepatitis, nephritis, and pancreatitis, mice infected with scrapie were found to have PrP^{SC} in the liver, kidneys, and pancreas, organs that do not contain scrapie prion in typical scrapie-infected mice. In another study,[46] naturally infected sheep with scrapie were also infected with maedi-visna virus, the European strain of ovine progressive pneumonia virus. In that study, PrP^{SC} was found in the mammary gland of sheep with lymphoid follicular mastitis (ie, hard bag), a common clinical sign of infection with the ovine progressive pneumonia virus. A common disease of sheep in the United States, ovine progressive pneumonia is characterized by lymphocytic infiltration of the lungs and mammary gland; joints and brain tissue may also be affected. Weight loss is a common sign of this disease, and when the brain is affected (the condition called visna), signs can mimic those of scrapie.

It is widely accepted that previous premises contamination can be a source of scrapie infection. Anecdotal accounts abound of flock depopulation and premises decontamination, followed by recurrence of disease in repopulated infection-free sheep. An early experiment[46] in which scrapie-contaminated material was buried for 3 years indicated that it remained infectious when unearthed.[47] In another study,[48] it was found that prions released in soil rich in phyllosilicates (such as clay) would be strongly adsorbed but could remain active. This may increase the risk of infection for ruminants grazing contaminated pastures or exposed to contaminated groundwater. Researchers believe that the infectious agent of scrapie has persisted in regions of Iceland for at least 16 years,[49] and its persistence has been proposed as being related to high concentrations of iron and a high iron-to-manganese ratio in the forage of those areas.[50] Carriage of prions by grass mites has also been reported,[51] but the importance of this finding is unknown.

Disease signs—Classic scrapie, which results from ingestion of PrP^{SC} by a susceptible sheep carrying the 136/171AAQQ genotype (the most common genotype in the United States), is a long-term, progressive, and debilitating neurologic illness that is believed to be uniformly fatal. Clinical signs may be noticed from 18 months to 5 years after exposure and include progressive weight loss with no concurrent loss in appetite, progressive ataxia, fine head tremors (most apparent in the ears), and cutaneous hypersensitivity. In an earlier study,[23] only about 70% of naturally exposed sheep with clinical signs of scrapie had pruritis (the clinical signs of which give rise to the disease's name); pruritis was not observed in any of the experimentally inoculated sheep with the AVQQ genotype.[23] Behavioral changes are often detected, with sheep assuming a vacant, fixed stare or suddenly becoming aggressive. Signs of hypersensitivity are often elicited by rubbing or scratching the sheep's back, which induces the sheep to throw its head back, make chewing motions and lick at the air, or compulsively nibble at the limbs below the carpus.

Ataxia is first detected when sheep are running. The hind limbs of sheep appear to be uncoordinated with the forelimbs, and affected animals adopt a bunny-hopping gait. Sheep often have a high-stepping gait in the forelimbs, resembling a prancing horse. As signs worsen, the hindquarters may sway when the sheep is standing.

Clinical signs last from 1 to > 3 months; sheep generally become recumbent because of weakness and incoordination. If helped up, an affected sheep may be able to remain standing for hours, but may not be able

to rise unassisted if it falls or lies down. Death follows within 1 to 2 weeks of a sheep becoming unable to right itself. Blindness, resembling that seen with polioencephalomalacia, occasionally develops.

Differential diagnoses include other diseases characterized by chronic weight loss: caseous lymphadenitis, abomasal emptying disease, Johne's disease, ovine progressive pneumonia (visna), dentition problems, and meningitis. The clinical signs of scrapie can vary, depending on the sheep's genotype and the strain of scrapie involved. Sheep with genotype 136/154/171 AVRRQQ that were orally inoculated with a brain homogenate from 7 domestic scrapie-infected sheep died after an abnormally short incubation time of approximately 1 year.[23] In those sheep, clinical signs varied from none (ie, sudden death) to being found unable to rise in the absence of previous ataxia and dying in 2 to 3 days to a substantially more rapid clinical course of the classic manifestation, lasting no longer than 3 weeks. Weight loss and signs of pruritis were not observed. Most veterinarians or producers would not recognize sheep with these signs as having scrapie. A complete necropsy should be performed on any sheep that dies unexpectedly, including evaluation and submission of the brain for immunohistochemical testing for scrapie.

Diagnosis—The pathologic changes associated with scrapie are confined to the CNS and include vacuolation, neuronal loss, astrocytosis, and accumulation of amyloid plaques. However, because histologic change can be inapparent, diagnosis of the disease in the United States relies on immunohistochemical testing to reveal the presence of PrP^{SC} in brain or lymphoid tissue.[52] The necessity for this type of testing was particularly evident in the study[23] involving inoculated 136/171 AVQQ-genotype sheep. Sheep do not mount an immune response against the abnormal prion, and at present, no sensitive or completely reliable test for diagnosis during the preclinical stages of the disease is available.

Detection of PrP^{SC} in spleen; retropharyngeal, mesenteric, and prescapular lymph nodes[53]; third-eyelid lymphoid tissue[54]; tonsil[55]; tongue[56]; retina[57]; rectal ring tissue[58]; spinal fluid[59]; and blood[19,60] prior to appearance of PrP^{SC} in the brain or of clinical signs has been reported. Deposition of PrP^{SC} in certain tissues enables detection of subclinical disease by biopsy in some instances. Detection of PrP^{SC} has been reported[h] in 76% of tonsils examined and 57% of lymphoid tissue specimens collected from the third eyelid of infected sheep. A small number of sheep in which the brain contains PrP^{SC} do not have detectable PrP^{SC} in the lymph nodes, and that number may be influenced by sheep genotype or scrapie strain.[35,61] The Nor98 strain, for example, has not been found in lymphoid tissues.[35]

The palatine tonsil has been used for biopsy and diagnosis,[55,62] but obtaining samples is not practical for use in live animals. Biopsy of lymph follicles of the third eyelid, although simpler and validated as a diagnostic test, yields a high percentage of unreadable samples because follicles may not be present in adequate numbers in as many as 40% to 60% of adult sheep.[h] Biopsy of other lymphoid tissues, such as mandibular lymph nodes and rectal mucosa, has not been validated as a diagnostic technique. However, biopsy of those sites may be useful diagnostically because examination of several tissues is likely to improve the chances of diagnosis. It has not been established at what time in the course of the disease the agent will consistently appear in these tissues, but it may be as early as 14 months after exposure. This interval likely depends on the age of the sheep at exposure and on the genotype or strain of scrapie because the incubation time in sheep with the 136V genotype appears to be shorter.[23]

Thorough histologic examination of the third eyelid has revealed that most lymphoid follicles are in the lamina propria of protuberances on the palpebral surface, rather than on the bulbar surface. Instillation of histamine into the conjunctival sac temporarily inflames the lymphoid follicles and may make them easier to sample. Administration of xylazine (0.1 mg/lb, IV) for restraint and topical administration of an anesthetic facilitates biopsy of third-eyelid lymphoid tissue and enables easy sampling of the mandibular lymph node and rectal-anal mucosa.

Regarding rectal biopsy, lymphoid tissue is located between the folds of the entire circumference of the rectal mucosa, extending to a point approximately 1 cm cranial to the mucocutaneous junction at the entry to the rectum. A small specimen of this tissue can easily be obtained from even an unsedated sheep.[58,62] Lymphoid tissue collected from either the eyelid or rectum should be laid on a small piece of sponge to keep it flat; sponge and adherent tissue can be placed in formalin solution without the tissue curling. For disinfection, instruments should be soaked in 2.5N NaOH or another disinfectant with activity against abnormal prions[63,i] for at least 24 hours before rinsing and sterilization in an autoclave.

In summary, scrapie is a reportable disease with an active and ongoing eradication program. The disease may cause clinical signs that are subtle and nonspecific, but most sheep with scrapie have one or more of the following signs: pruritis, weight loss without appetite loss, ataxia, weakness, head tremors, and cutaneous hypersensitivity. Early clinical signs may be restricted to fine tremors of the head that progress during a 1- to 3-month interval to noticeable head tremors, incoordination, and recumbency. Diagnosis is made on the basis of results of immunohistochemical staining of the obex and other parts of the brain or lymphoid tissue for PrP^{SC}. At present, no highly sensitive test is available for use in live animals; however, biopsy of lymphoid tissue from the third eyelid, rectum, or mandibular lymph node may reveal sheep with scrapie before clinical signs develop. Recent development of a test capable of detecting PrP^{SC} in blood[60] yields hope that a simple, cost-effective, and sensitive blood test for preclinical diagnosis of scrapie will be available in the future.

In the United States, sheep of blackface breeds (chiefly Suffolks) are responsible for 96% of scrapie diagnoses. Whiteface sheep comprise only a small percentage (4%) of cases, and the disease has yet to be reported in whiteface range sheep in the western United States. Genetics play a role in the susceptibility of sheep to scrapie, with the codons 136, 154, and 171 being chiefly involved. Sheep with codon 136VV genotypes are more susceptible to scrapie than those with

the 136AV genotype; sheep with the 136AA genotype are less susceptible. Sheep with codon 171RR are the most resistant to scrapie, followed by sheep with the QR genotype. Sheep with the QQ genotype are the least resistant. Codon 154H confers resistance, but is rare in US sheep. At least 12 sheep with the 171RR genotype have been diagnosed with scrapie in the European Union on the basis of an ELISA test that is not presently used in the United States.

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