Contents:

Scrapie in Sheep: A Transmissible Spongiform Encephalopathy

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Introduction

Scrapie is a transmissible, fatal, degenerative disorder of the central nervous system that affects sheep and goats. It belongs to a family of neurodegenerative diseases in mammals known as transmissible spongiform encephalopathies (TSEs), which includes bovine spongiform encephalopathy (BSE) in cattle, Creutzfeldt-Jakob Disease (CJD) in humans, and chronic wasting disease in deer and elk (Johnson and Gibbs, 1998). The focus of this review is on scrapie, which affects most sheep-producing countries in the world.

Symptoms
Scrapie is an insidious, fatal disease characterized by a long incubation period and neurodegeneration. After primary infection with scrapie has occurred, an incubation stage of at least one year typically precedes the development of overt physical symptoms. Clinical signs of scrapie in sheep (Dickinson, 1976) begin with mildly impaired social behavior, in which affected animals become nervous, confused, or anxious and separate themselves from the flock. Physical manifestation of the disease usually involves intense itching caused by local irritation of the skin. Scrapie acquired its name because, during this stage of the disease, affected sheep rub themselves against fence posts, buildings, and feeders and bite at their legs, belly, or rump in an attempt to relieve the itching, thereby scraping off their wool and causing skin abrasions.

As the disease progresses, locomotor incoordination becomes apparent in affected animals. Ataxia, especially of the hind limbs, is often accompanied by muscular tremors; consequently, scrapie-infected animals walk with a characteristic swaying of the hindquarters and a high-stepping gait of the forelimbs. Motor incapacitation could be accompanied by visual impairment (Clark, 1980), causing animals to run into fixed objects, stumble, and fall. Changes in fleece pigmentation (Laplanche et al., 1999) and facial hair color (Capucchio et al., 2001) are sometimes observed in scrapie-infected sheep, and these are probably caused by altered metabolism. Although affected animals typically have a normal appetite, weight loss and emaciation occur because prehension, chewing, and swallowing become impaired. Overt clinical signs usually last for one to three months before death (Clark, 1980).

The clinical presentation of scrapie varies widely among breeds of sheep and individual animals, particularly with respect to the development of intense scratching and locomotor incoordination. While Suffolk sheep typically show signs of both itching and motor incapacitation (Dickinson et al., 1965), only one of these symptoms typically dominates the clinical course in some other breeds of sheep. In Icelandic sheep, scrapie presents as ataxia and trembling with little itching (Pálsson and Sigurdsson, 1958). In contrast, the hill sheep of northern India exhibit severe itching and persistent rubbing without an extended period of locomotor incoordination (Zlotnik and Katiyar, 1961).

Response to scrapie infection also varies among individual animals of the same breed. Clark (1980) reported that only 31% of affected Rambouillet animals in the USDA Scrapie Field Trial at Mission, TX exhibited itching, while the remaining cases progressed directly to the stages of ataxia and trembling. In Norway, where 94% of scrapie cases involve the Rygja breed, approximately half of the infected sheep exhibit itching, while the other animals primarily show symptoms of incoordination (Ulvund, 1996). Furthermore, not all scrapie-infected sheep display clinical signs before death. Clark and Moar (1992) reported that 16% of the scrapie-positive animals examined on the Shetland Islands between 1985 and 1991 were found dead without exhibiting prior clinical symptoms. Thus, the physical manifestation of scrapie in sheep is widely variable, but death is inevitable for all infected animals.

Prevalence
The exact incidence of prevalence is difficult to ascertain because of inconsistent postmortem diagnosis and the lack of validated preclinical diagnostic tests. Furthermore, producers are reluctant to report suspect cases in order to protect their reputation and livelihood. Hoinville et al. (2000) estimate that only 13% of British farmers who suspect that they own scrapie-infected animals have reported them to the Ministry of Agriculture, Fisheries, and Food (MAFF), even though notification of

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suspect cases has been compulsory within the European Union since 1993 (Schreuder et al., 1993). Additionally, scrapie cases could be among deaths caused by unidentified illness. In a Scottish study (Clark, 1991), postmortem histopathological examinations of sheep that had been found dead without previously showing signs of disease revealed that 21% were infected with scrapie. All of these factors should be taken into account when considering scrapie prevalence statistics.

The first case of scrapie was reported in 1732 in England (McGowan, 1922). In the subsequent decades, scrapie endemically affected flocks in several countries due to intercontinental movement of affected sheep. In 1947, scrapie was first reported in the United States in animals imported from Canada that originated in the United Kingdom. Since this time, over 1,000 flocks have been infected with scrapie in the United States (APHIS, 2001a), and the number of reported scrapie cases is steadily increasing (Wineland et al., 1998). During the first half of 2001, 69 confirmed cases of scrapie were reported in the United States (APHIS, 2001b).

Today, the worldwide incidence of scrapie remains obscure, but it appears to be more prevalent in the northern hemisphere than in the southern hemisphere (Laplanche et al., 1999). Following the introduction and subsequent eradication of scrapie in Australia and New Zealand during 1952 (Brash, 1952; Bull and Murnane, 1958), extensive protective measures were implemented in these countries to prevent the importation of scrapie (MacDiarmid, 1996). Australia and New Zealand are now widely recognized as scrapie-free. In contrast, European countries have a relatively high incidence of scrapie-infected flocks. In Great Britain, the country with which scrapie is most commonly associated, 128 positive cases were reported during the first half of 2001 (DEFRA, 2001). In a recent survey (Hoinville et al., 2000), 14.9% of British sheep producers owning more than 30 breeding ewes reported that scrapie had probably affected their flock, and the prevalence of infected sheep in this survey was consistent with abattoir data from another study (Simmons et al., 2000). In the Netherlands, it has been estimated that 3.8 to 8.4% of flocks are infected with scrapie

(Schreuder et al., 1993). Although the Scandinavian countries of Denmark, Finland, and Sweden are at low risk for scrapie (Laplanche et al., 1999), there was recently a surge in the number of scrapie cases in Norway (Ulvund, 1996). Therefore, scrapie appears to be concentrated in particular geographic regions; whether this is due to the actual prevalence of scrapie or the absence of reporting in some areas is unknown. It is also important to note that the incidence of scrapie might be influenced by breed specificity or genetic predisposition (see below).

Economic Significance
In the United States, the American Sheep Industry Association estimates that scrapie costs the industry between $20 and $25 million annually (NIAA, 2001). This financial loss is due to decreased productivity of scrapie-infected flocks, lost income from potential exports, and increased disposal costs for offal. Annual mortalities in scrapie-infected flocks typically range from 3 to 5%, but in some cases annual mortalities can be greater than 10 or 20% (Detwiler, 1992). In scrapie-affected flocks, the number of infected animals increases and the age of scrapie onset decreases after several years, making these flocks economically unviable. The presence of scrapie in the US also prevents the exportation of breeding stock, semen, and embryos to many other countries.

The disposal of scrapie-infected carcasses is also of concern because the inclusion of TSE-infected protein in feedstuffs is probably one mode of horizontal scrapie transmission. Because the scrapie agent is extremely resistant to sterilization processes, including high temperatures, ultraviolet light, and suspension in formalin (Outram, 1976), rendering procedures that inactivate the scrapie agent are being investigated (Taylor et al., 1995, 1997, 1998, 1999; Schreuder et al., 1998a).

The scrapie disease has also economically impacted the scrapie-free countries of Australia and New Zealand. Most importantly, there has been damage to genetic improvement schemes where scrapie avoidance has limited the choice of potential breeding stock. Very few new bloodlines have been imported into Australia and New Zealand in the last 40 years (MacDiarmid,

1996).

In addition, the introduction of scrapie into Australia or New Zealand would severely impact biopharmaceutical manufacturers, a major component of the Australasian economy (MacDiarmid, 1996). The use of postmortem tissues derived from sheep and cattle for the production of biopharmaceuticals, including vaccines, has led to the generation of TSEs in some recipients. In Scotland, a scrapie infection occurred in 1937 after sheep were innoculated with a contaminated vaccine against louping-ill, which had been prepared in a 0.35% formalin suspension of ovine brain, spinal cord, and spleen tissues (Gordon, 1946). More recently, administration of a commercial vaccine against *Mycoplasma agalactiae*, which was prepared with ovine brain and mammary gland homogenates, was identified as the culprit for the sudden outbreak of scrapie in Italy in 1997 and 1998 (Agrimi et al., 1999; Caramelli et al., 2001).

Etiology of Scrapie

The current model for scrapie pathogenesis involves the interaction between a transmissible agent and the genetic makeup of the host. A novel proteinaceous infectious particle, called the prion protein (Pruisner, 1982), is thought to be the sole transmissible agent of scrapie (Pruisner et al., 1998). The natural transmission of prion proteins probably occurs by multiple pathogenic mechanisms, but the physical manifestation of the disease is also influenced by the host genetic makeup. The gene encoding prion protein contains several polymorphisms that are associated with scrapie susceptibility in sheep. Thus, an awareness of the interaction between the infectious prion agent and the host prion genotype appears to be critical for understanding scrapie etiology.

Infection with Prions
The only consistent characteristic of scrapie, as well as all TSEs, is the presence of an abnormal, aggregated form of a sialoglycoprotein called prion protein (PrP), which is tethered to neuronal and lymphocytic surfaces by a glycosylphosphatidylinositol (GPI) anchor. Upon scrapie infection, the normal cellular conformer (PrP^C), which consists of 42% a-helix and 3% ß-sheet, is posttranslationally converted into an abnormal scrapie isoform (PrP^{SC}),

which consists of 30% a-helix and 43% ß-sheet (Pan et al., 1993). This alteration in tertiary structure involves a templating process in which PrP^{SC} acts as a catalyst in the conversion of endogenous PrP^{C} into PrP^{SC} (Pruisner et al., 1990). Molecular chaperones (DebBurman et al., 1997) or partially unfolded PrP intermediates (Morillas et al., 2001) might aid in PrP^{SC} formation.

The mechanism by which PrP^{SC} subsequently induces pathological alterations in nervous tissue remains obscure. Immediately following exposure to the infectious agent, the conversion of PrP^{C} to PrP^{SC} occurs in gut-associated lymphoid tissues and in the spleen (Jeffrey et al., 2000), where PrP^{SC} incubates for several months before spreading to the central nervous system (Jeffrey et al., 2001). The exact mode of neuroinvasion by which PrP^{SC} translocates to the brain has not been elucidated, but a great deal of attention has focused on the implication of follicular dendritic cells and B-lymphocytes in PrP^{SC} transmission to the central nervous system (Klein et al., 1997; Brown et al., 1999a). However, recent evidence downplays the necessity of these factors in scrapie neuroinvasion (Shlomchik et al., 2001); dendritic cells from PrP^{SC}-infected splenic tissue can migrate to the central nervous system in the absence of additional lymphoid components (Aucouturier et al., 2001). Van Keulen et al. (1999) suggested that PrP^{SC} simply diffuses from gut-associated lymphoid tissues to the central nervous system by way of the enteric nervous system, and PrP^{SC} has been detected in enteric and autonomic ganglia of the gastrointestinal tract early in the incubation period (McBride et al., 2001). This hypothesis that neuroinvasion of the scrapie agent occurs directly within PrP^{SC}-containing tissues is supported by the observation that noradrenergic nerve endings lie close to PrP^{SC}-accumulating cells in the spleen (Bencsik et al., 2001).

Aggregation of PrP^{SC} molecules occurs upon PrP^{SC} invasion of the central nervous system, leading to the formation of prion rods, or scrapie-associated fibrils (SAF), in the brain (Rubenstein et al., 1987). Microglial cell activation occurs in response to abnormal PrP^{SC} deposition in the central nervous system (Williams et al., 1997; Giese et al., 1998). Subsequently, the intra-

cellular free calcium concentration increases in microglial cells (Herms et al., 1997), and tyrosine kinase signal transduction cascades are activated (Combs et al., 1999). The secretion of reactive oxygen species (Brown et al., 1996) and proinflammatory cytokines (Peyrin et al., 1999) by microglia seems to be critical for disease pathogenesis. Reactive oxygen species such as the superoxide anion are essential for the mediation of PrP^{SC}-induced neurotoxicity (Brown et al., 1996). Nerve cell death occurs by apoptosis (Giese et al., 1995; Lucassen et al., 1995), resulting in vacuolar formations that are characteristic of scrapie-positive animals. Astroglial cell proliferation, another attribute of scrapie-infected brain tissue, is induced by cytokines interleukin-1 and interleukin-6, which are also released from PrP^{SC}-activated microglia (Hafiz and Brown, 2000).

The normal physiological function of PrP^{C} is poorly understood, but experimental alteration of the gene for PrP^{C} in mice has provided insight into the normal cellular role of prion proteins. Mice lacking PrP^{C} generally exhibit normal behavior and development (Büeler et al., 1992), although there are reports of increased locomotor activity (Roesler et al., 1999) and greater susceptibility to seizures (Walz et al., 1999). Evidence exists that PrP^{C} may be involved in nucleic acid metabolism (Gabus et al., 2001), protection against neuronal apoptosis (Bounhar et al., 2001), resistance to oxidative stress via copper binding (Brown et al., 1999b, 2001; Klamt et al., 2001), signal transduction in neurons (Mouillet-Richard et al., 2000), mediation of neuritogenesis through laminin binding (Graner et al., 2000), T-cell activation (Cashman et al., 1990), regulation of intracellular free calcium levels (Whatley et al., 1995), and promotion of sleep continuity (Tobler et al., 1997).

Although the role of PrP^{C} in normal cellular physiology is ambiguous, its involvement in scrapie is well documented. Mice lacking PrP^{C} are resistant to scrapie infection (Büeler et al., 1993), and the presence of PrP^{C} in the host is necessary for scrapie infection to occur (Brandner et al., 1996). However, unresolved issues regarding the role of prions in scrapie remain. How did PrP^{SC} originally adopt its deviant structure, which is required for infectivity? Are there other players involved in scrapie infection? These questions are the basis for

ongoing discussions about infectious agents in the scrapie disease.

Genetic Influence

Scrapie susceptibility is influenced by the genetic makeup of infected sheep. The ovine prion protein gene (*PRNP*), which contains three exons and is over 20 Kb long (Westaway et al., 1994), is known to affect scrapie susceptibility in sheep. Exon 3 of the ovine *PRNP* gene contains the entire protein-coding sequence for PrP as well as a downstream 3'-untranslated region. Polymorphic variants in the protein-coding region of the *PRNP* gene, particularly at codons 136, 154, and 171, are associated with the incidence of scrapie in several breeds of sheep. Amino acid substitutions at these positions facilitate the conversion of PrP^{C} to PrP^{SC} in vitro (Bossers et al., 2000). In addition, polymorphisms in the 3'-untranslated region of ovine *PRNP*, such as an *Eco*RI restriction fragment length polymorphism (Hunter et al., 1991), have been associated with modified protein synthesis and disease progression (Goldmann et al., 1999).

In many breeds, including the Bleu du Maine, Cheviot, Flemish, Herdwick, Île-de-France, Romanov, Rygja, Scottish Halfbred, Shetland, Swaledale, Swifter, and Texel, the *PRNP* allele encoding valine at codon 136 (V_{136}) is associated with an extremely high risk of scrapie (Maciulis et al., 1992; Laplanche et al., 1993; Goldmann et al., 1994; Hunter et al., 1993, 1994, 1996, 1997a; Belt et al., 1995; Clouscard et al., 1995; Bossers et al., 1996; Tranulis et al., 1999). In scrapie-affected flocks of these breeds, infected animals typically carry V_{136} on least one *PRNP* allele. In a study of Scottish Cheviot sheep (Hunter et al., 1996), none of the scrapie-positive animals was homozygous for the wild-type codon (AA_{136}); however, 77% of infected sheep were VV_{136} and 23% were VA_{136}. Codon 136 also affects scrapie incubation time; sheep with the genotype VV_{136} have a shorter incubation time than VA_{136} animals (Goldmann et al., 1994; Hunter et al., 1996; Elsen et al., 1998).

The V_{136} codon is very rare in British, Irish, Japanese, and US Suffolk sheep (Hunter et al., 1994, 1997b; Ikeda et al., 1995; O'Rourke et al., 1996; O'Doherty et al., 2000) as well as in Lacuanes (Clouscard et al., 1995), Poll Dorsets (Hunter et al.,

1994), Soays (Hunter et al., 1997a), and Southdowns (Tyler et al., 1999). In these breeds, scrapie susceptibility is typically determined by PRNP codon 171. Three polymorphisms at codon 171 [glutamine (Q_{171}), arginine (R_{171}) and histidine (H_{171})] have been identified (Goldmann et al., 1990; Laplanche et al., 1993; Belt et al., 1995; O'Rourke et al., 1996). In studies of scrapie-positive Cheviot, Lacaune, Poll Dorset, Romanov, Soay, Southdown, and Suffolk sheep (Westaway et al., 1994; Clouscard et al., 1995; Ikeda et al., 1995; Hunter et al., 1997a, 1997b; Elsen et al., 1998; Tyler et al., 1999), infected animals were homozygous for glutamine at codon 171 (QQ_{171}). Sheep homozygous for arginine (RR_{171}) or heterozygous (QR_{171}) at PRNP codon 171 did not develop scrapie from either natural or experimental challenge in several studies (Laplanche et al., 1993; Goldmann et al., 1994; Westaway et al., 1994; O'Rourke et al., 1996, 1997; Hunter et al., 1997a; Elsen et al., 1998). These analyses suggest a relationship between the PRNP QQ_{171} genotype and scrapie susceptibility in Suffolk sheep. However, this association is not absolute, as there are several reported cases of QR_{171} animals with scrapie (Hunter et al., 1997b; Junghans et al., 1998; Tranulis et al., 1999), and a scrapie-positive Suffolk sheep with the RR_{171} genotype was found in Japan (Ikeda et al., 1995).

The H_{171} mutation has been identified in the Belclare, Corriedale, Galway, Mayo Blackface, Suffolk, and Texel breeds (Belt et al., 1995; Ikeda et al., 1995; O'Rourke et al., 1996; Hunter et al., 1997a; O'Doherty et al., 2001). While the H_{171} mutation appears to be associated with a high incidence of scrapie in the Corriedale breed (Ikeda et al., 1995), H_{171} in Texels confers neither resistance nor susceptibility to scrapie (Belt et al., 1995), and all Suffolk animals carrying the H_{171} mutation have been scrapie-free (O'Rourke et al., 1996; Hunter et al., 1997a).

The PRNP allele encoding arginine at codon 154 (R_{154}) is very frequent in sheep, but a histidine at codon 154 (H_{154}) has also been identified in the Belclare, Donegal Blackface, Galway, Herdwick, Icelandic, Mayo Blackface, Poll Dorset, Romanov, Rygja, Scottish Halfbred, Shetland, Suffolk, Swaledale, Texel, and Wicklow Cheviot breeds (Laplanche et al., 1993; Belt et al., 1995; Ikeda et al., 1995; Hunter et al., 1997a; Elsen et al., 1998, 1999; Thorgeirsdottir et al., 1999; Tranulis et al., 1999; O'Doherty et al., 2001). Although R_{154} is not associated with scrapie susceptibility or resistance, the presence of H_{154} confers scrapie resistance by overriding the Q_{171} mutation in some cases (Laplanche et al., 1993; Elsen et al., 1998, 1999; Thorgeirsdottir et al., 1999). In a study of Herdwick, Poll Dorset, Scottish Halfbred, Shetland, Swaledale sheep, the H_{154} mutation was identified only in scrapie-free animals (Hunter et al., 1997a). However, scrapie-positive animals with the genotype HH_{154} have been identified in Norway (Tranulis et al., 1999) and France (Elsen et al., 1998).

Recently, genotypes at all three PRNP codons have been taken into consideration when examining genetic predisposition to scrapie. The presence of a scrapie-susceptible genotype at any of the three codons renders an animal susceptible to scrapie. Therefore, the most resistant genotype is $AA_{136}RR_{154}RR_{171}$ (Belt et al., 1995; Bossers et al., 1996; Hunter et al., 1997a; Elsen et al., 1998, 1999; Thorgeirsdottir et al., 1999). Only one scrapie-positive animal with the genotype $AA_{136}RR_{154}RR_{171}$ has been identified (Ikeda et al., 1995). In addition, carriers of the $A_{136}H_{154}Q_{171}$ allele appear to be nearly as resistant to scrapie as $A_{136}R_{154}R_{171}$ carriers (Hunter et al., 1997a; Elsen et al., 1998, 1999; Thorgeirsdottir et al., 1999). The most susceptible genotype is $VV_{136}RR_{154}QQ_{171}$ (Bossers et al., 1996; Hunter et al., 1997a; Elsen et al., 1998, 1999; Thorgeirsdottir et al., 1999). However, it should be noted that while certain genotypes are susceptible to scrapie, the actual incidence of scrapie is dependent on other risk factors as well, such as exposure to the agent and dose of infection.

A PRNP-related gene called PRND (Moore et al., 1999), which encodes for the doppel protein (Dpl), has recently been identified. The topological structure of Dpl is very similar to that of PrP^C; it is an α-helical, GPI-anchored glycoprotein that is attached to the extracellular surface of cell membranes (Silverman et al., 2000; Mo et al., 2001). However, studies thus far have not identified a role for Dpl in scrapie; disease development and PrP^{SC} formation appear to occur normally in the absence of Dpl (Behrens et al., 2001). Dpl is upregulated in the central nervous system of PrP-deficient mice during Purkinje cell death and ataxia (Moore et al., 1999), which can be reversed upon introduction of PrP^C (Nishida et al., 1999). These apparent antagonistic roles of PrP^C and Dpl are supported by a recent observation that Dpl intensifies oxidative damage (Wong et al., 2001), which is in contrast to the normal cellular role of PrP^C as an antioxidant (Brown et al., 1999b, 2001; Klamt et al., 2001). Although Dpl has not been implicated in TSE development, the close relationship of Dpl and PrP^C justifies further investigation of Dpl in these diseases. To date, six polymorphic variants in the human PRND gene have been reported and are not associated with CJD susceptibility (Mead et al., 2000; Peoc'h et al., 2000). The ovine PRND sequence was recently described (Tranulis et al., 2001), and this sequence will serve as a tool for investigating the role of Dpl in scrapie development in sheep.

There is evidence that natural scrapie in Île-de-France sheep is influenced by the MHC (OLA) locus (Millot et al., 1985, 1988); however, scrapie was not associated with any of the OLA-linked markers tested in a study of Cheviot sheep (Hunter et al., 1996). In mice, MHC expression increases in neurons and astrocytes following scrapie infection (Duguid and Trzepacz, 1993). Differential expression of other genes in neural tissues following scrapie infection in mice has also been documented (Kenward et al., 1994; Lazarini et al., 1994; Doh-ura et al., 1995; Dandoy-Dron et al., 1998; Kim et al., 1999; Riemer et al., 2000). In addition, Miele et al. (2001) reported that the expression of the erythroid differentiation-related factor (EDRF) gene in the spleen decreases as scrapie progresses, which is the first report of differential gene expression in extraneural tissues. While the expression of these genes is altered during scrapie infection, there is no clear evidence that they are directly involved in disease manifestation.

Natural Transmission
In addition to the transfer of scrapie-susceptible or scrapie-resistant prion alleles to offspring, parental transmission of the infective

scrapie agent PrP^{SC} to progeny is an important means of disease propagation within a flock. Although there is no evidence for paternal transmission through semen (Wrathall, 1997), maternal transmission is thought to be one of the strongest contributors to scrapie dissemination (Pálsson, 1979). Progeny from scrapie-infected ewes are more likely to become clinically infected than unrelated offspring from scrapie-free dams (Dickinson et al., 1974; Hourrigan et al., 1979). However, it is difficult to determine whether this familial pattern of scrapie infectivity is due to genetic susceptibility, genuine maternal transmission in utero, or post-partum lateral transmission from ewe to lamb.

Although embryo transfer studies have primarily investigated the feasibility of salvaging valuable genetic material from infected animals, these studies have also provided insight into the maternal transmission of scrapie during gestation. Two groups have transferred embryos from scrapie-positive donor ewes to scrapie-free recipients to determine whether embryos harbor the infectious agent. Foster et al. (1992, 1996) have consistently found that scrapie develops in embryos from infected donor ewes; 23% of embryo transfer progeny in the earlier study and 50% of offspring from the later study subsequently developed scrapie. In contrast, Foote et al. (1993) successfully transferred 67 embryos from a scrapie-infected flock to scrapie-free recipients without scrapie transmission. However, this study has been criticized (Detwiler et al., 1996) because only 30 to 61% of donor ewes were diagnosed with scrapie, and only 9.5% of animals in the positive control group were infected. In a more recent study by the same group (Wang et al., 2001), 52 embryo-transfer progeny from scrapie-positive donors were successfully transferred to scrapie-free recipients without scrapie transmission.

These two groups also disagree on whether maternal transmission occurs through placental or fetal membranes in utero; both groups have also transferred embryos from scrapie-negative donors to scrapie-positive ewes. While Foster et al. (1996) found that 75% of these embryo-transfer progeny subsequently developed scrapie, all 25 offspring in the Foote et al. (1993) study remained scrapie-free during the 60-month

investigation. The infectious PrP^{SC} protein has been detected in placental membranes, including the caruncular endometrium and cotyledonary chorioallantois, from scrapie-positive ewes (Pattison et al., 1972, 1974; Race et al., 1998; Tuo et al., 2001); however, PrP^{SC} has not been found in fetal tissues and fluids (Tuo et al., 2001). Therefore, fetal exposure to PrP^{SC} in utero is probably not a primary mode of transmission because the fetus and the PrP^{SC}-positive tissues are physically separated by PrP^{SC}-negative amniotic fluids.

Although maternal transmission of PrP^{SC} does not appear to occur in utero, it is widely accepted that scrapie transmission occurs during the perinatal period. The longer that lambs remain in contact with their infected mothers after parturition, the more likely they are to develop scrapie (Hourrigan et al., 1979). Because the scrapie agent has not been identified in colostrum or mammary glands of scrapie-positive ewes (Hadlow et al., 1982), it is unlikely that transmission occurs through the milk. However, the expulsion of infected placental membranes and fluids during parturition may expose newborn lambs to the infectious scrapie agent PrP^{SC}. Given the relative stability of PrP^{SC} in the natural environment, the accumulation of PrP^{SC} in lambing barns over time would provide unexposed ewes and lambs a chance to come in contact with the infectious material. This model of transmission is supported by the observation that unrelated lambs housed in the same mothering pen as scrapie-infected ewes show an increased probability of developing the disease (Detwiler, 1992). Furthermore, approximately 89% of scrapie-infected animals are between the ages of 1.5 and 4.5 years (Dickinson et al., 1964); this fits the hypothesis that scrapie infection occurs primarily during lambing, followed by an incubation period of at least 18 months before clinical manifestation and death.

While recent evidence indicates that natural transmission occurs via parturition, the possibility that scrapie is transmitted by other mechanisms cannot be ruled out. Scrapie appears to be transmitted horizontally among sheep (Brotherston et al., 1968; Dickinson et al., 1974); however, the precise mode of horizontal transmission by which PrP^{SC} is disseminated through a flock is not

well understood. In preclinically scrapie-infected animals, the infectious agent is first detected in lymphoid tissues draining the digestive tract, including the retropharyngeal and mesenteric-portal lymph nodes (Hadlow et al., 1982), the tonsils (Schreuder et al., 1998b), and the ileal Peyer's patches (Heggebo et al., 2000). These results indicate that the ailmentary tract is a major route of entry for the scrapie agent.

Ingestion of the infectious agent could take place on scrapie-contaminated pastures, but exposure though shared bedding, water, feed, and pen surfaces or direct contact between flockmates during confinement are more likely to occur (Pálsson et al., 1979). Even though the primary mode of horizontal transmission is unknown, each of these routes is plausible because PrP^{SC} is relatively resistant to degradation, as demonstrated by Brown and Gadjusek (1991).

Tissues of scrapie-infected animals harboring the infectious agent PrP^{SC} are likely to be the primary sources of infectivity. Although PrP^{SC} is found in high concentrations in nervous tissues such as the brain (Race et al., 1998), the central nervous system is not exposed to the exterior where transmission could occur. Instead, excretions of PrP^{SC} in saliva, nasal discharge, and feces are more likely to be vehicles of transmission for the scrapie agent; additionally, skin scarification has been suggested as a possible PrP^{SC} source in sheep (Taylor et al., 1996). Salivary glands of scrapie-infected sheep do not contain PrP^{SC} (Herrmann et al., 2000), but small amounts of an infectious agent have been found in nasal mucosa, which was probably derived from the tonsils and retropharyngeal lymph nodes (Hadlow et al., 1982). The presence of the scrapie agent in the proximal colon (Hadlow et al., 1982) and the rectal nervous tissue (van Keulen et al., 1999) indicates that PrP^{SC} could be sloughed from intestinal wall into the feces; however, the scrapie agent has not been detected in fecal matter (Hourrigan et al., 1979).

Sheep with scrapie-resistant genotypes could harbor subclinical infections and transmit the PrP^{SC} agent to animals with scrapie-susceptible genotypes. Although Hill et al. (2000) recently demonstrated that the accumulation of a hamster PrP^{SC} strain in the brains of mice could occur without the development of clinical signs

during a normal lifespan, no such cases have been reported in sheep. Clearly, an understanding of the interaction between the infectious PrP^{SC} agent and the host genotype is important.

Control and Regulation of Scrapie

The lack of a validated live-animal diagnostic test for scrapie, combined with the long incubation period and variable expression of clinical signs in affected sheep, has made it difficult to employ regulatory measures to control this disease. The development of sensitive and reliable diagnostic tools will contribute greatly to the control and regulation of scrapie.

Diagnostic Techniques
Traditionally, scrapie diagnosis has involved the identification of clinical symptoms followed by confirmation using postmortem examination techniques (Fraser, 1976). The primary method of postmortem confirmation has been through histopathological examination of brain sections for neuronal degeneration, spongiform vacuolation, and astroglial cell proliferation. Additionally, the presence of SAF in brain tissue can be used to diagnose scrapie-positive animals (Merz et al., 1981). Because these neurological changes occur during advanced stages of the disease, it is not possible to diagnose preclinically infected animals using histological techniques. Other drawbacks to histopathology include the variable severity of neuronal vacuolation exhibited by different breeds of sheep, the natural autolysis of brain tissue within a few hours after death, and the presence of neurological changes in apparently normal sheep (Fraser, 1976).

The discovery that prion proteins are essential for scrapie manifestation (Pruisner et al., 1990) has led to the development of detection systems capable of identifying the PrP^{SC} molecule in tissues from scrapie-infected animals. Immunohistochemistry (IHC) on paraffin-embedded brain sections, which involves antibody staining of PrP^{SC}, is useful for verifying the presence of scrapie in questionable cases (Miller et al., 1993). Furthermore, IHC has been successfully used in the preclinical diagnosis of scrapie-positive animals that were erroneously diagnosed as scrapie-negative using histopatho-

logical techniques (Hamir et al., 2001; Kim et al., 2001; Ryder et al., 2001). However, IHC on brain tissue is a postmortem diagnostic technique; this test is not useful for determining the prevalence of scrapie in a population or for establishing policies to control the disease in live animals.

Recent efforts have focused on developing efficient PrP^{SC}-detection systems for accessible tissues from living sheep. The identification of PrP^{SC} in extraneural tissues of scrapie-infected sheep has provided several potential sources of PrP^{SC}-infected tissues. Although most studies have focused on testing lymphoreticular tissues for the presence of PrP^{SC} (Ikegami et al., 1991; van Keulen et al., 1996), blood from infected animals has been used in capillary immunoelectrophoresis assays (Schmerr et al., 1999). Tonsillar biopsy followed by IHC detection of PrP^{SC} (Schreuder et al., 1998b) initially showed potential as a preclinical diagnostic test; however, tonsil collection requires the administration of a general anaesthetic. The IHC detection of PrP^{SC} in the third eyelid, or nictitating membrane, of infected animals (O'Rourke et al., 1998) currently shows the most promise in preclinical scrapie diagnosis. The third eyelid is more accessible than the tonsils, and only a local anaesthetic is required for tissue collection. In a preliminary screening of preclinically infected sheep (O'Rourke et al., 2000), the results of the third-eyelid test were consistent with the scrapie status for 41 of 42 clinically suspect cases with confirmed scrapie and 174 of 175 scrapie-negative sheep. This test is useful for animals 14 months of age or older, assuming that the infection was acquired at birth (O'Rourke, 2001). The third-eyelid test is currently the focus of large-scale validation studies for use as a preclinical diagnostic tool that could be used in the control and regulation of scrapie worldwide.

Regulatory Measures
The United States Department of Agriculture (USDA) began regulating scrapie in 1952, after the US Secretary of Agriculture declared a state of emergency because of a scrapie outbreak in a flock in California (Detwiler et al., 1996). Despite ongoing efforts to eliminate scrapie from US flocks, the disease has continued to spread through the US sheep industry. In 1992, the USDA's Animal and Plant Health

Inspection Service (APHIS) established the current scrapie eradication program, the Voluntary Scrapie Flock Certification Program. Producers enrolled in the certification program are required to officially identify all animals older than one year of age, maintain complete records on every animal, and report suspect animals to health officials. Because scrapie has a long incubation period, participating flocks must be monitored for five years before being certified as scrapie-free. As of July 2, 2001, the program had only 781 participating flocks (APHIS, 2001b), of which 55 were certified scrapie-free; however, there are an estimated 66,000 sheep operations nationwide (NASS, 2001). Because the Voluntary Scrapie Flock Certification Program is not mandatory for all sheep producers, scrapie has continued to spread through US sheep flocks.

In an attempt to eliminate scrapie from the US by 2010, APHIS implemented the National Scrapie Eradication Program on November 19, 2001. All flocks that are not enrolled in the Voluntary Scrapie Flock Certification Program are required to participate in the new plan. The federal government allocated $10 million in the 2001 budget for the program, and the entire project will require an estimated $100 million (USDA, 2000). These funds will be used for animal identification, slaughter surveillance, and the validation of a live animal test for scrapie (USDA, 2001). Detailed and up-to-date information on this federal program is available online (www.animalagriculture.org/scrapie).

Under the National Scrapie Eradication Program, the primary responsibility of producers will be to use permanent ear tags or tattoos to identify all breeding sheep over 18 months of age, scrapie-exposed animals, and show sheep. In addition, a Certificate of Veterinary Inspection must accompany all breeding animals that enter into interstate commerce. The new program will also incorporate a slaughter surveillance plan, developed by APHIS's Centers for Epidemiology and Animal Health, which calls for the testing of 11,300 sheep at 25 plants in 14 states for scrapie using IHC of the brain stem. The data collected from this abattoir investigation will be useful in determining the prevalence of scrapie in the US. Eventually, scrapie-positive animals

will be traced to their flock of origin, and owners of infected flocks will be required to prepare a scrapie eradication plan for that flock. Another aspect of the National Scrapie Eradication Program is the validation of the third-eyelid test. Because this test will confirm whether or not living animals are infected with scrapie, it will be useful for identifying and removing only infected animals from the flock.

In the European Union (EU), scrapie has been a notifiable disease since 1993 (Schreuder et al., 1993). Further surveillance requirements were put forth in a 2001 EU Commission Regulation (EU, 2001). These requirements included the implementation of a compulsory slaughter scheme for all suspect animals and confirmatory diagnosis by histopathological examination or IHC detection. All EU-member countries were required to implement annual monitoring programs and provide compensation to owners for lost animals. Strict regulations on the import and export of live animals, embryos, ova, and semen were also described in the decision, and the feeding of all mammalian-derived protein to ruminants was prohibited.

In addition to these policies, the MAFF in England and agricultural administrations in Scotland and Wales are currently developing a National Scrapie Plan for Great Britain. During the first phase of the program, animals with genetic resistance to scrapie will be selectively bred. The Scrapie Information Group in Great Britain has developed a five-class scoring system (R1 to R5), that relates to the risk of scrapie infection in animals and their first generation progeny (Dawson et al., 1998). Scores correspond to specific genotypes; for example, the R1 classification corresponds to the $AA_{136}RR_{154}RR_{171}$ genotype for low scrapie susceptibility while the R5 category corresponds to the highly susceptible genotypes $VV_{136}RR_{154}QQ_{171}$, $AV_{136}RR_{154}QQ_{171}$, and $AV_{136}RR_{154}QH_{171}$. The Scrapie Information Group advises that animals with scores of R4 or R5 not be used for breeding.

Although Australia and New Zealand are widely recognized as being scrapie-free, these countries are taking measures to ensure that TSEs are not introduced into their herds or flocks (Turner, 1997). In 1996, the World Health Organization (WHO) advised that countries with no cases of TSE prohibit the feeding of meat and bone meal to livestock; subsequently, the Agriculture and Resource Management Council of Australia and New Zealand imposed a ban on feeding ruminant-derived protein. In 1997, the Australian Monitoring and Surveillance Program for TSEs was established, following the recommendation of the Office of International Epizooties (OIE). Under this program, veterinarians conduct field investigations and histopathological brain inspections to determine whether animals exhibiting signs of nervous disease are infected with TSE. The Australian Monitoring and Surveillance Program was structured so that there is a 90% probability of identifying a 1% incidence of TSE among nervous conditions affecting Australian sheep and cattle. It is important that Australia and New Zealand participate in these quality assurance programs to prove that they remain scrapie-free.

Conclusion

Clearly, controlling scrapie is vital to the future success of sheep operations. Because the complete biological mechanism of scrapie pathogenesis has not been elucidated, efforts at developing therapeutic drugs for treatment of clinical signs of the disease have been hindered. The identification of prion proteins and their crucial role in TSEs has led to the development of genetic tests for scrapie susceptibility and resistance. Given the currently available data, genetic selection will play a crucial role in the eradication of scrapie in the US. Therefore, breeding programs should incorporate criteria such as PRNP-resistant genotypes in animal selection. While genetic selection may be helpful in controlling and eliminating clinical disease within flocks at the present time, the future validation of reliable live-animal diagnostic tests will expedite the eradication of scrapie worldwide.

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