SCRAPIE: A NEURO DEGENERATIVE DISEASE IN SHEEP - A REVIEW

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ABSTRACT

Scrapie is a unique disease whose etiology is through protein infection rather than a biological agent. Genetic polymorphism at 136, 154 and 171 codons provide the susceptibility/resistance to the disease outbreak. PCR-SSCP has been found to be a robust technique for the identification of genetic variants because of SNP in the ORF (open reading frame) of PrP gene efficiently at low cost, evidenced by novel DNA sequences resulted from the SSCP variants. Prevalence of susceptible alleles at 136, 154 and 171 codons however did not result into the scrapie disease clinical symptoms in indigenous sheep breeds. Polymorphism at other loci in indigenous sheep could have played significant role in prevention of disease symptoms in indigenous sheep. The traditional feeding and management exclusively on range grazing without any concentrate supplementation having any animal protein might be responsible for the protection of animals from scrapie disease infection despite presence of susceptible alleles in the indigenous sheep animals.

Key words: Neuro degenerative, PRNP, Scrapie, Sheep.

1. Scrapie

Scrapie is a rare endemic brain disease of sheep and goats, an animal suffering from the condition scrapes itself up against posts to relieve an itch, damaging its coat and itself. Scrapie belongs to the most intriguing group of diseases, the prion diseases, comprising slowly developing fatal neurodegenerative conditions in sheep and other animal species. The disease was first described in sheep in Great Britain and Western Europe over 250 years ago (McGowan, 1922). Scrapie was introduced into the United States in 1947 (APHIS, 2001) from Great Britain via Canada. Scrapie had a significant impact on animal productivity.

2. Clinical signs and pathology

Typical clinical signs of scrapie in sheep (Dickinson, 1976) start insidiously with mildly impaired social behaviour, followed by locomotor incoordination or ataxia with trembling. Extreme itching may cause the animal to rub the wool from its sides and rear quarters. The appetite remains normal but the animal continues to lose body condition. Some show incoordination in movements with ovine progressive pneumonia, listeriosis, rabies external parasite and toxins may have some of the similar clinical signs. Pruritis can result from the animal attempting to relieve what seems to be an intense itching by scratching against fence posts or by biting the affected area and these clinical signs can last from 2 weeks to 6 months (Clark and Moar, 1992). Sheep apparently with scrapie and such a short clinical course that they are simply ‘found dead’.

3. Causative agent

Prusiner coined the word “prion” as a name for the infectious agent, by combining first two syllables of the words proteinaceous and infectious (Prusiner, 1982). In general usage, prion can refer to both the theoretical unit of infection and the specific protein (e.g., PrP) that is thought to be the infective agent, whether or not it is in an infective state. Prions are believed to infect and propagate by refolding abnormally into a structure which is able to convert normal molecules of the protein into the abnormally structured form. All known prions induce the formation of an amyloid fold, in which the protein polymerizes into a fiber with a core consisting of tightly packed beta sheets (Fig. 1).

Prions have the same system of organization as other proteins. They may have up to four levels of organization. The primary structure of a protein is the amino acid sequence that makes up the protein.

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The secondary structure of a protein is the local organization of the primary sequence of the protein -helical or -sheet formations. The tertiary structure of a protein is the overall shape of the protein, caused by interactions between the various local structures. Some proteins have a quaternary level of organization, which is defined by the interactions between the tertiary structures of two or more protein subunits.

Prions have one characteristic that makes them unique i.e. they can exist in two different conformations at the level of secondary structure. There is an alpha-helical portion of secondary structure in a normal prion that is refolded into beta-sheet formation in an aberrant prion. The primary structure of the aberrant prion remains the same, but its secondary structure is different (Fig 2). The protein that prions are made of is found throughout the body, even in healthy people and animals. However, the prion protein found in infectious material has a different structure and is resistant to proteases, the enzymes in the body that can normally break down proteins. The normal form of the protein is called PrPc, while the infectious form is called PrPsc - the C refers to ‘cellular’ or ‘common’ PrP, while the SC refers to ‘scrapie’, a prion disease occurring in sheep. While PrPc is structurally well-defined, PrPsc is certainly polydisperse and defined at a relatively poor level. PrP can be induced to fold into other more-or-less well-defined isomers in vitro, and their relationship to the form(s) that are pathogenic in vivo is not yet clear.

4. Scrapie-modified form of the prion protein in scrapie diagnosis

The abnormal disease-associated form of PrP is used both to diagnose a TSE at post-mortem and to search for infected tissues within the body of an animal. There are two methods for detection of PrPsc. Firstly, the PrP fibrils themselves can be visualized under the electron microscope and are referred to as scrapie-associated fibrils (SAF) (Dawson and Carter, 1987;) or prion rods. Secondly, the protein can be detected either on blots made from electrophoresis gels or in sections of tissue by means of PrP-specific antibodies (Western blots or immunocytochemistry) (Mohri et al., 1992). Classic scrapie may also show the widespread accumulation of PrPsc in peripheral tissues. Although early studies of a typical scrapie did not show PrPsc or infectivity outside the brain, recent data indicate that peripheral tissues from naturally infected animals can harbor infectivity either in the presence or absence of PrPsc (Andréoletti et al. 2011). However, whether this infectivity is established before or after the agent has propagated in the central nervous system is unknown. PrP Sc accumulation in the brain was usually associated with a more severe clinical disease (Konold et al., 2010).

5. Genetics of prion

A gene for the normal protein (PrP gene) has been isolated by (Oesch, et al., 1985). Some prion diseases can be inherited, and in all inherited cases there is a mutation in the PrP gene. Many different PrP mutations have been identified and it is thought that the mutations somehow make PrPc more likely to spontaneously change into the abnormal PrPsc form. Prion diseases are the only known diseases that can be sporadic, genetic, or infectious.

It should be noted that the same gene is responsible for spongiform encephalo pathies which are not known to be transmissible, as well as some non-neurological diseases. Whereas, some require a mutation for transmission to occur, and there are respective mutations which can prevent or protect against transmission for most of the TSEs (e.g. mutations leading to total absence of the PrP gene or heterozygosity at codon 129 of the same gene). Familial forms of prion disease are caused by inherited mutations in the PrP gene. This gene provides instructions for making a protein called prion protein (PrP).

6. Genetic polymorphism in prp gene

In sheep, transmission and the development of clinical disease have been reported to be influenced by the host’s genotype susceptibility or resistance to the classical form of scrapie is associated with polymorphisms in the PrP gene at codons 136, 154 and 171 (Hunter et al., 1994; Wylie et al., 2000). These three codons are located in a part of the protein that may undergo structural changes during the conversion from PrPc to PrPsc. At codon 136, alanine (A) is linked to scrapie resistance and valine (V) is associated with susceptibility (Hunter et al., 1997a). At codon 154, histidine (H) is associated with resistance and arginine (R) with susceptibility. At codon 171,
arginine (R) is linked to resistance, while glutamine (Q) and histidine (H) are linked to susceptibility. Within the sheep population, susceptibility to particular strains of TSE has been shown to be heavily affected by polymorphisms of the prion protein gene of the sheep (Hoinville, 1996, Goldmann, 2008).

It has been proposed that the genetic control of susceptibility to scrapie is either closely linked to, or identical to, the prion protein (PrP) gene (Hunter et al., 1994). The ovine PrP gene contains three exons and is over 20 kb (Wylie et al., 2000). In many breeds, including the Cheviot, Swaledale, Île-de-France, Shetland, Scottish Halfbred, and Bleu du Maine, the PrP allele encoding valine at codon 136 (V$_{136}$) is associated with an extremely high risk of scrapie, with most scrapie-positive sheep of these breeds, either naturally or experimentally infected, possessing the V$_{136}$ codon (Clouscard et al., 1995; Goldmann et al., 1994). However, the V$_{136}$ variant is rare in British and US Suffolk (Goldmann et al., 1994; Hunter et al., 1994) and at a very low frequency in Japanese Suffolk (Ikeda et al., 1995). In contrast to other breeds, variation at codon 171 has been associated with scrapie susceptibility in Suffolk (Wylie et al., 2000). Three amino acid variants at codon 171 [glutamine (Q$_{171}$), arginine (R$_{171}$), and histidine (H$_{171}$)] have been identified (Goldmann et al., 1994). In previous studies (O’Rourke et al., 1996; Wylie et al., 2000), Suffolk sheep that were scrapie positive were all homozygous for glutamine at codon 171 (QQ$_{171}$). Also, sheep homozygous for arginine (RR$_{171}$) or heterozygous (QR$_{171}$) did not develop scrapie from either natural or experimental challenge in several studies (Goldmann et al., 1994). These analyses suggest a relationship between the PRPN QQ$_{171}$ genotype and scrapie susceptibility in Suffolk sheep. However, this association is not absolute, as 2 of 64 scrapie-positive animals were QR$_{171}$ in one study (Hunter et al., 1997) and a scrapie-positive Suffolk sheep with the RR$_{171}$ genotype was found in Japan (Ikeda et al., 1995).

An extensive study of a scrapie epidemic in a closed flock of Romanov has been presented (Elsen et al., 1999). Over a 4-year period, 1,015 animals were exposed to scrapie, resulting in 304 deaths. The most resistant PrP allele was ARR (codons 136/
154/171), with no ARR carriers developing scrapie during the epidemic. The VRQ allele was highly susceptible, with 76% of the scrapie-positive animals possessing at least one copy of this allele. Selection for ARR or AHQ carriers may be a method for increasing scrapie resistance within a flock. However, they noted that these animals may still be susceptible to other scrapie strains, and it is possible that these healthy animals may transmit the scrapie agent.

These associations of scrapie with PrP genotype at either codon 136 or codon 171 may not be breed-dependent. Scrapie has been described in two different groups of Romanov sheep. In one of these, scrapie was associated with V_{136} alleles (Laplanche et al., 1993) however, in a different group of Romanovs; the association of disease was with QQ_{171} genotypes (Clouscard et al., 1995). The difference between the two groups of Romanov is intriguing. The scrapie outbreak described in (Clouscard et al. 1995) and associated with QQ_{171} may have resulted not from a ‘natural’ infection but from some form of coincident ‘contamination’ associated with a challenge of the animals with nematode larvae. The strains of scrapie, the infecting dose or the route of infection may have been different in each Romanov group and may have influenced precisely which PrP genotypes became affected by disease. This experiment also raises the possibility that nematode larvae play a role in spreading scrapie between sheep and between flocks.

In Texel sheep in the Netherlands (Belt et al., 1995), in a study involving 34 sheep from 18 different flocks, many different genotypes were affected by scrapie: W_{136}R_{154}Q_{171} (9%), VA_{136}R_{154}Q_{171} (35%), VA_{136}R_{154}H_{171} (44%), VA_{136}R_{154}R_{171} (3%) and AA_{136}R_{154}Q_{171} (9%). Single-flock studies tend to give simpler disease association with codon 136 or codon 171 or both together, either because of the genetic make-up of the flock or because only a single scrapie source is acting (Hunter et al., 1997b). Sheep from many flocks may be affected by as many strains of scrapie having different PrP codon ‘tropisms’. Most studies so far have agreed on the protective effect associated with R_{171} especially when homozygous (Laplanche et al., 1993; Westaway et al., 1994); however, there is a recent report from Japan of a single scrapie-affected Suffolk sheep of R_{171} genotype (Ikeda et al., 1995). It may be that Japanese scrapie targets PrP genotypes differently from European and US scrapie, but the sheep may also be different genetically. The same study (Ikeda et al., 1995) reported a link of R_{171} with a particular series of EcoRI and HindIII restriction fragment length polymorphisms (RFLP) not found so far in British Suffolks (Hunter et al., 1997a). The implications of this finding are not clear at the moment; nor is the disease linkage (if any) of alleles encoding H_{171} which have been found in both scrapie-affected and healthy sheep at low frequencies.

In Indian sheep breeds genetic polymorphism at 136, 154 and 171 codons provide the susceptibility/resistance to the disease outbreak. At codon 136 two genotypes AA and VV were observed in Karnah sheep breed with frequencies of 90.91% and 9.09% respectively. In Malpura, Garole and Mandya sheep breeds only AA genotypes were present. At codon 154 three genotypes RR, RH and HH were observed. The frequencies of these genotypes were 40.00%, 40.00% and 20.00% respectively in Mandya sheep. In Karnah sheep breed RR and RH genotypes were present in 90.91 and 9.09 percent animals respectively. In Malpura and Garole sheep breeds all the genotypes were of RR type. At codon 171 two genotypes QQ and QR were having frequencies of 54.55 and 45.45% respectively in Karnah sheep breed (Choudhary et al., 2007). In Malpura, Garole and Mandya sheep breeds all the genotypes were of QQ type. PCR-SSCP has been found to be a robust technique for the identification of genetic variants because of SNP in the ORF (open reading frame) of PrP gene efficiently at low cost, evidenced by novel DNA sequences resulted from the SSCP variants. Prevalence of susceptible alleles at 136, 154 and 171 codons however did not result into the scrapie disease clinical symptoms in indigenous sheep breeds. Polymorphism at other loci in indigenous sheep could have played significant role in prevention of disease symptoms in indigenous sheep (Choudhary et al., 2012). The traditional feeding and management exclusively on range grazing without any concentrate supplementation having any animal protein might be responsible for the protection of animals from scrapie disease infection despite presence of susceptible alleles in the indigenous sheep animals (Gupta et al., 2007).
7. Involvement of other genes in scrapie control

Parry (1984) believed that natural scrapie was caused by a recessive gene. In another study, showing the importance of the Sip gene in control of natural scrapie (Foster and Dickinson, 1988), the results were interpreted to suggest that, although the sA allele of Sip was dominant in controlling experimental scrapie, it was recessive in the case of the natural disease. Most sheep PrP geneticists would probably now say that Parry’s recessive gene is likely to be PrP, which, in the Suffolk breed which he studied, is linked to scrapie incidence in a recessive manner - PrP QQ
animals are at greatest risk of scrapie. The apparent conflict in the idea proposed by Alan Dickinson (Foster and Dickinson, 1988) between dominant susceptibility in experimental disease and ‘recessive’ susceptibility in natural disease can be at least partially resolved by addressing the unusual characteristics of SSBP/l, the scrapie source used to define the Sip locus. This source targets sheep according to PrP codon 136 genotype, with V136 acting in a dominant manner, and with such a minor effect from the codon 171 genotype that it is not obvious when only Sip genotype is considered (Goldmann et al., 1994). In outbreaks in which natural scrapie also targets codon 136, it does so within the QQ genotype group (recessive). Within the QQ group, the allele V136R154Q171 is dominant, as there are, in many outbreaks, twice as many VA136RR154QQ171 scrapie cases as there are VV136RR154QQ171 (Hunter et al., 1994). This effect is not reliant on the numbers of each allele within the affected flock.

Genes known to be linked to incidence of other diseases, such as the major histocompatibility complex (MHC), may also have some influence on scrapie. Natural scrapie incidence in Ile--de-France sheep has been said to be controlled by a recessive gene called Scr (Millot et al., 1988), which is linked to the OLA (ovine lymphocyte antigen) complex (sheep equivalent of MHC) in Ile-de-France sheep. However, an examination of sheep class I lymphocyte antigens in NPU Cheviot sheep found no linkage of scrapie with any of the markers tested (Hunter, 1996). This area warrants further investigation.

In sheep, several polymorphisms in the open reading frame of PrP are associated with differences in the phenotypic expression of prion diseases, such as in the incubation period, pathology and clinical signs. It has been confirmed that the polymorphisms of prion protein gene (PRNP) at codons 136, 154, and 171 have strong relationship with scrapie in sheep.

In sheep, variation in the PrP gene has been identified at a number of codons, but polymorphism at some codons is rare and only three codons (136, 154, and 171) have a reported linkage with the incidence of scrapie (Baylis and Goldmann, 2004). The ovine PrP polymorphism at codons 136, 154, and 171 were strongly associated with susceptibility or resistance to classical scrapie in sheep (Goldmann, 2008). Various PCR-based approaches have been used to determine ovine PrP sequences at codons 136, 154, and 171. These include direct DNA sequencing (Tranulis et al., 1999.), RFLP (Hunter et al., 1993; Ikeda et al., 1995), allele-specific oligonucleotide hybridization (Ishiguro et al., 1998), primer extension assay (Vaccari et al., 2004) and PCR-SSCP (Choudhary et al., 2007).

REFERENCES


