ARTICLES

Scrapie: Report of an outbreak and brief review

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Abstract

An outbreak of scrapie in western Canada is described. The disease was confirmed in seven sheep, all originating from the same flock; six were Suffolk ewes and one was a Hampshire ewe. The main clinical signs were pruritus with a positive "nibbling reflex", weight loss and seizures precipitated by handling or excitement. At presentation four ewes were between 35 and 38 months of age; two were approximately four years old and the oldest was six years old. No evidence of scrapie was seen in six goats on the original farm. The clinical signs, epizootiology, pathology, and pathogenesis of the disease are reviewed.

Résumé

La tremblante du mouton : épidémie et revue

Une épidémie de tremblante du mouton dans l'Ouest canadien est décrite. La maladie fut confirmée chez sept moutons qui originaient tous du même élevage; six de ceux-ci étaient des brebis Suffolk tandis que l'autre était de race Hampshire. Les principaux signes cliniques furent du prurit, un réflexe de « grignotement » positif, de la perte de poids et des convulsions précipitées par l'excitation ou la manipulation des brebis. Au moment de la présentation, quatre brebis étaient âgées de 35 à 38 mois; deux étaient âgées approximativement de quatre ans et la plus vieille avait six ans. Cette maladie ne fut pas identifiée chez six chèvres présentes dans l'élevage. Les signes cliniques, l'épizootiologie, la pathologie et la pathogénèse de la maladie sont revues.

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Introduction

Scrapie is an infectious, degenerative disease of the central nervous system of sheep and goats. The disease has been reported from most of the sheep-rearing areas of the world, but is most prevalent in western Europe (1). Authorities in New Zealand and Australia have been particularly vigilant in preventing the disease becoming established in their national flocks (2). No breed of sheep has been shown to be resistant to scrapie, but manifestation of clinical signs is subject to genetic control (3). The agent of scrapie has not been completely defined; it produces no obvious immune response; it is highly resistant to conventional antimicrobial agents; it may be similar to the very small viruses of plants (4–7).

Hadlow (8) drew attention to the clinical, epidemiological and histopathological similarities of scrapie and kuru, a degenerative disease of the nervous system affecting members of the Fore tribes of New Guinea (9). The close resemblance between kuru and Creutzfeldt-Jakob disease of man, first described in the 1920's, has also been recognized (10). These three diseases, together with transmissible encephalopathy of mink (11), transmissible spongiform encephalopathy of mule deer and elk (12,13), and the Gerstmann-Straussler syndrome of man (14) have been classified as subacute spongiform viral encephalopathies (5,6). The recently described bovine spongiform encephalopathy is pathologically very similar to this group of diseases (15).

In Canada, scrapie is a reportable disease. It was first recorded in this country in 1938 in a three-year-old Suffolk ewe recently imported from Britain (16). The clinical disease was confirmed in a goat in 1974 (17). Prior to 1981, a compulsory slaughter policy was in place but this was replaced by one of restricted slaughter of maternal female relatives of affected animals. Between 1981 and 1986, 17 outbreaks occurred and 1924 sheep from 94 flocks were slaughtered with compensation (18).

We report herein an outbreak of scrapie in western Canada. The disease was confirmed in five sheep from one flock over a period of three months. A further case occurred in a ewe born in this flock but sold as a lamb to a neighboring flock. Approximately one year after the original affected flock had been slaughtered, scrapie was diagnosed in a ewe born in the primary flock but kept in a small isolated experimental flock.

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History and clinical signs

The main flock consisted of approximately 120 ewes, 12 rams, five adult female goats and one adult male goat. There were approximately 60 Suffolk, 50 Hampshire, eight Columbia, and four Corriedale ewes.

TABLE 1
Breeds and ages of confirmed scrapie cases

Sheep No.	Breed	Date of birth	Age at presentation (months)
74M[a]	Suffolk	80-2-10	72
46P	Suffolk	82-1-26	47
12R	Suffolk	83-1-17	35
18R	Hampshire	83-1-19	38
26R	Suffolk	83-1-19	49
36R	Suffolk	83-1-20	36
39R	Suffolk	83-1-20	36

[a]Dam of 46P

In addition there were five Suffolk, four Hampshire, and three Columbia rams.

In December 1985, a four-year-old purebred Suffolk ewe (46P, Table 1) was admitted to the Western College of Veterinary Medicine with a history of weight loss over several months although her appetite had remained good. For three to four days before admission the ewe had appeared uncomfortable, holding her back slightly arched.

On clinical examination, the ewe was reasonably bright but quite thin; she weighed 72 kg and the transverse processes of the lumbar vertebrae were easily palpated. Her fleece was poor with evidence of "wool break", i.e. the wool was easily separated from the skin. She had a respiratory rate of 15–20 breaths/minute, a heart rate of 90–100 beats/minute, and a rectal temperature of 39.1°C. No abnormal respiratory sounds were detected on auscultation but a spontaneous cough was present. The ewe appeared to enjoy rubbing against fixed objects and people. An area of wool loss was noted on the right hind leg. On rubbing the skin in this area and along her back, the ewe would make nibbling, smacking movements with her lips. She would frequently bite at her forelegs when lying down. Careful examination of the fleece and skin failed to reveal any parasites. Ataxia of the hind legs was not observed, but the ewe walked with a high "goose-stepping" action of her forelegs.

One day after admission the ewe became depressed; her respiratory effort increased, and her rectal temperature was 39.9°C. Crackles and wheezes were heard on auscultation of the chest, and she was drooling excessively. She had difficulty rising and was unsteady on her feet. Fine muscle tremors and fasciculations were noted at this time. The ewe continued to deteriorate, developing severe dyspnea, and died two days after admission.

A clinical diagnosis of scrapie with terminal pneumonia was made. Blood samples collected on admission and submitted for routine hematology and serum biochemistry showed no significant findings.

A routine necropsy was performed. The carcass was emaciated with serous atrophy of the fat stores. Bronchopneumonia, from which *Pasteurella haemolytica* and *Pasteurella multocida* were isolated, was present in the cranioventral areas of both lungs. In addition, membranoproliferative glomerulonephrosis was noted histologically. The brain and spinal cord were removed and fixed in 10% neutral buffered formalin. The neurohistological examination of this and all other suspected cases was performed at the Agriculture Canada, Animal Diseases Research Institute (Nepean). Degeneration of the neurons, marked neuronal vacuolation, and diffuse gliosis were seen on histological examination of the brain, changes consistent with a diagnosis of scrapie.

Shortly after the initial case was diagnosed, Agriculture Canada Food Production and Inspection Branch veterinarians were notified about another Suffolk ewe (12R, Table 1), which was showing clinical signs similar to those of the first case. This animal had been born on the farm from which the first case had originated but had been sold as a lamb. Histological examination of the brain confirmed the clinical diagnosis of scrapie.

Over a ten week period, the Agriculture Canada veterinarians identified eight animals in the suspect flock which showed clinical signs suggestive of scrapie, but the disease was confirmed in only four. Three of the four were diagnosed within seven weeks of the first case being identified. Three were Suffolk ewes and the fourth was a Hampshire ewe. One, 74M, was the dam of the index case, 46P (Table 1). The flock was destroyed in March 1986 and the owner was compensated. No evidence of scrapie was noted in the goats present on the farm; they were also destroyed.

In March 1987, one of the authors (Petrie) was asked to examine a Suffolk ewe which was a member of a small isolated experimental flock. It had been identified by the attendants because it was thinner than others in the group despite being fed a good diet. Furthermore, on attempting to catch this ewe she had collapsed into lateral recumbency, and exhibited paddling and horizontal nystagmus. The ewe regained consciousness within one to two minutes.

On clinical examination, the ewe was extremely thin, dull, but interested in her surroundings. She was observed rubbing her hindquarters against the wall of the pen and nibbling at the water bowl. Examination of her fleece, revealed no areas of wool loss, but a heavy burden of biting lice (*Damalinia ovis*) and keds (*Melophagus ovinus*) was present. There was also evidence of wool-break. If light hand pressure was applied to her poll, the ewe would react against this pressure with vigorous force. Pressure applied to either side of the chest also provoked a reaction towards the applied pressure. Handling or excitement would occa-

sionally precipitate seizures. She was destroyed and scrapie was confirmed histopathologically.

The ear tattoo indicated that this animal (26R, Table 1) had been born in the original affected flock. Examination of the breeding records of the affected animals revealed that five of the seven histologically confirmed cases were born within a four-day period in January 1983 (Table 1). The two exceptions were the first case identified (46P) and her dam (74M). Thus, at the time of presentation, four animals (12R, 18R, 36R and 39R) were approximately three years old; two (46P and 26R) were four years old; the oldest (74M) was six years old. Apart from the mother-daughter relationship of 74M and 46P, it was not possible to establish a familial pattern among affected animals with the information available and before the flock was slaughtered.

Discussion and Review

This outbreak of scrapie is unusual in the number of cases which occurred over a very short period in a flock in which the disease had never previously been diagnosed. In the three-month period before the flock was slaughtered, 3.5% of the sheep developed clinical signs. The clinical diagnosis is usually based on pruritus, incoordination and ataxia, weight loss of prolonged duration, and the age of affected animals, but must be confirmed by histological examination of the brain. The disease must be differentiated from other causes of pruritus, such as lice or mange mites, or rarely pseudorabies, and those conditions causing incoordination and ataxia, such as spinal or brain abscesses, migrating parasites, trauma, or visna.

Clinical signs

For convenience of description, the clinical signs of scrapie have been grouped into three major syndromes: pruritus, alterations in behavior, and alterations in gait (19). In the classical pruritic syndrome, affected sheep rub against fixed objects, resulting in wool loss especially from the hindquarters, around the base of the tail, and along the chest wall. The poll is another predilection site. Affected animals appear to derive pleasure from this action, frequently making nibbling or smacking movements of the lips, or chewing on fixed objects whilst rubbing. The "nibbling reflex" can be elicited by massaging affected animals along the back or in the areas of wool loss. Biting at the forelegs as occurred in the index case is probably a manifestation of the pruritus, as is movement directed towards applied pressure. As a result of self-traumatization, the skin in areas denuded of wool is often thickened and multiple small pustules may be present.

Hyperesthesia is a common clinical finding in scrapie. Affected sheep may appear much more alert than normal and may also exhibit whole body tremors when approached. Moreover, seizures may be precipitated by excitement or handling as occurred with 26R. In contrast, some affected sheep become increasingly dull and obtuse as the disease progresses.

Incoordination, weakness, and imbalance of the hindlimbs may be one of the first indications of scrapie. This is most commonly observed when an affected animal is forced to change direction quickly. The weakness and incoordination are progressive, causing increasing difficulty in rising. Hypermetria, demonstrated by the high-stepping gait of the index case and indicative of cerebellar damage, is a frequent clinical sign.

In some breeds or strains of sheep, one clinical syndrome, either pruritus (20) or incoordination (21), predominates, but the majority of affected Suffolk sheep exhibit both pruritus and incoordination (22). Once clinical signs develop, death is inevitable. The duration of clinical disease is difficult to state with certainty because of the insidious onset, but varies from one to eight months (1). As the disease progresses, most affected animals lose weight, frequently despite an apparently good appetite, but some sheep actually gain weight (23). Obesity has been observed in certain strains of inbred mice infected with specific strains of the scrapie agent (24).

Visual impairment as the result of a retinopathy, characterized by scattered accumulations of lipid between the pigment epithelium and the layer of rods and cones (25), has been recognized in a small proportion of sheep with natural scrapie (26,67). A similar retinopathy has been demonstrated in rodents with scrapie (27–29).

Pathology

Gross lesions are not visible in the brain and confirmation of scrapie depends on histological examination. Diagnosis is based on vacuolation of the neurons in the medulla, pons, and midbrain. Interstitial spongy degeneration is often found in the same regions. Nonspecific hypertrophy of the astrocytes can be demonstrated by gold impregnation techniques (30,31). Recently, focal deposits of amyloid within the walls of the blood vessels of the cerebrum and cerebellum were demonstrated by light microscopy in 11 of 20 sheep with naturally occurring scrapie (32). Prior to that report, cerebral amyloidosis had been recorded only rarely in sheep with scrapie (23), although frequently observed with certain scrapie agent-inbred mouse combinations (33).

Unique fibrils structurally resembling amyloid fibrils were first identified by electron microscopy in the brain tissue of mice and hamsters experimentally infected with scrapie (34). These scrapie-associated fibrils (SAF) have been demonstrated in the brain tissue of naturally occurring cases of scrapie in sheep, and their presence may be a useful additional aid for the pathological diagnosis of scrapie (35,36). Their demonstration was not attempted in this outbreak. Scrapie-associated fibrils have been identified in natural and experimental cases of Creutzfeldt-Jakob disease, the Gerstmann-Straussler syndrome, and kuru, and are specific for these diseases (37–39). They have also been observed in brain tissue from cases of bovine spongiform encephalopathy (15).

Scrapie-associated fibrils are probably identical to the aggregated prions described by Prusiner and colleagues (40–42). They are closely associated with infectivity of the scrapie agent (43–45), but appear to be a product of infection rather than the infective agent

itself (46,47). The major protein of SAF, or prion protein, is encoded by a host gene which is found in many mammalian tissues (48,49), but the protein itself is formed primarily in neurons (50). Notwithstanding, SAF can also be demonstrated in the spleen and lymph nodes of naturally and experimentally infected sheep and mice (38,51,52) although there are reports to the contrary (47,49). The SAF protein produced by scrapie-affected animals is distinguished from the protein produced in normal neuronal tissue by the resistance of the former to protease digestion (53). Under the action of detergents, the SAF protein from scrapie-affected tissue aggregates into amyloid rods whereas the protein from normal neuronal tissue is completely solubilized (41,53).

Epizootiology and transmission

The age distribution of the affected animals in the present outbreak fits the normal pattern of scrapie. The majority of cases occur between two and four years of age and natural scrapie has been recognized only rarely in animals less than 18 months old (1,54). The upper age limit of clinical disease depends on the animal's lifespan, with the disease occasionally developing in animals more than ten years old (1). In affected flocks, a proportion of infected sheep may be culled for commercial reasons before clinical signs develop (54). The annual prevalence in affected flocks is often as low as 1–2%, but can be as high as 10–15%. Within a specific age group, i.e. three-year-olds, the incidence in heavily affected flocks can be up to 50% (22).

In naturally acquired scrapie, the incubation period is taken to be the age at which clinical signs are first noted and varies between approximately 18 months and five years (55). An incubation period of approximately two and one-half years was observed following the accidental transmission of scrapie by a contaminated sheep brain louping ill vaccine (56). Experimental infection of Cheviot sheep has revealed two phenotypes, one in which the average incubation period following subcutaneous inoculation was ten months (range 4–25 months), and one which could only be infected intracerebrally and in which the incubation period varied from 18 months through to old age (54).

The incubation period in both mice and sheep is controlled by one major gene known as the *sinc* (*s*crapie *inc*ubation period) gene in mice and the *sip* (*s*crapie *i*ncubation *p*eriod) gene in sheep (57,58). The *sinc* gene has two known alleles allowing the possibility of three mouse genotypes (59). The *sinc* gene and the gene which codes for the major SAF protein are closely linked (60,61). It has also been shown in mice that the incubation period of certain strains of the scrapie agent can exceed the natural lifespan of the mice (62).

Breeding trials with Cheviot, Herdwick, and Scottish Blackface breeds of sheep have shown that susceptibility to scrapie is inherited as a dominant trait (54,63,64). Resistance should not be regarded as absolute, as sheep apparently resistant to one strain of the agent readily succumb when infected with another strain (57). Although there is no evidence that infection is transmitted in semen (65), rams can influence the genetic susceptibility of a flock (19). Indeed, it has been argued that scrapie is an inherited disease (66).

A striking feature of the present outbreak was the births of five of the seven affected ewes during a four-day period in January 1983, suggesting that they were all exposed to the scrapie agent at the same time. In an affected flock, lambs born to ewes clinically affected with scrapie are twice as likely to develop clinical disease as those lambs born to non-affected ewes (1). Furthermore, a strong familial tendency to succumb to scrapie is well recognized, especially in the Suffolk breed (22). In the only familial relationship observed in the present outbreak, the dam of the index case did not develop clinical signs until after her daughter had died. The brain of the dam of one (12R) of the five affected ewes born in January 1983 was examined histologically but was negative for scrapie. The outcome of the remaining four dams was not established.

The route and timing of the transfer of infection from dam to offspring is not known with certainty, but the early removal of lambs after birth from a contaminated environment greatly reduces the incidence of clinical disease (1). The scrapie agent has not been found in fetuses from clinically affected ewes (67) and preliminary results of embryo transfer experiments indicate that no transovarian or intrauterine transmission of infection occurs (65). However, fetal membranes of affected ewes and goats are highly infectious (1,68).

In the present outbreak, the ewes were closely confined in a small barn for lambing, affording ample opportunity for contamination of bedding by infected fetal membranes and the early infection of lambs born into such an environment. The transfer of infection in nasal and oropharyngeal secretions from an infected ewe to the young lamb is also a possibility, as the scrapie agent has been demonstrated in the nasal mucosa, tonsil, and retropharyngeal lymph nodes of affected ewes and goats (67,69). Transmission through colostrum or milk is unlikely as infectivity has not been demonstrated in either colostrum from infected ewes or the mammary tissue of infected ewes and goats (67,69).

Confirmation that lateral transmission of scrapie can occur was hindered because of the uncertainty that test animals were not harboring infection before exposure to sheep with clinical scrapie (54). The long-term trial at Mission, Texas conducted by Hourrigan and colleagues (1) has now provided convincing evidence of lateral transmission; five of 140 previously unexposed sheep developed scrapie when placed in close contact with clinically affected and scrapie-exposed sheep. The period from exposure to the development of clinical signs varied from just over five years to seven and three-quarter years and at onset of clinical signs the ages of the five animals ranged from six to eight and one-half years. Subsequently, 22% of the progeny of the previously unexposed sheep developed clinical scrapie. The average age at the onset of clinical signs of this group was three and one-half years (1).

Pathogenesis

The successful transmission of scrapie to mice in 1960 (70) has enabled many aspects of the disease to be

studied in detail, even though the etiological agent has not been defined. Based upon the length of the incubation period and histological profile of the brain lesions in experimentally infected mice, at least 20 strains and three major classes of the scrapie agent have been identified (71–73). It should be noted that this mouse bioassay system is quite time-consuming with incubation periods varying between 6 and 18 months (67,73) and inoculated mice having to be observed for a minimum of two years (74).

The lymphoreticular system plays a major role in the pathogenesis of scrapie. Following inoculation of mice with brain tissue from clinically affected sheep, the scrapie agent was initially detected in the spleen, peripheral lymph nodes, thymus, intestines, and salivary glands; the spinal cord and brain were the last organs to become infected (74). Later studies have shown that the agent can only be found in the central nervous system (CNS) after a fixed proportion of the incubation period, approximately 35%, has elapsed (75,76).

After infection by any peripheral route, i.e. subcutaneously, intraperitoneally or intravenously, the spleen is the primary site of agent replication, at least in rodents (75–79). Splenectomy or asplenia prolongs the incubation period but does not prevent the inexorable development of disease (77), suggesting that other organs, such as visceral lymph nodes, can act as primary sites of agent multiplication. After replication in the spleen, the agent is thought to gain access to the CNS by migrating intra-axonally along the visceral autonomic nerves (59,77,79). Primary infection of the CNS is restricted to a segment of the thoracic spinal cord (75,79). From this site, infection migrates both cranially and caudally at a predictable rate of 0.5 to 1.0 mm per day. In the brain, infection also progresses rostrally from the medulla (78). This proposed intra-axonal transport of the scrapie agent is supported by the demonstration of infectivity in the contralateral tectal region of the brain following intraocular inoculation (79,80).

The possibility of hematogenous spread to the CNS cannot be excluded. Despite reports to the contrary (74,81), the infective agent has been demonstrated in blood and serum of scrapie-infected mice and hamsters (82,83) and in experimental Creutzfeldt-Jakob disease (84). In the latter report, the agent was associated with the buffy coat and low density splenic lymphocytes.

Experimental infection of goats revealed a temporal distribution of infectivity similar to that in mice but over a longer time period (85). The agent was detected in the peripheral lymph nodes, spleen and tonsils of goats killed 12 to 24 months after subcutaneous inoculation. In the CNS, infectivity was demonstrated in the spinal cord, medulla oblongata and midbrain of a goat killed 32 months after inoculation. Late in the infection, the agent could be identified only in the CNS, pituitary and adrenal glands. In sheep that had been exposed to clinically affected animals from birth, the infectious agent was first demonstrated in the retropharyngeal and mesenteric portal lymph nodes, spleen, distal small intestine, and colon when they were 10–14 months old. Infection of the CNS was first identified in a clinically normal sheep killed at 25 months of age. Highest titers were found in the diencephalon, midbrain, medulla oblongata and cerebellar cortex (67).

These findings suggest that natural infection occurs by the oral route with initial infection of the pharyngeal lymphatic tissue before disseminated lymphoreticular replication, or that initial infection and replication occur in the distal small intestine and proximal colon with subsequent spread to the spleen and visceral lymphatic tissue. It is not known if the agent replicates in the mucosa of the intestine or in the associated lymphatic tissue (67). Apart from the demonstration of infectivity in placental tissue (1,68), and the possible excretion of the agent in nasal secretions (67), other routes of agent shedding are unknown. Given its presence in intestinal tissue (67), fecal excretion seems the most obvious route, but the agent has not been demonstrated in feces (1,67).

Gajdusek (6,86) has recently put forward a hypothesis for the pathogenesis of scrapie and other closely related diseases of the CNS that result in the formation of neurofibrillary tangles, amyloid fibrils, scrapie-associated fibrils, and amyloid plaques. All of these pathological products are chemically and immunologically related to the 10 nm neurofilaments of neurons. Gajdusek suggests that the infectious agents of scrapie, Creutzfeldt-Jakob disease, and kuru either interfere with the production of neurofilaments leading to an accumulation of neurofilament in the perikaryon and lysis of the neuron, or "switch-on" the production of abnormal neurofilaments which form amyloid fibrils and amyloid plaques.

Identification of preclinical cases

The lack of any detectable immunological response to the scrapie agent (87), or any other suitable marker, has impeded the identification of infected, but preclinical sheep. Increased serum concentrations of IgG_2 have been reported in a high proportion of natural cases of scrapie but this response is not specific (88). The recent report that four of eight sheep with clinical scrapie had IgG autoantibodies to a 62 kDa neurofilament protein (89) should be further explored. In addition, although brain SAF are of no diagnostic value in the live animal, if they could be readily detected in non-neural tissue (35), or if antibodies to SAF were produced at some stage of the infection, the identification of preclinical cases would be facilitated. Unfortunately, antibodies to the protease-resistant protein of SAF were not found in the plasma of scrapie-infected mice before or during clinical disease (90).

The flock involved in the present outbreak had been established by purchases of purebred stock from several flocks over a number of years, but the source of the infection was not identified, despite extremely thorough investigations. This failure illustrates the difficulty in attempting to control a disease which not only has a very long incubation period but also a genetic component controlling expression of clinical signs and for which no serological test is currently available.

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