

Scrapie cases in Northern Cyprus

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Abstract: Scrapie is a fatal neurodegenerative disease affecting small ruminants that causes economic losses in many countries. It is categorized together with other fatal neurodegenerative diseases as a transmissible spongiform encephalopathy. The aim of this study was to investigate clinical cases and the cause of death in a flock of sheep from Northern Cyprus (Turkish Republic of Northern Cyprus) suspected of having scrapie. Brains from 4 sheep showing clinical signs of classical scrapie, out of a flock of 200 animals, were analyzed by histopathological examination and infective prion enzyme-linked immunosorbent assay (ELISA). The 4 affected sheep were 2–3 years of age and initially showed signs of salivation. Further clinical signs included hyperirritability, excitability, grinding of teeth, scratching, head tremor, ataxia, weakness of the hind limbs, and paresis. Infective prion protein was detected by ELISA in all 4 sheep brains, and many well-defined vacuoles of different sizes were seen in the neuropil of the gray matter from the medulla oblongata, obex, caudal cerebral pedunculi, and the proximal part of medulla spinalis. After the diagnosis of scrapie, all of the animals in the flock were euthanized and buried to eradicate the disease according to EU rules. This study describes classical scrapie in sheep for the first time in Northern Cyprus.

Key words: Scrapie, sheep, brain, ELISA, Northern Cyprus

1. Introduction

Scrapie is a fatal neurodegenerative disease affecting small ruminants (1–3). It causes economic losses in many countries because of animal deaths and control programs and has been categorized as a transmissible spongiform encephalopathy (TSE). Amongst the TSEs, particular attention has been focused on scrapie since it was assumed that bovine spongiform encephalopathy (BSE) originated in cattle fed with scrapie contaminated feed (3–5). It has therefore been a model for prion diseases seen in several animal species and humans. There is no evidence, however, that scrapie affects human health, although the BSE agent was found in sheep (1–7). The central nervous system is the primary tissue affected by scrapie, and diverse clinical symptoms can occur (1–3,5). Scrapie can be subdivided in to classical and atypical forms. The atypical form was first identified in Norway in 1998 and has been designated as “scrapie Nor98” (8). Classical scrapie is an infectious disease of sheep and goats that has been known for 250 years. It was originally reported in the UK, but after the introduction of wide-scale scrapie surveillance in EU countries in 2002, it has now been reported in many other countries with the exception of Australia and New

Zealand. While there have been no reported scrapie cases in Turkey, it is not considered free of disease by the OIE. Classical scrapie affects sheep with a certain genotype that have been exposed to the infectious agent. Geographical region, flock size, flock type, and sheep movement between herds have been found to be risk factors for the occurrence of classical scrapie (9,10). Atypical scrapie occurs in sheep resistant to classical scrapie, and as natural transmission from sheep to sheep has not yet been proven, it therefore occurs at a very low level in farms across the European Union (10).

Reports have indicated the presence of scrapie in the Republic of Cyprus since 1985 (11,12); however, there has been no report of the existence of scrapie in Northern Cyprus until now. The aim of this study was to investigate the presence of scrapie cases in the sheep population in the Gazimağusa district of Northern Cyprus (Turkish Republic of Northern Cyprus).

2. Materials and methods

2.1. Necropsy and histopathology

Four sheep were euthanized and systemic necropsy was performed. The brains were examined grossly for the

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presence of any pathological changes. The whole brains, including the medulla oblongata and proximal part of medulla spinalis, were removed and vertically split into halves. For histopathology, samples of other organs and half of the brain were placed into 10% buffered formalin for 6–7 h. Slides were prepared from the samples kept in buffered formalin using conventional methods as previously described (13) and examined microscopically. Briefly, 1 cm of tissues from the brain, cerebellum, medulla oblongata (obex), and proximal part of medulla spinalis were taken and kept in 10% buffered formalin saline. The smaller-sized samples were taken from tissues, put into the same solution, and kept for 7–8 days. These tissues were processed routinely and embedded in paraffin. Paraffin sections were cut at a thickness of 4–5 μm and were stained with hematoxylin and eosin (H&E). The second half of the brain was kept for the extraction of prion to use in the enzyme-linked immunosorbent assay (ELISA). The samples for prion analysis were transported at 4–8 $^{\circ}\text{C}$ to the laboratory within 1 day.

2.2. Prion extraction and ELISA

Prion extraction was performed from the obex of the medulla oblongata using a commercial test kit (Bio-Rad, Ref: 355-1100). The 350-mg tissue samples were homogenized using a RiboLyser (Hybaid-FP120HY-230) and the procedure was performed as described by the manufacturer. For the detection of infective prion protein, the Platelia BSE test kit (BSE-Scrapie Detection Kit, Platelia, Bio-Rad, Ref: 355-1103) was used as described by the manufacturer. Positive and negative controls were used in parallel as supplied in the kit.

3. Results

3.1. Description of cases and samples

The clinical cases investigated in this study were from a flock of 200 sheep in the Gazimağusa district of Northern Cyprus. Cases first began in 1998 after the introduction of newly purchased sheep into the flock that showed signs of neurological disorders similar to scrapie. The veterinarian involved suspected scrapie, but no laboratory testing was performed. Subsequently, 20 sheep died in 2000 and 80 sheep died between 2000 and 2002. All were clinically suspected of scrapie but were not tested for scrapie. After their recent deaths, 4 sheep showing clinical signs of scrapie were investigated for laboratory confirmation of the disease.

3.2. Clinical findings and ELISA

All 4 affected sheep were 2–3 years of age and initially showed signs of salivation. The disease then progressed to show signs of changes in behavior, hyperexcitability, running away, grinding of teeth, pruritus, rubbing, tail-biting, and motor function abnormalities (head tremor, ataxia, weakness of the hind limbs, and paresis). Pruritus

was pronounced on the head, tail, shoulder, and lateral abdomen, causing scratching with hind limbs and resulting in localized skin lesions and alopecia. The sheep tended to scrape their heads along walls and gates. Chronic emaciation occurred although the appetite appeared to be normal. Milk production was increased. Most of the affected sheep died within 2–3 months after showing clinical signs. The 4 sheep investigated in this study, however, were euthanized and infective prion protein was detected by ELISA in their brains. The negative control sample showed no sign of infective prion protein presence.

3.3. Necropsies and histopathology

No gross lesion was observed in any of the 4 sheep brains examined. Slight hyperemia was observed in the liver, lungs, and brains. Cerebrospinal fluid was increased in 2 of the sheep. In histopathological examinations, numerous well-defined small vacuolar changes of different sizes were seen in the neuropil of the gray matter of the medulla oblongata, obex, caudal cerebral pedunculi, and midbrain (Figure 1). Similar vacuoles were also seen, particularly in motor-neurons localized in the obex. The vacuoles in the perikarya of the neurons were either single and large or few and small (Figure 2). An increase in the number of neuroglial cells was also noted. In addition, vacuolar changes were also seen in the pyramidal cells and neuropil in the stratum pyramidale of the cerebral cortex and in the neuropil and motor-neurons in the proximal part of the medulla spinalis. Slight lymphocyte infiltration around the blood vessels and gliosis was also seen in the aforementioned areas.

4. Discussion

Scrapie has been reported in many countries and is still causing economic losses to sheep farmers worldwide. Until now, however, there have been no reports of the presence of scrapie in sheep from the north of Cyprus.

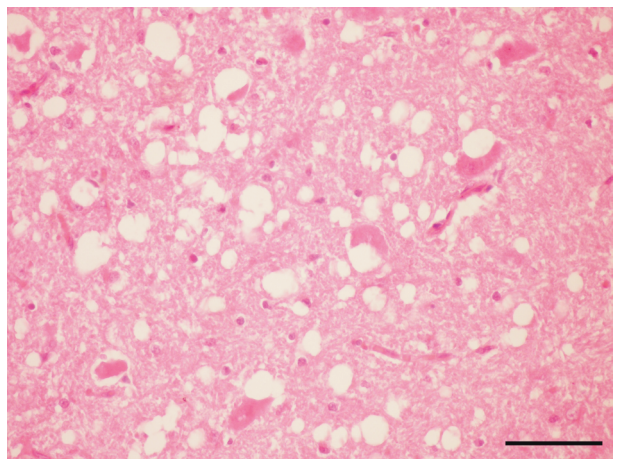


Figure 1. Numerous vacuoles seen in the neurons and neuropils in the medulla oblongata. H&E; bar = 100 μm

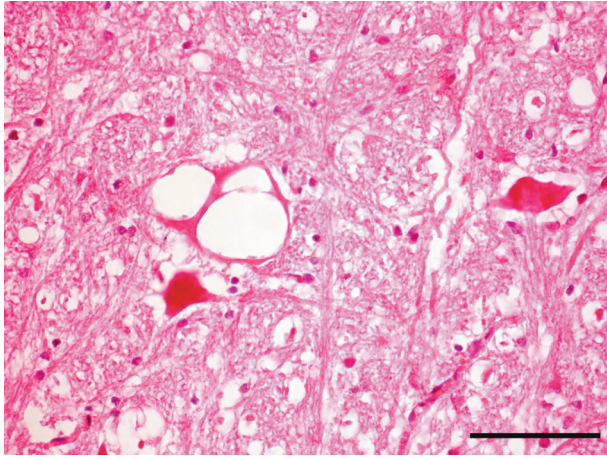


Figure 2. Multiple vacuolar changes seen in the neuron in the obex. H&E; bar = 50 µm.

There have been outbreaks of classical scrapie in the south of Cyprus since 1985 (11), and in the following 4 years it was diagnosed in 23 flocks (12,14). In this time, 356 out of 957 sheep examined showed the histopathological lesions unique for classical scrapie (11,12). We have reported the first classical scrapie cases in Northern Cyprus.

One of the most important modes of transboundary dissemination of scrapie is the movement of animals from flocks that have previously had cases of scrapie. This is a more complicated issue in cases of subclinical signs of scrapie because there is no laboratory test to diagnose the disease in a living but nonclinical animal (1,2,8,14). We think the cases of scrapie identified in this study were due to this mode of transmission, since there have been cases in the south of Cyprus and the clinical signs were observed 10–11 months after new animals were brought into the flock.

It has been shown that classical scrapie is linked to the *PrP* gene, and that atypical scrapie (Nor98) can be seen in sheep carrying different genes (8,15). This finding has been used to discriminate classical scrapie from atypical scrapie. In 2005, the European Food Safety Authority (EFSA) published 3 documents regarding the tests for TSEs and discrimination of classical and atypical scrapie (15–17). ELISA tests have been used for the detection of TSEs, with the Bio-Rad Platelia test being recommended for the detection of both types of scrapie in sheep (16,17). Both western blot and ELISA tests have been used for the detection of classical and atypical scrapie. In 2005, data about atypical scrapie from 13 countries were analyzed (16,18) and the results showed that 95% of the atypical scrapie cases were detected by the Bio-Rad Platelia/TeSeE rapid test. It has been reported that the western blot missed 1 of the 3 atypical scrapie cases in the fallen stock, indicating potential differences in the diagnostic

sensitivities between these 2 screening methods (19). In this study, the Bio-Rad Platelia/TeSeE rapid test was used to detect infective prion in the brains of sheep.

The criteria proposed by the EFSA and findings of published papers indicate that atypical scrapie cases have been characterized by the distribution of pathological changes and deposition of the prion protein (PrP^{Sc}), which is generally found in the cerebellum and has not been detected in peripheral tissues (16,17).

The clinical signs of atypical scrapie can be different or less pronounced than the signs observed in classical scrapie (8). Ataxia, anxiety, and loss of body condition were observed in Norwegian sheep with atypical scrapie (8,20–22). In Ireland, weight loss and nervous behavior have been reported (8,23). In the Falkland Islands, the single diagnosed animal displayed abnormal behavior with persistent biting at its leg and “collapsing episodes” when it tried to run (24). In the UK, ataxia and delayed repositioning of the hind limbs were reported in 2 sheep (25). Pruritus has not been observed in the atypical scrapie cases apart from in one of the suspected animals in the UK, which displayed a positive scratch response (25). In this study, all of the sheep showed pruritus and other neurological signs, which fit the criteria of classical scrapie published by EFSA.

The majority of atypical scrapie cases have been seen in older sheep. Out of 84 cases in different countries, the mean age was 6.5 years (8). In German scrapie cases, 60% of the animals were over 5 years old (20). However, peak incidence was also found in animals 4–5 years old with classical scrapie in the UK (26). The high numbers of classical scrapie cases were found in the sheep 18–36 months old in European countries (16). In this study, the age of the sheep analyzed was between 2 and 3 years old, which is the age of most sheep with classical scrapie. The sheep that died in the flock, but were not analyzed in this study, had an age range of 2–4 years.

Vacuolizations in the cerebrum, cerebellum, and medulla oblongata have been very important findings in the histopathological diagnosis of scrapie and other TSE infections. Vacuolization comprises 2 separate features, a microcytic vacuolization of the gray matter of the neuropil (spongiosis or spongiform change) and characteristic single large, or multiple, vacuoles in the perikaryon of neurons (5,27). The vacuolar changes observed in the sheep in this study were characteristic of TSE infections and similar to those seen in goat and sheep scrapie cases (27–29). These vacuoles were localized to the gray matter neuropil and were well-defined small vacuoles. These vacuolar changes were also seen in the perikarya of neurons as either large and singular or small and multiple. The intensity of the vacuoles were varied. They were more intense in the neuropil of the obex, caudal cerebral

pedunculi, and midbrain. Many vacuoles were also seen in the neurons of the obex. These results are consistent with results obtained from scrapie reported in sheep and goats. Histopathological findings in this study reflect classical scrapie, which has been described by EFSA and other investigators (27,28).

Early identification of scrapie cases in the flock affects the success of control programs in scrapie eradication. Therefore, active surveillance of TSEs in small ruminants has been going on as an EU regulatory requirement since 2002. EU member states test according to minimum requirements and non-EU member states were free to establish their own programs. The prevalences of atypical and classical scrapie have been studied in 20 European countries and low prevalence has been found in the surveillance program targeting sheep older than 18 months (slaughtered and fallen stock) (15). In this study, after the diagnosis of scrapie, all animals in the flock were

euthanized and buried to eradicate disease according to EU rules.

In the UK, atypical scrapie was found in around 0.1% of both abattoir and fallen stock surveys, and this figure did not change significantly between surveys and years (30). The prevalence of atypical scrapie in European countries was found to be 2–6 cases per 10,000 and atypical scrapie has been reported in most European countries as well as in the Falkland Islands, Canada, and USA (8,15). Classical scrapie has been reported in 20 countries and atypical scrapie has been reported in 14 countries. The number of classical scrapie cases varied in each country but at least one case has been reported from each country every year (15).

In conclusion, scrapie cases have been found in the north of Cyprus (Turkish Republic of Northern Cyprus). Early identification and epidemiological studies are necessary in Cyprus in order to control the spread of disease in the sheep and goat populations.

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