A Survey of More Than 11 Years of Neurologic Diseases of Ruminants with Special Reference to Transmissible Spongiform Encephalopathies (TSEs) in Greece

S. LEONTIDES1,5, V. PSYCHAS1, S. ARGYROUDIS2, A. GIANNATI-STEFANOU3, E. PASCHALERI-PAPADOPOLOU3, T. MANOUSIS4 and T. SKLAVIADIS4

Addresses of authors: 1Laboratory of Pathology and 2Clinic of Farm Animal Medicine, Faculty of Veterinary Medicine, Aristotle University, 54006 Thessaloniki and 3Veterinary Research Institute, National Agricultural Research Foundation, 26th October 66, 54627 Thessaloniki, Greece; 4Laboratory of Pharmacology, Faculty of Pharmaceutical Sciences, Aristotle University, 54006 Thessaloniki, Greece; 5Corresponding author: Box 400, Aristotle University, 54006 Thessaloniki, Greece, Tel: +30 31 994531, Fax: +30 31 994532

With 5 figures and 1 table

(Received for publication May 17, 1999)

Summary

The first cases of scrapie were detected in Greece in a flock of sheep in October 1986. All the animals of the affected flock and all sheep in two flocks that were in contact were killed and buried. A systematic investigation of all available cases with signs indicating a neurological disease started in sheep and goats in late 1986, as well as in cattle in 1989. The investigation was based on clinical examination, necropsy or macroscopical examination of the brain and viscera, and histological examination of the brain in all animals except those with coenurosis. Histological examinations of specimens from the spinal cord and other tissues, and if considered necessary bacteriological, toxicological and serological examinations were also carried out. In October 1997, scrapie was diagnosed in sheep of a second flock (a mixed flock of sheep and goats), grazing in a pasture close to the place where scrapie was initially detected. All animals of the second flock were also killed and buried. Diagnosis in the first flock was based on clinical signs and histological lesions, and in the second immunoblotting was also used. Distinctive lesions of scrapie were found in the brain and/or the spinal cord of eight sheep with clinical signs from the two flocks. The lesions were revealed in the brain stem and/or in the cervical spinal cord, and tended to be symmetrical. In one sheep, severe lesions in the cortex of cerebral hemispheres and of the cerebellum were also found. In the brain of two sheep from the second flock the pathological isoform of PrP protein was detected. Despite the eradication scheme applied, scrapie in sheep reappeared after 11 years in a place close to where it occurred initially. This may indicate that the effectiveness of the eradication scheme implemented was not adequate and additional approaches may be needed.

Introduction

Scrapie, the prototype of the group of diseases referred to as chronic or subacute transmissible spongiform encephalopathies (TSEs), has been present as a natural disease in Europe since at least the middle of the 18th century and remains endemic in parts of Europe, North America and elsewhere where sheep and goats (to a smaller extent) are raised (Mitchell and Stamp, 1983; Jubb and Huxtable, 1993; Radostits et al., 1994; Smith and Sherman, 1994; Summers et al., 1995; Aiello, 1998).

The first cases of scrapie were detected in Greece in a flock of sheep in Central Macedonia (Northern Greece) in October 1986. A preliminary report followed in the 4th Hellenic Veterinary
Congress (Argyroudis et al., 1987). The diagnosis was based on the clinical signs and the histological lesions of the brain.

**Materials and Methods**

A more systematic investigation of all available cases with signs indicative of neurological disease started in sheep and goats in late 1986, as well as in cattle in 1989. The investigation was based on: (a) a clinical examination of individual animals and of the flock or herd, (b) necropsy or macroscopical examination of the brain and often of the viscera as well (when only the head and/or viscera were available), (c) histological examination of the brain in all animals except those with coenurosis or of specimens from the spinal cord and other tissues as well, and (d) bacteriological, toxicological and serological examinations, if considered necessary.

For histological examination, the brain and in some cases samples from two spinal cord segments at the level of vertebrae C3 and L3, as well as samples from other tissues were fixed in neutral buffered 10% formalin. Subsequently the brain was sliced transversely through the longitudinal axis at 3–4 mm intervals, after the cerebellum was removed by cutting its peduncles. The cerebellum was sliced transversely starting from the fissura prima to the middle peduncles at the same intervals as described above. Eight coronal slices were selected as follows: medulla at the obex and at the caudal cerebellar peduncles including the trapezoidal body, middle of the pons, mesencephalon through the rostral colliculi just posterior to the pineal body, middle transverse section of the cerebellum as above, diencephalon at the mamillary body and at the hypophyseal infundibulum–optic tract levels, and frontal cortex rostral to the corpus callosum. Cross slices from the spinal cord segments and specimens from tissue samples were also prepared. The slices and tissue specimens were processed and embedded in paraffin, and sections of 4–6 μm thick were stained with haematoxylin and eosin.

Bacteriological examinations of the brain for *Listeria monocytogenes* were carried out on primary culture and after cold enrichment.

Serological examinations in sheep and goats for antibodies to maedi-visna and caprine arthritis-encephalitis virus infection respectively, were performed with agar-gel immunodiffusion tests at flock level.

For the biochemical identification of PrP<sup>sc</sup> a standard purification protocol was applied (Sklaviadis et al., 1989). Purified material was also subjected to partial proteolysis by proteinase K.

**Results**

The neurological diseases diagnosed, the number of animals affected by each disease, and the number of flocks or herds from which the affected animals originated from October 1986 to the end of 1997 are shown in Table 1. Diseases affecting only the nervous system or the nervous system along with other systems are included in the table. Diseases causing neurological signs without affecting the nervous system, such as parturient paresis or ketosis, were excluded.

The sheep of the first (1986) affected flock (300 animals) were grazing in a restricted pasture close to an urban area along with the sheep of two smaller flocks (both about 150 animals). Clinical signs indicative of scrapie or of any neurological disease were not observed in the sheep of the two smaller flocks or in the sheep or goats of several other flocks that

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Number of sheep/flocks</th>
<th>Number of goats/flocks</th>
<th>Number of cattle/herds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scrapie</td>
<td>8/2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Listeriosis</td>
<td>13/11</td>
<td>37/25</td>
<td>2/2</td>
</tr>
<tr>
<td>PEM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3/2</td>
<td>7/3</td>
<td>7/5</td>
</tr>
<tr>
<td>Coenurosis</td>
<td>34/14</td>
<td>1/1</td>
<td>–</td>
</tr>
<tr>
<td>Visna</td>
<td>53/13</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MCF&lt;sup&gt;b&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
<td>19/10</td>
</tr>
</tbody>
</table>

<sup>a</sup> PEM, polioencephalomalacia. <sup>b</sup> MCF, malignant catarrhal fever.
pastured in neighbouring areas (about 3 km away). All animals of the affected flock and of the two other sheep flocks grazing in the same pasture (in-contact or exposed flocks) were killed and buried.

Scrapie in the sheep of the second affected flock was diagnosed in October 1997, i.e. 11 years after the first cases of the disease were diagnosed in the country. The flock was a mixed flock of sheep and goats (500 sheep and 50 goats), grazing in an area located close (about 3 km) to the place where the sheep of the flock affected in 1986 pastured. All sheep and goats of this flock were also killed and buried.

The sheep of flocks affected in 1986 and 1997 were offspring of crosses of hellenic milking breeds. The goats of the mixed flock (second) were of the indigenous hellenic breed (Capra prisca) or offspring of crosses with other milking breeds such as Alpine or Maltese. The predominant clinical signs observed in sheep were intense pruritus starting from the base of the tail and extending to the whole body, extensive wool loss, scratches or abrasions of the skin and progressive emaciation. In advanced stages of the disease, ataxia, intermittent head nodding and apathy were also detected. The disease was always fatal several months after clinical signs appeared.

Six and three adult sheep from the first (1986) and second (1997) flock respectively were necropsied. Five sheep of the first flock and all three sheep of the second exhibited clinical signs indicative of scrapie. Seven of these sheep were euthanized, while one ewe (first flock) died of pregnancy toxaemia. The ninth sheep, a 3-year-old ram (first flock) born to a ewe affected with scrapie and kept in confinement, did not reveal any suspicious signs and died of obstructive urolithiasis. Gross lesions were not detected in the euthanized sheep.

Histological examination revealed distinctive lesions of scrapie in the brain and/or spinal cord of eight sheep with clinical signs, while unequivocal lesions of the disease were not found in the 3-year-old ram. The lesions observed were vacuolation of neuronal perikaryon (Fig. 1) and other forms of neuronal degeneration (shrinkage and hyperchromasia or chromatolysis), as well as vacuolation of the gray matter neuropil (spongiform change or status spongiosus) accompanied normally by an increase in number and size of glial nuclei (Fig. 2) that seemed to be predominantly nuclei of astrocytes (astrocytosis). These lesions were bilateral and usually symmetrical, and were detected mainly in the thalamic regions, the mesencephalon, the pons and the medulla, as well as in the cervical spinal cord. The lesions were more consistently found

Fig. 1. Medulla, reticular formation. Vacuolation (multilocular) of neuronal perikaryon. H&E × 185.
in the medullary reticular formation and raphe nuclei, the dorsal nucleus of the vagus, the cuneate and vestibular nuclei, the substantia nigra, the dorsomedial and ventral thalamic nuclei and the spinal trigeminal nucleus, as well as in the intermediate substance and the ventral horn. Severe lesions in the cortex of the cerebral hemispheres and cerebellum were also found in one sheep from the second flock (1997). These lesions had a laminar distribution in the isocortex affecting mainly the internal pyramidal layer (layer V) of the superior and ventrolateral frontal gyri, and the parietal cortex (to a lesser extent), also involving the pyramidal layer of the allocortex of hippocampus (to an even smaller degree). The lesions consisted of shrinkage and cytoplasmic vacuolation mainly of large pyramidal cell bodies in the isocortex and of pyramidal cell bodies in the allocortex, with vacuolation of the surrounding neuropil (Fig. 3). The lesions in the cerebellum were focal and most severe in the lingula. Severe neuropil vacuolation in the granular and molecular layers with degeneration and loss of Purkinje as well as loss of granule cells was detected. Proliferation of Bergmann's cell nuclei in the molecular layer was evident (Fig. 4).

Brain stems from scrapie-affected sheep from the second flock (1997) were used in order to detect the pathological isoform of PrP protein and the resistance to proteinase K digestion (Fig. 5). Immunostaining of two cases revealed full length (lanes 1 and 2) and proteinase K digested (lanes+) PrP\textsuperscript{\textsc{sc}}.

**Discussion**

The histological lesions consistent with scrapie (Zlotnik, 1958; Pattison, 1988; Toumazos, 1988, 1991; Jubb and Huxtable, 1993; CEC, 1994; Storts, 1995; Summers et al., 1995; Wood et al., 1997) and the identification of the pathological isoform of PrP protein confirmed that the sheep in the two flocks were infected with scrapie. The re-appearance of the disease in sheep after 11 years, in a flock pasturing in a close or neighbouring area where scrapie in sheep appeared initially, may indicate that the effectiveness of the eradication scheme applied was inadequate.

The ambitious aim to eliminate scrapie or even to minimize the prevalence of the disease in the country may need the implementation of additional approaches. These approaches should
be based on the following principles: (a) continuous and intensive monitoring of the incidence of scrapie in the small ruminant population of the country by means of histopathological and biochemical (when suitable) examination of specimens from all clinically suspect cases. (b) In
flocks where the incidence of infection is considered high (>5% per year) the use of an eradication programme consisting of destruction of all animals in the flock as well as in contact or exposed flocks should be implemented. (c) In flocks where the disease is sporadic clinically suspect cases should be destroyed and examined. If found affected, their family lines should be immediately eliminated. (d) Rigorous regulation of movement of animals among affected and apparently disease-free flocks and areas should be applied. (e) In the affected flocks and areas alteration of herd-resistance by selection of genetically resistant parent stock genotypes should begin as soon as possible. These principles are expected to improve not only the cost/benefit ratio of the control programme but also the flock owners’ compliance which has sometimes been problematic when eradication methods were implemented. Analogous programmes for the control of scrapie have been implemented or scheduled in other countries (Mitchell and Stamp, 1983; Radostits et al., 1994; Smith and Sherman, 1994; Aiello, 1998; G. Neophytou, Ministry of Agriculture, Veterinary Services, Nicosia, Cyprus, personal communication).

Furthermore, study of the pathology of natural ovine or possibly caprine scrapie will provide more information about the topographical distribution and pattern of lesions in hellenic milking breeds, which is very limited at present.

Acknowledgments

We wish to thank Drs E. C. Appleby and R. M. Barlow of the Royal Veterinary College, London University, for kindly examining the brain sections from our first cases of scrapie in sheep and confirming our diagnosis.

References


