NATURAL SCRAPIE AND PrP GENOTYPE IN A FLOCK OF MASSESE SHEEP IN ITALY

I. Barbieri1, R. Mattioli2, D. Gelmetti1, L. Gibelli1, M. Tamba1, G. Vecchi1, G. Lombardi1, L. Capucci1
1Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna - Brescia
2Azienda Sanitaria Locale - Bologna Nord - Italy

Introduction
Scrapie is a fatal neurodegenerative disease in sheep and goats that occurs naturally and belongs to the group of disorders known as transmissible spongiform encephalopathies (TSE) or Prion diseases. Prion diseases manifest as infectious, sporadic and/or inherited disorders. They are characterised by the accumulation of protease-resistant isoform of the host encoded prion protein (PrP) in the brain of affected animals and humans.

In Italy, scrapie has sporadically been observed since 1977. Recently the disease has gained more attention following a sudden increase of reported cases starting from 1996, especially in goats, probably as a consequence to the use of contaminated vaccine against contagious agalactia, which raise the hypothesis of a iatrogenic origin of the infection (1).

Several polymorphisms in the gene of PrP are associated with the incidence, susceptibility and pathology of the disease. In particular, amino acid codons 136, 154 and to a minor extent 171, seem to be mostly involved in determining scrapie susceptibility of sheep. This finding has been use to write a “Guidance on the use of PrP genotyping as an aid to the control of clinical scrapie” (4).

In spite of the well es tablished correlation between occurrence of scrapie in sheep and their genotype, only few genetic studies have been carried out on the several breeds of sheep reared in Italy.

Here, we report on a case of scrapie in a flock of Massese sheep which was found to be affected by scrapie during an epidemiological survey on neurological disease of sheep carried out on 23 flocks in Emilia Romagna region during 1996 (5). The flock of Massese sheep, a breed quite common in the regions of central Italy, was founded using sheep coming from two regions: a group belonged to a flock in Emilia Romagna and the second was moved from a flock from Tuscany. No data are available from the original flocks. Interestingly, overt cases of scrapie were only observed in the first group while the latter was claimed by the farmer as scrapie-free since no cases has never been observed. In spite of the limited information available, the data show that sheep homozygous glutamine at codon 171 (QQ171) are highly susceptible to scrapie.

Material and methods
Sheep
The study was carried out on three groups of animals. Six sheep belonging to the Emilia Romagna group (A) all affected by clinical scrapie. Nine sheep, the last remaining of group A, moved in an isolated area and observed for three years. In September 1999 blood was collected from 50 sheep of different age belonging to the Toscana group (B).

Genotyping
High molecular weight DNA was extracted both from blood and brain samples and amplification reaction were performed in a 50 µl reaction mixture containing 50-100 ng of purified DNA and the primers p8 (+) 5’-ATGGTGAAGGCCACATAGGC-3’ and p9 (-) 5’-CTCTATTTCCTCATAGTAGATAG-3’ which refer respectively to bp 72-92 and bp 818-842 of the PrP sheep sequence. Detection of polymorphisms at codons 136, 154 and 171 was performed with the use of sequencing primers p1 (+) 5’-GCAACCGCTATCCACCTCAG-3’ and p2 (-) 5’-CACAGTCAAGCAACACACAG-3’ corresponding respectively to bp 217-236 and bp 626-645 of the PrP sheep sequence. DNA Sequencing was carried out on both strands of the PCR products using DNA an ABI Prism 310 Genetic Analyzer.

Results
Between March and October 1996, 6 sheep from the group from Emilia Romagna died or had to be killed after showing clinical signs of scrapie. The average age of these sheep was 2,5 ± 0,5 years. The clinical period of the disease ranged between 0,5 to 3 months. Immunohistochemistry and western blot analysis performed on brain homogenates showed that all the animals were positive to PrP-res (data not shown).

All 6 sheep had a genotype ARQ/ARQ (Figure 1). The last 9 sheep of the scrapie affected group were isolated when 2,5 - 3,5 years old. Three of these sheep died when older that 4 years without showing overt symptoms of scrapie. However, one sheep resulted clinically positive to PrP-res both by immunohistochemistry and western blot, while the second one was positive only by western blot but...
showing PrP-res levels significantly lower than the former. Both this sheep belonged to the group of 3 animals with ARQ/VRQ genotype. The third sheep, with an ARR/ARR genotype, resulted PrP-res negative. Among other sheep that are still alive, one has an ARQ/ARQ genotype and 4 have an ARQ/ARR (figure 1).

As regards sheep apparently scrapie free belonging to the Toscana group, genotyping analysis are reported in figure 2 where results have been grouped in relation to their age.

Figure 2: genotypes of the Toscana sheep according to their age

<table>
<thead>
<tr>
<th>N° of sheep</th>
<th>&gt; 4 years</th>
<th>4-2 years</th>
<th>&lt; 2 years</th>
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Genotype ARQ/ARQ was absent in the animals older than 4 years but it was 25% in the group 4-2 years old and 28% in the group < 2 years old. Genotype ARR/ARR was 30% in the oldest group, 8% in the medium group and 16% in the youngest group. Heterozygous genotype ARR/ARQ was 75% in the oldest group, 67% in the medium group and 48% in the youngest group. The allele VRQ was never found in this sheep but two animals were ARQ/ARQ.

Discussion
Massese breed is typical of the regions of central Italy with some flocks also present in Emilia Romagna region where often they have been crossed with local breeds. The flock object of this study was established with sheep coming from two regions and our interest in it was due to the observation of scrapie in the sheep from Emilia Romagna but not in those from Toscana (5). In spite of the limited information available, the results obtained allows to draw some conclusions.

From the general point of view, PrP genotyping showed the presence in Massese sheep of at least 4 PrP alleles, ARQ, ARR, VRQ and AHR due to polymorphisms at codon 136 (A or V), codon 154 (R or H) and codon 171 (R or Q) of PrP gene. Actually, alleles VRQ and AHR were found in only 5% and 3% of sheep respectively, while ARQ and ARR were the main alleles present in 77% and 66% of sheep respectively. Interestingly, the less frequent alleles were group specific, with the VRQ found in 20% (3 out of 15) of the Emilia Romagna sheep and the AHR in 4% (2 out of 50) of the Toscana sheep. Therefore, the genetic data confirm the history of the flock setting up, suggesting that Emilia Romagna Massese sheep have been crossed with a valine breed.

As regards to the association between genotype and scrapie susceptibility, the data clearly showed that sheep with genotype ARQ/ARQ were the most susceptible followed by those with VRQ/ARQ. Therefore, Massese breed seems to belong to those breeds, for example Lacune sheep in France (3) and Suffolk sheep in Great Britain and Japan (7, 6) where scrapie incidence is strongly associated with codon 171, where QQ171 animals are at particularly high risk. It is worth to note, even if the limited number of animals does not allow to draw firm conclusions, that V136 (VRQ/ARQ) seems to decrease the susceptibility to scrapie. In fact, we found 2 out of 3 of the VRQ/ARQ sheep PrPsc positive even if they never showed overt signs of scrapie. This observation reminds the finding in Suffolk sheep in Japan where four apparently healthy sheep resulted then PrPsc positive and all were VRQ/ARQ (6).

A final consideration can be done on the frequency of the genotypes found in the massese sheep belonging to the Toscana line. When the genotypes were grouped in relation to the age the ARQ/ARQ resulted not present in sheep older than 4 years while its frequency was 27% in younger sheep. Considering that ARQ/ARQ genotype resulted at particularly high risk in the massese sheep belonging to the Emilia Romagna line, this observation suggests that also the line of Toscana animals could be affected by scrapie, either clinically less obvious or not accurately reported. A similar picture has been recently described by Baylis et al. and defined as the "scrapie signature" (2). In a comparative study based on the PrP genotyping of flocks free and affected by scrapie, they found in the former ones a consistent low frequency of the susceptible genotype in animals older than 4 in respect to the younger ones. Of course, this finding is directly related to the fact that the average age of death due to scrapie in an affected flock is 2-3 years. This observation indicate that PrP genotyping could be thought also to look for the "scrapie signature", that is to mark a flock as suspected of scrapie even when no or short clinical observation are available.

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References