The distribution of protease-resistant prion protein (PrPSc) and its correlation with histological findings in seven major sites of the brain in three sheep with natural scrapie are presented. Although sheep scrapie has been known for over 200 years (Hunter, 1979), it was first histopathologically confirmed in Cyprus in 1985 (Toumazos, 1988). We report here for the first time, the identification of PrPSc in scrapie-affected animals from this region using Western blot analysis.

Three sheep (designated A, B and C) were selected, having been positively identified for scrapie using classical histological methods (Toumazos, 1991). All sheep were 3-year-old females belonging to a mixed breed (Chios, Friesian and local Cypriot races). Sheep A and C showed severe clinical signs, while those in sheep B were moderate. Coronal tissue blocks were removed from the cortex, the medulla, the mesencephalon, the paraterminal body, the thalamus, the hippocampus and the cerebellum of each animal and divided into two halves. One half of each block was fixed in 10% formal saline for histopathological analysis. Serial sections of 6–7 μm were stained with haematoxylin and eosin (Luna et al., 1968) for light microscopy, and the lesions were graded mild (+), moderate (++) and severe (+++), as previously described (Toumazos, 1991). The other half of each block was frozen immediately at –70°C for immunoblotting studies. For this purpose, PrPSc-enriched material was prepared using the purification protocol of Sklaviadis et al. (1989), followed by treatment with proteinase K (5 μg/mL per 0.5 g equivalent brain, 37°C, 1 h). Digestion with proteinase K under the above conditions leads to the reduction of the PrP protein (33–35 kDa molecular weight) to the proteinase resistant form, PrPSc (27–30 kDa), which is indicative of TSE infection. The samples were run on a 13% SDS-PAGE gel and immunoblotted (Towbin et al., 1979) with a rabbit anti-PrP polyclonal antibody, 78 295, which was a generous gift from Dr R. Kascsak (Institute for Basic Research in Developmental Disabilities, Staten Island, NY, USA). This was followed by incubation with alkaline phosphatase-labelled goat anti-rabbit antibody and development Nitro Blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP). The Western blot obtained from each sample was then graded with respect to the intensity of staining as mild (+), moderate (++) and severe (+++) using scanning densitometry. The allelic fingerprint at codons 136, 154 and 171 for the PrP gene from all three sheep were determined using a variation of the method of Laplanche et al. (1993).
No macroscopic lesions were observed in the three brain samples at necropsy. A compilation of histopathological description in terms of spongiosis and vacuolation (Fig. 1), along with Western blot findings (Fig. 2) from all seven areas of the brain under study is provided in Table I. Although all three animals presented lesions, both spongiosis and vacuolation were of mild degree in all areas tested from brain A. In brain B, the lesions were moderate of degree, whereas brain C exhibited severe spongiosis and vacuolation. These differences did not show any association with the genotype of the sheep. On the contrary, PrP<sup>Sc</sup> deposition (Fig. 2 and Table I) was seen to be consistently moderate to severe in all cases. This was particularly impressive in the case of brain A, where the histological changes were only mild. PrP<sup>Sc</sup> levels in the cerebellum and medulla from all three cases were consistently high, whereas mild-to-moderate PrP<sup>Sc</sup> expression was seen in the hippocampus. Tests for allelic variations of the PrP gene showed that sheep A and sheep B were homozygous with the ARQ/ARQ genotype which is associated with high disease incidence, while sheep C was heterozygous with the ARQ/AHQ genotype, which is less frequent in scrapie-affected sheep.

In conclusion, all three Cyprus sheep that were histologically positive for scrapie were also confirmed positive by Western blotting. PrP<sup>Sc</sup> deposition did not correlate with severity of spongiosis, vacuolation or clinical signs, but was elevated in the medulla, the cerebellum and the mesencephalon, with consistently high amounts in the medulla. The finding of high amounts of PrP<sup>Sc</sup> in brain A in spite of mild histopathological changes, is consistent with the view that immunochromatographic tests such as the Western blot analysis may provide a reliable tool along with others that are available, to identify pre-clinical cases of scrapie. Similar reports have indicated the usefulness of Western blotting in the diagnosis of pre-clinical scrapie-infected mice and natural scrapie in France and in the UK (Doi et al., 1988; Madec et al., 1997; Cooley et al., 1998). However, additional studies are required before verifying such a claim. A major epidemiological study will provide the material necessary for an extended genotypic, histological and biochemical profile of scrapie cases in Cyprus.

### Table I

<table>
<thead>
<tr>
<th></th>
<th>Brain A</th>
<th>Brain B</th>
<th>Brain C</th>
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<tr>
<td></td>
<td>S  V  P</td>
<td>S  V  P</td>
<td>S  V  P</td>
</tr>
<tr>
<td>Cortex</td>
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<td>+++  +++  +</td>
<td>+  +  +</td>
</tr>
<tr>
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<tr>
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<tr>
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<tr>
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</tbody>
</table>

S, Spongiosis; V, Vacuolation; P, PrP<sup>Sc</sup>; ++++, Severe; ++, Moderate; +, Mild; –, No lesions.
ACKNOWLEDGEMENTS

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REFERENCES


Fig 2. PrPSc distribution in Western blots. 0.16 g tissue equivalent per lane. PK-treated samples indicated with a cross (+); M indicates molecular weight markers; 1, cortex; 2, hippocampus; 3, mesencephalon; 4, paraterminal body; 5, cerebellum; 6, medulla; 7, thalamus.


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