

Supplementary Methods

Scrapie infection challenge

TSE-free New Zealand Polled-Dorset sheep (kindly provided by Hugh Simons VLA- Weybridge-GB) were used for intracerebral inoculation. Infectious material was derived from a VRQ/VRQ genotype sheep naturally infected by scrapie (Langlade isolate). Natural infection challenge was performed using Romanov sheep born and bred in the Langlade flock, where a natural scrapie epidemic has been occurring at a high incidence since 1993¹². Oral infection challenge was performed on new-born lambs. Twelve-hour-old VRQ/VRQ (n=14) and ARR/ARR (n=20) lambs were exposed to a dose of 5 g of infected brain through natural suckling. Infectious material was pooled brain tissue from ARQ/VRQ sheep affected by the Langlade isolate.

Tissue processing and IHC detection

All procedures were performed as previously described⁴. Briefly, samples were fixed in neutral-buffered 10% formalin (4% formaldehyde) for 4 to 10 days before paraffin embedding. After deparaffinisation, tissue sections, 2 µm thick, were incubated for 30 min at room temperature in 98% formic acid (Merck, Gradignan, France) before autoclaving (121°C) in 10 mM citrate buffer (pH 6.1) for 5 min. PrP^{Sc} IHC detection was first performed using 8G8 antibody raised against the human recombinant PrP protein and specifically recognising the 95-108 amino acid sequence (SQWNKP) of the PrP protein. Positive muscle spindles were also immunolabelled using 2G11, SAF84 and BAR 233 antibodies, which are respectively raised against synthetic peptide (ovine PrP sequence 146-182)⁴, acid formic-treated hamster SAF and recombinant ovine protein (sequence 141-152).

For each sample a negative serum control was performed in which the primary antibody was either omitted or replaced by purified mouse Ig2a serum. In addition, anti-PrP monoclonal antibodies were replaced by isotype-matched monoclonal antibodies irrelevant to the tissue under investigation. PrP^{Sc}/S100 double labelling was performed as previously described⁴, using 2G11 and a rabbit anti-bovine S100 serum (1/100 diluted -DAKO, Z 0311). For double labelling, cross-reactivity controls were performed, in order to verify the absence of inter-species reactivity of secondary antibodies toward primary antibodies. The absence of affinity between the two secondary antibodies was also checked.

Paraffin-Embedded Tissue Blot (PET blot)

PET blot was realized using sections from positive muscles and control muscles (ARR/ARR animal) following a published protocol¹³. Briefly, sections were collected on 0.45 µm nitrocellulose membrane before drying and deparaffinisation. PK (Roche ref 1373200) digestion (250 µg/ml, 2 h, 55°C) was performed before denaturation in guanidium isothiocyanate solution (3 M, 10 min, at room temperature). Immunodetection was carried out using SHa-31 monoclonal antibody (4 µg/ml), which binds the 145-152 sequence of PrP(YEDRYRE), followed by application of an alkaline phosphatase-coupled secondary antibody (Dako ref D0314 – 1/500 diluted). Enzymatic activity was revealed using NBT/BCIP substrate chromogen.

A negative control, in which the primary antibody was omitted, was included for each sample. Serial sections from each tissue sample, 4 µm thick for PET blot and 2 µm thick for IHC, were respectively collected onto membrane or glass slides before performing PrP^{Sc} immunodetection by both methods. Results obtained with both methods were then compared. This experimental design allowed identification of the nature of PET blot PrP^{Sc}-positive structures (using shape and location of labelling on tissue section).

ELISA method

ELISA measurements were performed using a modified version of the Bio-Rad test (Platelia®BSE, TeSeE®) currently used for the *post mortem* diagnosis of BSE in cattle and originally developed at the CEA¹⁴. This new kit uses two different monoclonal antibodies allowing much more sensitive detection of denatured sheep PrP than the current TeSeE® detection kit optimised for the detection

Prior to detection, PrP^{Sc} was purified and concentrated from tissue homogenate using the TeSeE® purification kit following the manufacturer's instructions. This procedure involves a PK treatment (0.4 µg/mg of tissue) and a centrifugation step to concentrate PrP^{Sc}. The corresponding pellet is denatured at 100°C in the presence of a mixture of a chaotropic agent and a detergent. After five-fold dilution in an appropriate buffer, denatured PrP^{Sc} is successively reacted with capture antibody and tracer antibody (detection kit).

Western blot preceded by immunopurification

Denatured PrP^{Sc} was concentrated by reaction with a monoclonal antibody (SHa-31) immobilised on a Sepharose 4B gel and released from the gel by a denaturing treatment. Briefly, 4 X 500 µl of 10% muscle homogenate from VRQ/VRQ and orally exposed ARR/ARR lambs (180 dpe) were submitted to PrP^{Sc} purification as described above (TeSeE® purification kit) including a treatment with an increased concentration of PK (2 µg/mg of tissue). For both samples, the four pellets dissolved in Laemmli buffer were diluted 10-fold in 10 mM Tris pH 7.4, 10 mM EDTA, 1% DOC and 0.5% NP40 and reacted for 2.5 hours at room temperature with 50 µl of SHa-31 coupled to Sepharose 4B. PrP was removed from the gel with 50 µl of Laemmli buffer (10 min at 100°C). Samples were loaded undiluted (equivalent to 80 mg of tissue) and diluted 1/5 in Laemmli buffer (equivalent to 16 mg of tissue) on a 12% acrylamide gel, before electrophoresis and blotting. Loads equivalent to 0.02mg, 0.04 and 0.2 mg of a positive sheep brain were used as controls. Immunodetection was performed using SHa-31 conjugated to horseradish peroxidase (at 0.06 µg per ml). Peroxidase activity was revealed using ECL substrate (Pierce).

Langlade isolate titration and estimation of the relative amount of PrP^{Sc} in muscle

Obex from a VRQ/VRQ Langlade sheep clinically infected with scrapie was homogenised before IC inoculation of successive 1/10 dilutions in ovine VRQ PrP transgenic mice (tg338), considered as highly efficient for detection of sheep scrapie infectivity⁶. Infectious titre (10^{6.6} ID50 per g of tissue) was determined following Kärber's method.

The relative amount of PrP^{Sc} per mg of fresh tissue was measured using a quantitative ELISA in (i) successive dilutions from the obex that was titrated in tg338 mice, (ii) all positive muscles, and (iii) mesenteric lymph node and obex from animals with positive muscle samples (Fig 1c). Tests were calibrated with reference to a standard curve plotted using sheep recombinant PrP. Recombinant ovine VRQ-PrP was kindly provided by D. Marc (INRA PII, Nouzilly, France). It was used to calibrate absorbance measurements performed in each experiment and thus to compare inter-assay results and to quantify the relative amounts of ovine PrP^{Sc} in samples.

References

12. Elsen, J.M. *et al. Arch. Virol.* **144**, 431–445 (1999).
13. Schulz-Schaeffer, W.J. *et al. Am. J. Pathol.* **156**, 51–56 (2000).
14. Moynagh, J. & Schimmel, H. *Nature* **400**, 105 (1999).
15. Grassi, J. *et al. Arch. Virol. Suppl.* **16**, 197–205 (2000).