Atypical prion protein in sheep brain collected during the British scrapie-surveillance programme

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Scrapie of sheep and goats is the most common prion disease (or transmissible spongiform encephalopathy, TSE) of mammals and aggregates of abnormal, proteinase-resistant prion protein (PrPSc) are found in all naturally occurring prion diseases. During active surveillance of British sheep for TSEs, 29 201 sheep brain stem samples were collected from abattoirs and analysed for the presence of PrPSc. Of these samples, 54 were found to be positive by using an ELISA screening test, but 28 of these could not be confirmed initially by immunohistochemistry. These unconfirmed or atypical cases were generally found in PrP genotypes normally associated with relative resistance to clinical scrapie and further biochemical analysis revealed that they contained forms of PrPSc with a relatively protease-sensitive amyloid core, some resembling those of Nor98 scrapie. The presence of these atypical forms of protease-resistant PrP raises concerns that some TSE disorders of PrP metabolism previously may have escaped identification in the British sheep population.

INTRODUCTION

Prions are cellular proteins that can transfer metabolic and pathological phenotypes laterally between cells and organisms or vertically from parent to progeny (Prusiner, 1982; Wickner, 1994; Legname et al., 2004; Wickner et al., 2004). Frequently, the conversion of a normal cellular protein into a prion form involves aggregation, which changes its physico-chemical properties from those of a soluble protein into those of an amyloid, a sparingly-soluble, proteinase-resistant isoform rich in cross-β-pleated sheet structures (Dobson, 2005). Scrapie of sheep and goats is the most common prion disease of mammals and typically causes a progressive degeneration of the central nervous system (Fraser, 1976). The ovine cellular prion protein (oPrPc) is a glycosylphosphatidyl inositol-linked membrane glycoprotein with a molecular mass of 33–35 kDa and is normally widely expressed in the central nervous system, lymphoid and other tissues. During the development of scrapie, this protein accumulates in these tissues as a relatively proteinase K (PK)-resistant prion form (PrPSc) (Gilmour et al., 1986; van Keulen et al., 1995; Jeffrey et al., 1998; Hardt et al., 2000). Extraction of these tissues with mild non-denaturing detergents, limited proteolysis and differential ultracentrifugation allows the purification of fibril or rod-like aggregates of the PK-resistant, amyloid core structures of PrPSc, known as PrPres. PrPres has a molecular mass of 27–30 kDa and typically lacks the N-terminal 67–70 aa of oPrPc (Hope et al., 1986; Goldmann et al., 1990). Aggregates of PrP isofoms are common to all naturally occurring and most experimental prion diseases and, because they co-purify with high titres of infectivity, they are thought to represent at least one identity of an infectious agent formed from conformational isomers of PrP.

Conceptually, however, there may be other forms of prions in domestic animals (mammals) that do not have the amyloid properties of PrPres (or even its deleterious effects) and so may escape detection by screening methods (protease hydrolysis and analysis by Western blotting or ELISA) based on this single physical property of the abnormal protein. Even at the time of the first isolation of PrPres (Bolton et al., 1982; McKinley et al., 1983), doubts were raised over the quantitative correlation of infectivity and this PrP amyloid (Czub et al., 1986, 1988), and these reservations were reinforced by the finding that most abnormal PrP in infected brain was not PrP of 27–30 kDa but an isoform covalently identical to normal PrPc (Hope et al., 1986; Bolton et al., 1991). Subsequently, the introduction of analytical methods omitting PK has allowed quantification of this proteinase-sensitive component of PrPSc (Safar et al., 1998, 2005),

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Supplementary material is available in JGV Online.
and the use of several artificial transgenic models of transmissible, prion protein-related disorders has shown again the dissociation of infectivity titre and the classical, PK-resistant amyloid core of PrPSc (Barron et al., 2003). Recently, the in vitro generation of PrPSc failed to match the specific infectivity of an equivalent amount of naturally occurring PrPSc (Castilla et al., 2005). High-resolution amino acid sequencing of abnormal PrP isolated from human (Tagliavini et al., 1994) and mouse (Hope et al., 1988) brain has identified peptide fragments of PrPSc diagnostic for cleavage within the amyloid core of PrPSc, between residues ~140 and 170, and molecular analysis of scrapie strain Nor98 sheep brain has provided evidence recently for a similar co-existence of PrP core amyloid and less-stable PrPSc conformers in a naturally occurring sheep disease (Benestad et al., 2003; De Bosschere et al., 2004; Gavier-Widen et al., 2004; Onnasch et al., 2004).

This aspect of prion biology has taken on greater practical significance with the advent of rapid testing that relies on PrPSc detection for transmissible spongiform encephalopathy (TSE) surveillance in sheep. Prior to 2005, these tests had only been evaluated extensively for their performance in detecting PrPSc as a confirmatory marker for clinical disease in cattle and in one, in particular, the Bio-Rad Platelia test system, appeared to detect a PrP abnormality in apparently healthy sheep in the absence of the characteristic PK resistance of PrPSc. During 2002–2003, a programme of statutory EU surveillance for scrapie in sheep in Great Britain was conducted in the sheep population submitted for abattoir slaughter (Elliott et al., 2005) in which brain stems from cull sheep were screened for disease-associated PrPSc using the Bio-Rad Platelia test system (Moynagh & Schimmel, 1999; Grassi et al., 2001). From a total of 29 201 samples tested, 54 were reported to be positive in the screening assay. Of those available for testing, 24 were confirmed as scrapie by immunohistochemical (IHC) detection of disease-specific PrPSc deposition in fixed sections of the obex. A further 28 samples that were positive by screening, however, were negative by IHC examination at this limited anatomical site and thus could not be confirmed as scrapie cases. Here, we have described characterization of the prion protein associated with these cases.

METHODS

Tissue samples and control standards. Brain stems (caudal medulla) collected from sheep slaughtered at the abattoir for human consumption were screened by Bio-Rad Platelia ELISA for abnormal prion protein (PrPSc) as part of the EU statutory surveillance programme for scrapie in Great Britain. Evidence of TSE or, more accurately, a prion protein disorder in Bio-Rad Platelia ELISA-positive samples, was confirmed in some but not all cases by IHC detection of PrP in brain stem at the level of the obex. Our biochemical study used both IHC-confirmed and unclassified Bio-Rad Platelia ELISA-positive samples, as well as Bio-Rad Platelia ELISA-negative samples from this survey. The unclassified Bio-Rad Platelia ELISA-positive samples were also found to be negative by the Office International des Epizooties (OIE)-recognized scrapie-associated fibrils (SAF) Western blot method, which has been developed from a high-detergent PK protocol for purification of the protease-resistant core of PrPSc, PrPSc (Hope et al., 1986, 1988).

Negative and positive control reference materials (CRMs) were prepared, respectively, from pooled homogenates of brains of Bio-Rad Platelia ELISA-negative sheep (CRM-A) and pooled homogenates of brains of PrP IHC-confirmed, Bio-Rad Platelia ELISA-positive sheep (CRM-B). An unclassified CRM (CRM-C) was prepared from four IHC-negative, Bio-Rad Platelia ELISA-positive sheep. The genotypes of the sheep used in CRM-A were not determined, but CRM-B contained a limited number of genotypes known to be susceptible to clinical scrapie: ARQ/AHQ, ARQ/ARQ, ARQ/VRQ and VRQ/VRQ. Of the four CRM-C Bio-Rad Platelia ELISA-positive brains, two were of the AHQ/VRQ genotype, one was ARR/VRQ and the PrP genotype of the fourth was unknown. These positive, negative and unclassified CRMs were included daily as controls in each ELISA and Western blot procedure.

Genotyping of PrP codons 136, 154 and 171. PrP genotyping of the Bio-Rad Platelia ELISA-positive cases at the three codons associated with scrapie susceptibility and resistance (codons 136, 154 and 171) was undertaken as part of the British surveillance programme through partial sequencing of the PrP gene. DNA was extracted from 25 mg brain medulla tissue using the DNeasy 96 Tissue kit (Qiagen) and dissolved in a final volume of 150 μL. A hot-start PCR amplification was undertaken using 2.5 μL of this genomic DNA, 2× PCR master mix (Promega) and 30 pmol each of the primers 5’-ATGAGACACCACCACTACAGGGCT-3’ and 5’-CATTGGATGCTGACACCACCACTACAGGGCT-3’. After 40 cycles, the PCR product of 900 bp was treated with shrimp alkaline phosphatase and exonuclease I according to the manufacturer’s instructions (New England Biolabs). Cycle sequencing was undertaken with the reverse primer 5’-TCGCTCATTATCTGATGTCAGTTT-3’ using the BigDye terminator kit following the manufacturer’s instructions (PE Applied Biosystems). The cycle sequencing product was precipitated with ethanol and resuspended in 40 μL sample loading solution (PE Applied Biosystems) and 4 μL was loaded onto a 36 cm ABI Prism 377 DNA Sequencer gel. The full PrP ORF of sample DNAs from each case was independently sequenced and these genotypes were verified by a commercial contractor (Qiagen).

Bio-Rad Platelia ELISA. The Bio-Rad Platelia Purification and Detection kits provided the reagents for the extraction and PK hydrolysis of PrPSc in our study and the subsequent detection of residual PrPSc by a sandwich ELISA (Bio-Rad). The procedures set out in the manufacturer’s kit insert were followed. Briefly, caudal medulla (0-35 g) was homogenized in a mild detergent buffer, proteins including PrPSc were digested at 37 °C for 10 min using low concentrations of PK, and residual proteins including PrPSc were precipitated by using alcohol. Following low-speed centrifugation, the sample pellet was denatured and solubilized, diluted and used for the assay using a microtitre plate-based colorimetric immunoassay with two monoclonal antibodies (mAbs) (one for capture and one for detection). This ELISA system, originally approved for the confirmation of disease in clinical cases of cattle bovine spongiform encephalopathy (BSE) (Grassi et al., 2001; Moynagh & Schimmel, 1999), has recently been evaluated for its ability to detect scrapie, Nor98 and experimental BSE in sheep (EFSA, 2005).

The detergents, mAbs, PK activity and buffers are subject to commercial confidentiality, but a key feature of this kit is the element of controlled PK digestion. Elimination of normal PrPSc uses 4 μL of the kit PK reagent (ml homogenate) and this level is designated 1 x PK. The final concentration or protease activity of this reagent is undisclosed, but is probably at least 10 times less than concentrations of PK usually used to purify and characterize PrPSc (Hope et al., 1986). The ELISA
sample preparation was also performed without PK treatment (designated 0 × PK) or using 20 μl of the kit reagent (ml homogenate) −1 (5 × PK).

**Western blotting.** Brain stems (caudal medulla) were extracted and the extract was treated with PK and alcohol precipitated as described above for the preparation of ELISA samples (see above). For Western blotting, the sample pellet was boiled in Laemmli SDS-PAGE sample buffer and loaded (0-05 g equivalents per track) onto a gel cassette (Bio-Rad). Electrophoresis was carried out at room temperature at 200 V and the dye front was allowed to migrate for no more than 50 min to prevent loss of polypeptides of < 6 kDa running off the end of the gel. After electrophoresis, the gel proteins were blotted onto an activated PVDF membrane at 100 V for 1 h and, after rinsing and blocking, the membrane was incubated with either a mixture of mAbs SAF60 and BAR226 (specific for the core prion protein; referred to as the core blot) or the single mAb SAF34 (specific for the N-terminal octarepeat area; the N-blot) and PrP-related bands were visualized by using a horseradish peroxidase-coupled anti-mouse immunoglobulin secondary antibody conjugate (Bio-Rad) and the Amersham ECL developer kit. Each set of samples was flanked by MagicMark Western Protein Standards (eight proteins tagged with an IgG-binding sequence and covering the range 20–120 kDa; Invitrogen) to calibrate the blots for molecular mass.

mAb SAF60 binds to a linear peptide epitope sequence, YPNQVY, encoded by codons 160–165 of the oPrP gene, while BAR226 appears to be specific for an unidentified conformational epitope that is retained on Western blotting of the PK-resistant core of oPrPSc, encoded by codons ~90–233 of the ovine PrP gene [J. Grassi, personal communication, Commissariat a l’Énergie Atomique (CEA) Pharmacology and Immunology Unit, CEA/Saclay, Gif sur Yvette, France]. A mixture of mAbs SAF60 and BAR226 was used in the core blot. The mAb SAF34 binds to the octapeptide repeat sequence of the ovine PrP gene, typically represented by the sequence PH/QGGGG/G/WQQ which occurs five times in a tandem repeat encoded by codons 54–95 of the ovine PrP gene (Swiss-Prot accession number P23907; Goldmann et al., 1990). This single mAb was used in the N-blot.

**RESULTS**

**Source of tissues**

Brain stem samples for this biochemical study were collected by active surveillance of sheep culled at abattoirs throughout Great Britain during the period 2002–2003. Full details of this brain stem abattoir survey can be found in the Defra scrapie survey (available at http://www.defra.gov.uk/animalh/bse/othertses/scrapie/scrapiesurvey. pdf). Up to the end of August 2004, 80 067 animals had been examined under this EU initiative using the Bio-Rad Platelia ELISA and 126 were scored as positive after the initial screening test and retesting. However, only 56 of these cases could be confirmed using standard IHC detection of PrP in brain stem at the level of the obex, and the remaining 70 unclassified cases that were negative or inconclusive were investigated using the OIE SAF Western blot confirmatory test (OIE Handbook, 2004, in preparation). Prior to further biochemical investigations of these survey brain samples, the original Bio-Rad Platelia ELISA screening assay was repeated on residual caudal medulla samples of groups of confirmed (n = 22) and unconfirmed (n = 23) positive survey samples, a group of PrPSc-negative, genotype-matched control samples from a flock of New Zealand-derived scrapie-free sheep (n = 18), and CRMs prepared independently of the survey materials. In addition, we also processed these samples using the higher PK levels more conventionally used in the analysis and purification of PrPres (Hope et al., 1986). These data are shown in Fig. 2, and presented with additional PrP genotype information in Supplementary Fig. S1(a–d) (available in JGV Online).

**ELISA**

Prior to further biochemical investigations of these survey brain samples, the original Bio-Rad Platelia ELISA screening test (OIE Handbook, 2004). The IHC-confirmed, Bio-Rad Platelia ELISA-positive survey samples were all positive (n = 22) on retesting and all but two (both of low initial A450/620 signal) were strongly positive when processed using high levels of PK. Similarly, the IHC-unconfirmed, Bio-Rad Platelia ELISA-positive survey samples were all also positive on retesting, but had consistently lower A450/620 values and, in contrast to the ‘confirmed’ group, the majority (21/23) were classified as negative when assayed at the 5 × PK level (Fig. 2 and Supplementary Fig. 2, available in JGV Online). The
CRM-B and CRM-C standards gave values equivalent to the IHC-confirmed, Bio-Rad Platelia ELISA-positive survey samples and the IHC-unconfirmed, Bio-Rad Platelia ELISA-positive survey samples, respectively. All New Zealand-derived, scrapie-free samples and CRM-A samples (pooled negative-control material) were below the negative cut-off value of the kit when assayed at 1× and 5× PK levels.

**Western immunoblotting of sheep brain CRMs**

The immunoreactive PrP polypeptides in extracts of CRMs before and after low and high PK exposure are shown in Fig. 3. Normal PrPC, mostly present as the diglycosylated 33–35 kDa isoform, is abundant in brain and gave a strong signal in all three brain standards with both the N-blot and core blot mAbs. PrP C was destroyed even by limited (1× PK) proteolysis, as shown by the complete disappearance of bands from CRM-A (TSE-negative control) in both blots at high and low PK exposure levels (Fig. 3a and b; CRM-A).

CRM-B, the brain pool made up from PrP IHC-confirmed, clinical cases of scrapie, gave strong signals at both PK concentrations with the core blot mAbs and the characteristic 6–7 kDa band shift was observed by comparison of the signals in the 0× PK and 5× PK tracks (Fig. 3a). At the 1× PK level, the N-blot gave a good signal consistent with only partial cleavage of the N-terminal sequence at this low exposure, but, as expected from previous studies (Hope et al., 1986), the signal disappeared after 5× PK treatment (Fig. 3b).

CRM-C, the brain pool made from PrP IHC-negative, Bio-Rad Platelia ELISA-negative tissue, gave an almost identical PrP immunoreactive profile in both N-blot and core blot systems. At the 1× PK level, reduced but qualitatively similar banding to that seen without protease treatment was observed in the molecular mass range of PrPC using both N- and C-terminal PrP-specific mAbs. No 6–7 kDa band shift characteristic of the conversion of PrP Sc to its N-terminally truncated amyloid core subunit, PrP res, was observed at either level of protease exposure. At the 5× PK exposure level, no banding was seen with either mAb system (Fig. 3a and b).

**British survey samples**

Representative core and N-blots of the British survey samples are shown in Fig. 4, and the patterns obtained with a wider range of genotypes are provided as Supplementary Fig. S2 (available in JGV Online). In general, the survey IHC-confirmed, Bio-Rad Platelia ELISA-positive samples...
gave patterns resembling CRM-B (Fig. 4a) and the survey
IHC-unconfirmed, Bio-Rad Platelia ELISA-positive samples
gave patterns resembling CRM-C (Fig. 4b). In some cases,
intermediate banding patterns and the appearance of lower
molecular mass PrP immunoreactive bands migrating
between the lowest molecular mass standard (~20 kDa)
and the gel dye front were visualized in the core blots but not
in the N-blot system (see Fig. 4c, asterisks, and samples 3
and 5 in Supplementary Fig. S2).

By including the CRMs in each batch of analyses, we were
able to provide an internal control for variation in PK
hydrolysis conditions. By incorporating this quality con-
trol, we noted that the appearance and intensity of this
intermediate ~14 kDa (more precisely <20 kDa) band
seemed related both to the degree of proteolysis, as judged
by inter- and intra-analysis comparisons of the CRM
standards, and to the nature of the sample (genotype, Bio-
Rad Platelia response, IHC-confirmed or unconfirmed)
data not presented). This variability needs to be controlled
further to allow higher-resolution molecular phenotyping
of these classes of sheep PrP-related abnormalities but,
notwithstanding this operational constraint, the two groups
of IHC-confirmed and IHC-negative, Bio-Rad Platelia
ELISA-positive cases could clearly be differentiated on the
basis of the molecular phenotypes defined in Fig. 4(a and b).

**DISCUSSION**

About half of suspect TSE cases found by active surveillance
in Great Britain during 2002–2003 by rapid testing for
abnormal PrPrP in sheep brain stem have been characterized
by a form of the putative transmissible agent similar to that
originally described in rodent models of disease (McKinley
et al., 1983; Hope et al., 1986, 1988). This oPrPrPSc had the
same migration as PrPrPSc on SDS-polyacrylamide gels,
equivalent to a molecular mass of 33–35 kDa, but, unlike
PrPSc, oPrPrPSc aggregated into conformers that were rela-
tively resistant to proteolytic hydrolysis. PK treatment of
this abnormal prion protein in mild denaturing buffers
(PrPrPSc: enzyme ratio of approx. 1:50) cleaved the N-
terminal 60–70 aa from the more robust core structure to
leave a large fragment of PrP, PrPres (codons ~90–230, with
a molecular mass of 27–30 kDa), under conditions where
PrPSc was completely hydrolysed. Consequently, this PrPres
was seen even after stringent PK treatment (5×) using our
core blot system, but was not seen using the N-blot
molecular phenotyping system. No PrPrP signal was observed
in either blotting system using mild (1×) or stringent (5×)
PK digestion of PrPSc standards (CRM-A; Fig. 3a and b).
The characteristic molecular mass shift of PrPSc to PrPres
(7–8 kDa) was also seen in core blots but not in N-blots of
this phenotype (CRM-B; Fig. 3a and b, and Fig. 4a). This is
the molecular phenotype commonly associated with the case
definition of scrapie in small ruminants (OIE, 2004) and
these suspect cases were confirmed as TSE by PrP IHC
detection at the level of the obex and by an independent
Western blotting system (Prionics Check WB). The oPrPrP

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**Fig. 4.** PrP Western blots of British survey cases. Prion
proteins in Bio-Rad Platelia-positive cases confirmed (a, c) or
initially unconfirmed (b) by IHC detection at the level of the
obex. PrPs were visualized by mAbs SAF60/BAR226 (core
blot) or mAb SAF34 (N-blot) after no PK treatment (0), mild
PK treatment (1×) or stringent PK treatment (5×). Three
cases are illustrated with their genotypes: ARR/VRQ (a), AHQ/
AHQ (b) and ARR/VRQ (c). Further examples in other geno-
types are shown in Supplementary Fig. S2 (available in JGV
Online). The solid arrow represents the electrophoretic migra-
tion position of full-length PrPSc and PrPres (~33–35 kDa); the
dashed arrow is the position on the gel blot of the conventional
proteinase-resistant core fragment of PrPSc, PrPres (~27–
30 kDa). The dashed line indicates the dye front of the electrophoresis run.
gene is highly polymorphic and the relative susceptibility or resistance of a sheep to clinical scrapie depends, amongst other factors, on its PrP genotype (Hunter, 1997; Goldmann et al., 2005). The most common dimorphisms are at codons 136, 154 and 171 and common alleles are denoted by the amino acids (in single-letter code) encoded by codons 136, 154 and 171. For instance, the ARR allele comprises A136, R154 and R171, and the ARR/ ARR homozygous genotype is classed as the one most resistant to the development of scrapie (and experimental BSE). Conversely, the VRQ/ VRQ genotype is recognized to be at high risk of developing natural scrapie. The PrP genotypes at codons 136, 154 and 171 in these confirmed cases broadly matched those previously described in association with clinical cases of scrapie with almost all carrying either a VRQ or an ARQ allele (National Scrapie Plan, http://www.defra.gov.uk/animalh/bse/othertses/scrapie/nsp/index.html).

By contrast, the remaining half of the suspect TSE cases were characterized by a form of the putative transmissible agent that appeared to have a less stable PrP core structure. Under stringent PK digestion conditions (5 × n), these samples were completely destroyed and were indistinguishable from normal, genotype-matched PrPC controls by ELISA (Fig. 2) or by either type of Western blot (Fig. 3 and Fig. 4b). Using mild (1 × n) PK conditions, these samples gave a diffuse banding pattern similar to undigested PrP C in both core and N-blot systems with no apparent band shift. These cases clearly represent a prion protein abnormality, as no similar signal was seen by ELISA or Western blotting of normal PrPC controls after mild PK treatment (n > 100, Figs 2 and 3). There are some similarities between these cases and Nor98 and Nor98-like cases now described in Europe (Benestad et al., 2003; Buschmann et al., 2004; De Bosschere et al., 2004; Gavier-Widen et al., 2004; Madec et al., 2004; Orge et al., 2004; Onnasch et al., 2004; Moum et al., 2005), particularly their genotype distribution, which appears to favour AHQ and ARR (and AF141RQ; Moum et al., 2005) carriers (Fig. 1; Baylis & McIntyre, 2004). The L141F dimorphism typing of our samples is in progress and may provide further insight into these molecular phenotypes.

At the molecular level, these European cases are characterized by the accumulation in brain, particularly in the cerebral cortex and cerebellum, of a PrP immunoreactive peptide (or mixture of peptides) of molecular masses of 6–5–12 kDa in addition to higher molecular mass PrP C controls amyloid core markers for sheep scrapie. In the first case definition of Nor98, Benestad and colleagues used highly stringent PK digestion conditions (100 µg PK ml−1 for 30 min at 37 °C) and the mAb P4 against a peptide fragment defined by codons 84–104 to define a molecular profile where there was mostly 12 kDa peptide and some ~27–30/23–26 kDa banding, depending on the area of the brain sample; other mAbs were used to map these fragments and it was reported that, as expected, the molecular mass of the lowest band detected varied with the mAb used, although the data were not shown (Benestad et al., 2003). Two Irish cases and a case from Belgium showed a range of immunoreactive PrP C (12–30 kDa) in cerebellum and/or cerebrum when compared with Norwegian Nor98 using the mAbs BAR226/ SAF60 or 12F10/SAF60 (De Bosschere et al., 2004) or the Prionics Check WB (Onnasch et al., 2004), a system utilizing mAb 6H4, which is similar in specificity to SAF60. Seven Portuguese cases sampled from the brain stem and processed using a Bio-Rad kit (but otherwise undefined) also gave a profile of PrP C ranging from <20 to ~30 kDa (Orge et al., 2004). German sheep TSE cases in ARR/ ARR sheep had a more protease-sensitive PrP C than that of classical scrapie and a molecular mass range of <20–30 kDa was detected using the SAF tissue preparation procedure for Western blotting (Hope et al., 1986) and mAb L42 against codons 145–163 of oPrP (Harmeyer et al., 1998). A 6–5 kDa fragment observed in Swedish cases has been tentatively assigned to a 90–144 aa peptide (Ottinger et al., 2005) similar to that described in Gerstmann–Sträussler–Scheinker syndrome of humans (Tagliavini et al., 1994). Fig. 4(c) illustrates the same or a similar molecular phenotype with immunoreactive PrP bands at 27–30, 23–26, 18–21 and ~10 kDa seen using the core blot, but, consistent with a 90–144 aa-containing peptide, not using the N-blot in a confirmed case in an ARR/ VRQ sheep from the British survey. However, other cases from the British survey did not show a 6–12 kDa fragment, or in some cases any fragments, with either blotting system under the same conditions and so represent a wider range of sheep TSE phenotypes that requires further biological and biochemical definition to extend our understanding of mammalian prions.

ACKNOWLEDGEMENTS

This work reports the efforts of a large range of Defra and VLA administrators, veterinary field officers, veterinary pathologists, epidemiologists and VLA laboratory technicians as well as the authors of this manuscript and so we would like to thank everyone concerned for their perseverance and professionalism. This work also benefited considerably from the mAbs and interest of Drs Jacques Grassi and Jean-Phillipe Deslys and their colleagues at CEA, France, and the good will of their collaborators at Bio-Rad, France/UK.

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