Characterization of an unusual transmissible spongiform encephalopathy in goat by transmission in knock-in transgenic mice

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Bovine spongiform encephalopathy (BSE) is a fatal neurodegenerative disorder of cattle, and its transmission to humans through contaminated food is thought to be the cause of the variant form of Creutzfeldt–Jakob disease. BSE is believed to have spread from the recycling in cattle of ruminant tissue in meat and bone meal (MBM). However, during this time, sheep and goats were also exposed to BSE-contaminated MBM. Both sheep and goats are experimentally susceptible to BSE, and while there have been no reported natural BSE cases in sheep, two goat BSE field cases have been documented. While cases of BSE are rare in small ruminants, the existence of scrapie in both sheep and goats is well established. In the UK, during 2006–2007, a serious outbreak of clinical scrapie was detected in a large dairy goat herd. Subsequently, 200 goats were selected for post-mortem examination, one of which showed biochemical and immunohistochemical features of the disease-associated prion protein (PrP TSE ) which differed from all other infected goats. In the present study, we investigated this unusual case by performing transmission bioassays into a panel of mouse lines. Following characterization, we found that strain properties such as the ability to transmit to different mouse lines, lesion profile pattern, degree of PrP deposition in the brain and biochemical features of this unusual goat case were neither consistent with goat BSE nor with a goat scrapie herdmate control. However, our results suggest that this unusual case has BSE-like properties and highlights the need for continued surveillance.

INTRODUCTION

The transmissible spongiform encephalopathies (TSEs) are a group of fatal infectious neurodegenerative diseases that include scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in cervids and Creutzfeldt–Jakob disease (CJD) in humans. TSEs are characterized by the accumulation in the brain of disease-associated prion protein (PrP TSE ), which is a relatively protease-resistant conformational variant of the normal host encoded cellular prion protein (PrP C ). Due to the infectious nature of TSEs, these diseases can be transmitted via a number of different routes and, while TSEs tend to transmit more readily within species, they are able to transmit between different species, although this is dependent on both the TSE agent and the host.

The existence of naturally occurring classical scrapie in sheep and goats has been known for more than 200 years and is not considered a public health risk according to the opinion of the Scientific Panel on Biological Hazards on classification of atypical TSE cases in small ruminants (EFSA, 2005). However, the transmission of BSE to humans through contaminated food is thought to be the cause of the variant form of Creutzfeldt–Jakob disease (vCJD) (Bruce et al., 1997; Hill et al., 1997). This relationship reveals a potential risk of transmission of other ruminant TSEs to humans. During the BSE epidemic, both sheep and goats were exposed to BSE-contaminated meat and bone meal (MBM) (Baylis et al., 2002). Studies have shown that sheep and goats are experimentally susceptible to BSE (Baylis et al., 2002; Bellworthy et al., 2008; Foster et al., 1993; Houston et al., 2003; Stack et al., 2009a). While no natural cases of BSE have thus far been detected in sheep, two BSE cases in goats have been confirmed, one in France (Eloit et al., 2005) and the other in the UK (Spiropoulos et al., 2011). Furthermore, studies have shown the transmission of goat and/or sheep BSE into transgenic mice expressing human PrP and have found transmission of these isolates more efficient than cattle BSE (Padilla et al., 2011; Plinston et al., 2011).

BSE was first reported in 1987 (Wells et al., 1987) and, although it has been extensively characterized, the true
aetiology of infection remains unknown. Due to classical strain typing in mice (incubation time, lesion profiles, patterns of PrP staining in the brain) (Bruce, 1996; Bruce et al., 1997) and biochemical features of the proteinase K (PK)-resistant PrP in natural and experimental BSE (Collinge et al., 1996; Kuczius & Groschup, 1999; Kuczius et al., 1998), TSE disease in cattle was originally believed to be caused by a single prion strain, known as classical BSE (BSE-C). However, through increased TSE surveillance, two atypical BSE agents have also more recently been reported (Biacabe et al., 2004; Casalone et al., 2004; Jacobs et al., 2007; Stack et al., 2009b) and are identified as H-type BSE and bovine amyloidotic spongiform encephalopathy (BASE, also named L-type BSE). These atypical bovine TSEs can be distinguished by the electrophoretic positions of their PrP<sub>trans</sub> isoforms (Biacabe et al., 2004; Buschmann et al., 2006; Casalone et al., 2004; Jacobs et al., 2007) and different glycoform patterns.

Due to concerns that BSE may also infect sheep and goats, active surveillance of small ruminants has been considerably improved. Many countries now apply screening, confirmatory and discriminatory tests based on the post-mortem detection of PrP<sub>trans</sub> in the central nervous system. Following an outbreak of scrapie in a large goat herd in 2006–2007, the whole herd was culled and 200 goats were examined for PrP<sub>trans</sub> (González et al., 2009). One goat demonstrated biochemical and immunohistochemical (IHC) features of PrP<sub>trans</sub> different from all other 71 infected goats in the herd. This particular case was an eight-year-old Anglo-Nubian goat, homozygous for the caprine wild-type allele isoleucine at codon 142 (González et al., 2009). Notably, the existence of atypical scrapie was ruled out (González et al., 2009). Initial analysis of this goat case identified some similarities to BSE, such as a reduced P4 antibody signal following Western blotting and lack of 12B2 antibody staining of intracellular PrP<sub>trans</sub> deposits. However, further analysis with additional antibodies also showed distinct differences with experimental BSE in goats (González et al., 2009). In the present study, we aimed to characterize this unusual goat TSE (ref. V3698) by challenging knock-in (produced by gene targeting) bovine (Bov6) or human PrP Tg (HuTg) mice, as well as 129/Ola wild-type mice. One experimental goat BSE and a goat scrapie herdmate isolate (goat scrapie HM) were included as controls. Following neuropathological and biochemical analysis, we found that although V3698 presented some BSE-like properties, it differed from both experimental goat BSE and goat scrapie HM.

RESULTS

Transmissibility of V3698 differs from experimental goat BSE and goat scrapie

Using a panel of mouse lines (Bov6, HuTg and 129/Ola), animals were challenged intracerebrally with isolate V3698, experimental goat BSE or isolate V3981 from a goat herdmate with confirmed classical scrapie (González et al., 2009), referred to from this point on as goat scrapie HM. Following V3698 inoculations, a TSE was induced in 9/24 Bov6 and 1/22 129/Ola mice and was defined by the presence of PrP deposition (using immunohistochemistry) in the brain and/or vacuolar pathology (Table 1). In contrast, the majority of Bov6 mice (22/23) and almost 60% of 129/Ola mice (13/22) developed a TSE following challenge with experimental goat BSE. Following goat scrapie HM challenge, the majority of 129/Ola mice (20/24) were positive for TSE disease. Interestingly, however, 9/23 Bov6 mice were positive for TSE pathology (PrP deposition and/or vacuolar pathology). While the majority of TSE positive mice challenged with either experimental goat BSE or goat scrapie HM showed clinical signs of disease, this was not the case for V3698 (Table 1). Furthermore, TSE neuropathology occurred around 150 days later in mice challenged with V3698 as compared with either Bov6 mice challenged with experimental goat BSE or 129/Ola challenged with goat scrapie HM. Thus, our results demonstrated that V3698 attack rates were not consistent with either experimental goat BSE or goat scrapie HM. Full strain characteristics of V3698 are shown in Table S1, available in JGV Online.

Comparison of lesion profiles from previous TSE agents transmissible to Bov6 mice

The lesion profiles, which define areas of vacuolation and their degree of severity in the brain, were determined for Bov6 mice inoculated with V3698, experimental goat BSE and goat scrapie HM (Fig. 1a). Although the production of lesion profiles in preclinical mice is not standard practice, all Bov6 mice challenged with V3698 or goat scrapie HM which scored positively for vacuolation pathology were included to give an indication of vacuolation profile in comparison with experimental goat BSE. Lesion profiles of V3698 and experimental goat BSE were clearly distinct, indicating different TSE isolates (Fig. 1a). A higher degree of vacuolar pathology was observed in several brain regions (hippocampus, retrosomal cortex, cingulate and motor cortex, and cerebellar white matter) in Bov6 mice challenged with V3698. However, lesion profiles in Bov6 mice challenged with V3698 or goat scrapie HM were strikingly similar. To further investigate the vacular pattern of V3698, we also compared lesion profiles produced from previous experiments in which cattle isolates of BASE, BSE-C and H-type BSE had been transmitted to the Bov6 mice (Wilson et al., 2012a) (Fig. 1b–d). In these comparisons, V3698 was found to display a similar pattern of vacuolar targeting to that observed in Bov6 mice challenged with BASE, and was distinct from BSE-C and H-type BSE.

Bov6 Mice challenged with V3698 or goat scrapie show low levels of PrP deposition

Widespread extensive PrP deposition, detected using immunohistochemistry, was observed in Bov6
challenged with experimental goat BSE (Fig. 2a, b). In contrast, Bov6 mice challenged with V3698 showed very little PrP deposition, which was observed in the thalamus (Fig. 2c, d), midbrain and medulla. While the majority of 129/Ola challenged with goat scrapie HM showed heavy PrP deposition (Fig. 2e, f), only seven Bov6 mice showed PrP deposition. However, similarly to V3698, we observed very little PrP deposition in the Bov6 mice challenged with goat scrapie HM, which was also confined to the thalamus (Fig. 2g, h), midbrain and medulla. Due to the low levels of PrP deposition in Bov6 mice challenged with V3698 or goat scrapie HM, we could not conclude similarities or differences in PrP distribution pattern compared with experimental goat BSE or goat scrapie HM in 129/Ola mice. Using thioflavin-S fluorescence we were unable to detect PrP amyloid plaques in Bov6 mice challenged with experimental goat BSE, Bov6 mice challenged with V3698 or in 129/Ola challenged with goat scrapie HM (Fig. S1).

**Glial neuropathology was evident despite minimal visible PrP deposition**

To control for age-related effects, gliosis was first assessed in brain tissue obtained from a previous ageing study of Bov6 mice (Wilson et al., 2012a). Mild gliosis was present throughout the brains of aged Bov6 control mice (Fig. 3b, c). In contrast to gliosis due to ageing, an increase in the appearance of astrogliosis and microgliosis was clearly evident throughout the brains of Bov6 mice inoculated with V3698, experimental goat BSE and goat scrapie HM, and 129/Ola challenged with goat scrapie HM (all of which showed signs of TSE pathology), and was most evident in the thalamus (Fig. 3e, f, h, i, k, l, n, o). While areas of PrP deposition in the thalamus clearly correlated with gliosis in Bov6 mice challenged with experimental goat BSE and 129/Ola challenged with goat scrapie HM (Fig. 3g, m), Bov6 mice challenged with V3698 or goat scrapie HM showed gliosis despite the presence of very little visible PrP deposition (Fig. 3d, j). No PrP deposition was observed in aged Bov6 mice (Fig. 3a).

**Table 1. Bioassay results of primary passage of V3698, goat BSE and goat scrapie HM into both bovine and human PrP Tg mice and 129/Ola mice**

<table>
<thead>
<tr>
<th>TSE isolate</th>
<th>Mouse line</th>
<th>Survival*</th>
<th>Incubation†</th>
<th>Clinical signs</th>
<th>TSE pathology‡</th>
<th>Clin +/TSE path +§</th>
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</thead>
<tbody>
<tr>
<td>V3698</td>
<td>Bov6</td>
<td>598 ± 20</td>
<td>–</td>
<td>1/24</td>
<td>9/24</td>
<td>0</td>
</tr>
<tr>
<td>129/Ola</td>
<td>–</td>
<td>572</td>
<td>3/22</td>
<td>1/22</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HuMM</td>
<td>–</td>
<td>–</td>
<td>1/23</td>
<td>0/23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HuMV</td>
<td>–</td>
<td>–</td>
<td>8/24</td>
<td>0/24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HuVV</td>
<td>–</td>
<td>–</td>
<td>2/23</td>
<td>0/23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental Goat BSE (G8247)</td>
<td>Bov6</td>
<td>447 ± 14</td>
<td>453 ± 15</td>
<td>18/23</td>
<td>22/23</td>
<td>18</td>
</tr>
<tr>
<td>129/Ola</td>
<td>379 ± 18</td>
<td>385 ± 18</td>
<td>12/22</td>
<td>13/22</td>
<td>12</td>
<td></td>
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<tr>
<td>HuMM</td>
<td>625 ± 12</td>
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<td>3/24</td>
<td>10/24</td>
<td>0</td>
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<td>HuMV</td>
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<td>–</td>
<td>6/23</td>
<td>0/23</td>
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<tr>
<td>HuVV</td>
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<td>–</td>
<td>5/23</td>
<td>0/23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goat Scrapie HM (V3981)</td>
<td>Bov6</td>
<td>646 ± 12</td>
<td>–</td>
<td>0/23</td>
<td>9/23</td>
<td>–</td>
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<tr>
<td>129/Ola</td>
<td>439 ± 6</td>
<td>443 ± 6</td>
<td>18/24</td>
<td>20/24</td>
<td>18</td>
<td></td>
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<tr>
<td>HuMM</td>
<td>–</td>
<td>–</td>
<td>1/22</td>
<td>0/22</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>HuMV</td>
<td>–</td>
<td>–</td>
<td>3/24</td>
<td>0/24</td>
<td>–</td>
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</tr>
<tr>
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<td>–</td>
<td>–</td>
<td>1/24</td>
<td>0/24</td>
<td>–</td>
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</tr>
</tbody>
</table>

* Measured as days ± SEM and calculated from mice showing vacuolar pathology and/or PrP deposition.
† Measured as days ± SEM and calculated from mice showing both clinical signs of disease and neuropathology (vacuolar pathology and/or PrP deposition).
§ Number of animals positive for PrP deposition and/or vacuolar pathology.
§§ Number of animals with clinical signs and confirmed TSE pathology.

**Molecular profile of V3698 differs from goat scrapie HM and experimental goat BSE**

Brains from Bov6 mice challenged with V3698, experimental goat BSE and goat scrapie HM and also 129/Ola mice challenged with goat scrapie HM (2 or 3 brains per isolate) were examined for the presence of PrP TSE by Western blot (Fig. 4a, b). Brains from Bov6 mice challenged with BASE and BSE-C were also included for comparison. All brains selected for analysis showed moderate to intense PrP TSE deposition (by immunohistochemistry) in the brain, except for Bov6 mice challenged with V3698 and goat scrapie HM which showed very little PrP TSE deposition. Due to the low levels of PrP in these brains, precipitation of PrP using sodium phosphotungstic acid (NaPTA) was performed to concentrate all samples. Following Western blot analysis using anti-PrP Ab6H4, distinct PrP TSE profiles were observed between Bov6 mice
challenged with V3698 or experimental goat BSE and 129/Ola mice challenged with goat scrapie HM (Fig. 4a). However, similar PrPTSE profiles were observed between Bov6 brains challenged with V3698 or goat scrapie HM (Fig. 4c). The experimental goat BSE PrPTSE had a similar glycopattern to BSE-C with the characteristic heavier diglycosylated band. Indeed, previous studies have shown that glycoform pattern can be used to distinguish among different TSE strains (Baron et al., 2007; Béringue et al., 2007; Capobianco et al., 2007; Somerville et al., 1997, 2005). However, a number of phenotypic markers are different TSE strains (Baron et al., 2007; Béringue et al., 2007; Capobianco et al., 2007; Somerville et al., 1997, 2005). However, a number of phenotypic markers are

Fig. 1. Pattern of vacuolation observed in brains of Bov6 mice challenged with V3698, experimental goat BSE and goat scrapie HM (a). Pattern of vacuolation observed in brains of Bov6 mice challenged with V3698, BASE, classical BSE (BSE-C) and H-type BSE (b–d). Lesion profiles for BASE, BSE-C and H-type BSE (shown for comparison) originate from previously published work (Wilson et al., 2012a). A profile was produced from nine grey-matter areas (1, medulla; 2, cerebellum; 3, superior colliculus; 4, hypothalamus; 5, thalamus; 6, hippocampus; 7, septum; 8, retrosplenial cortex; 9, cingulate and motor cortex) and three white matter areas (1, cerebellar white matter; 2, midbrain white matter; 3, cerebral peduncle). Mean±SEM scores were taken from a minimum of seven mice per group and plotted against brain area.

Fig. 2. Comparative analysis of variation in intensity of PrPTSE in the thalamus region of brains from Bov6 mice challenged with experimental goat BSE, 456 days post-inoculation (p.i.) (a, b), V3698, 623 days p.i. (c, d), or goat scrapie HM, 680 days p.i. (g, h), and 129/Ola mice challenged with goat scrapie HM, 407 days p.i. (e, f), (b), (d) and (f) are higher magnification images of the boxed areas in (a), (c) and (e), respectively. Images obtained after staining with anti-PrP antibody 6H4 and counterstained with haematoxylin. Magnification as shown.
required to reliably discriminate between strains. Ratios of all three glycoforms (un-, mono- and diglycosylated), calculated by a Kodak 440CF digital imager, were similar between Bov6 mice brains challenged with BSE-C PrPTSE and experimental goat BSE PrPTSE. These differed from V3698, which showed a PrP glycoform ratio similar to BASE in Bov6 mice. All were distinct from 129/Ola mouse brains challenged with goat scrapie HM (Fig. 5). Western blot analysis was also performed using anti-PrP Ab12B2, which can be used to differentiate between BSE and classical scrapie (Jacobs et al., 2011; Jeffrey et al., 2006; Langeveld et al., 2006). A strong signal was detected only from the 129/Ola mouse brains challenged with goat scrapie HM (Fig. 4b, d).

**Transmission of experimental goat BSE into HuTg mice**

Previous studies have shown that experimental goat BSE and sheep BSE are transmissible to Tg mice expressing codon 129 methionine human PrP (HuTg mice) (Padilla et al., 2011; Plinston et al., 2011) and transmit to these
mice more efficiently than cattle BSE (Padilla et al., 2011). In the present study, we also found transmission of experimental goat BSE into HuTg mice (10/24 of the HuMM mice subgroup showed PrP deposition). Neuropathological analysis of these mice showed PrP deposition was plaque-like and restricted to the thalamus (Fig. 6) and midbrain. Despite the presence of PrP deposition, no HuTg mice were positive for vacuolation pathology, except for one, which had limited vacuolation in the cerebral and midbrain white matter. Due to the lack of overexpression of PrP in these mice, they may more closely represent what happens in nature and, thus, this finding is of particular concern. No transmission of either V3698 or goat scrapie HM was detected in the HuTg mice, which further suggests that V3698 is distinct from goat BSE (Table 1).

DISCUSSION

In this study, we examined the transmission characteristics, neuropathology and biochemical characteristics of an unusual TSE present in a goat, designated V3698. Initial investigation of this case revealed unusual biochemical and
immunohistochemical features of PrP^{TSE} compared with other scrapie-infected goats in the herd (González et al., 2009), which presented both similarities and differences to both goat BSE and previously studied isolates of goat scrapie. To help determine the nature of V3698 and improve our understanding of the diversity of goat TSEs, we performed transmission bioassays into a panel of mice. Along with V3698 we therefore also inoculated an experimental goat BSE isolate and goat scrapie HM control into gene-targeted bovine or human PrP Tg mice, as well as 129/Ola wild-type mice. As these transgenic mice are produced by gene replacement, they do not suffer from any adverse phenotypes which are associated with overexpression or ectopic expression of the transgene in standard transgenic lines, and express PrP^{c} at the same level as wild-type mice (Manson et al., 1999; Moore et al., 1998). Furthermore, previous studies have shown efficient transmission of TSE agents into gene-targeted transgenic PrP mouse lines, demonstrating that these mice do live long enough to show signs of infection, supporting the use of targeted mouse models to analyse TSE disease transmission (Bishop et al., 2006, 2010; Plinston et al., 2011). While caprine transgenic mouse lines may have been an alternative model system, as yet there are no available knock-in caprine transgenic mice. Furthermore, in this study, the use of bovine and human PrP knock-in transgenic lines allows direct comparison with archive data produced following inoculation of these lines with a wide range of TSE agents, including vCJD, BSE-C (Bishop et al., 2006), BASE and H-type BSE (Wilson et al., 2012a).

The data presented here show that transmission, neuropathology and biochemical properties of V3698 were not consistent with either experimental goat BSE or goat scrapie HM. Lesion profiles between V3698 and experimental goat BSE were clearly distinct, as were the amount of PrP deposition present in the brain and ratios of PrP^{TSE} glycoforms. Furthermore, lack of binding to an anti-PrP antibody (12B2) suggested that V3698 was not a classical scrapie strain. This was consistent with the original study of V3698 in which PrP^{TSE} in brain tissue, but not retropharyngeal lymph node, was shown to lack 12B2 binding (González et al., 2009). Interestingly, although goat scrapie HM-inoculated 129/Ola mice produce a strong PrP^{TSE} signal on immunoblots following probing with 12B2, indicating classical scrapie, the PrP^{TSE} in Bov6 mice inoculated with goat scrapie HM was negative for 12B2 binding. We have previously observed a strong 12B2 signal on immunoblots from Bov6 mice challenged with H-type BSE (data not shown), proving that Bov6 PrP^{TSE} can bind 12B2. These data are also consistent with studies showing that while labelling for classical BSE or BASE with 12B2 is minimal or absent, H-type BSE is strongly labelled with this antibody (Baron et al., 2011; Dudas et al., 2010; Jacobs et al., 2011). Unlike goat scrapie HM or experimental goat BSE, V3698 failed to transmit efficiently to 129/Ola mice. While evidence of scrapie transmission to cattle has been described by others (Cutlip et al., 1994; Konold et al., 2006), our previous studies have shown a lack of transmission of either classical or atypical scrapie into Bov6 mice, (Plinston et al., 2011; Wilson et al., 2012b). Importantly, all successful disease transmissions into Bov6 mice in our lab, as yet, have been from BSE-like strains (Bishop et al., 2006; Plinston et al., 2011; Wilson et al., 2012a). Hence, the observed transmissibility of the goat scrapie HM to Bov6 mice and lack of 12B2 binding is perplexing. As we have not previously challenged our Bov6 mice with other isolates of goat scrapie, we cannot rule out the possibility that scrapie in goats is transmissible to this mouse line. However, the results presented here may also suggest that the same agent has been isolated from both goat field samples in the Bov6 mice based on the lack of 12B2 reactivity, similarities in both lesion and molecular profile, the lack of PrP deposition, and the fact that both V3698 and the goat scrapie HM control came from the same herd. Indeed, it is widely accepted that animals carrying TSE infection in the field may be infected with more than one ‘strain’. Laboratory TSE strains represent isolates which have been selected from field cases following serial transmission in mice and probably represent selection from the field case of the isolate that replicates most efficiently in that mouse genotype. The similarities of V3698 and the goat scrapie HM inoculum in Bov6 mice may suggest that the same agent was isolated from both goats. However, the high transmission of goat scrapie HM, but not V3698, to 129/Ola mice indicates either a difference in the TSE agent, or the presence of more than one TSE agent that may have been differentially selected in the various mouse genotypes. Further transmission studies would need to be performed to determine whether this was the case.

Interestingly, we found that V3698 showed a number of similarities to earlier transmissions investigating the susceptibility of Bov6 mice to BASE (Wilson et al., 2012a). As previously discussed, V3698 failed to transmit efficiently into 129/Ola control mice, which was similar to prior studies with BASE (Wilson et al., 2012a). In contrast, and as was the case for experimental goat BSE in these studies, others have shown that classical cattle BSE transmits very efficiently to a variety of inbred mouse strains (Capobianco et al., 2007; Wilson et al., 2012a). Despite signs of neuropathology, we did not observe clinical signs in Bov6 mice challenged with V3698, which was also the case in Bov6 mice challenged with BASE (Wilson et al., 2012a). Indeed, several studies have shown that cattle infected with atypical BSE were found to be healthy at slaughter (Biacabe et al., 2004; Casalone et al., 2004). We also observed a similarity between the vacuolation pattern of V3698 and BASE in the Bov6 mice. However, note well that we observed properties of V3698 which were not consistent with BASE, such as the mobility of the unglycosylated PrP fragment, lack of prominent, widespread PrP deposition and amyloid plaques, as had been shown in previous studies involving Bov6 mice (Wilson et al., 2012a). As BASE has not been previously
transmitted into goats, the strain characteristics of this TSE in goats is unknown. Furthermore, the existence of BASE could be difficult to determine if mixed TSE infections were present in a single host. Therefore, further studies would be necessary to identify whether V3698 was BASE in a goat.

Although incubation periods or survival times are often used to distinguish among TSEs, they may not be as reliable during first passage, especially when a TSE is only diagnosed in a small number of animals due to low titre and/or limited transmission of specific TSE strains in certain mouse lines. Therefore, while we still observed transmission in 37% of Bov6 mice challenged with V3698, we included in this study incubation and survival times as an observation but did not rely on them to discriminate among TSE isolates.

As part of this study we also investigated the susceptibility of gene-targeted human PrP Tg mice to V3698, experimental goat BSE and goat scrapie HM. Approximately 40% of brains from HuMM Tg mice challenged with experimental goat BSE showed PrP deposition. Our results support previous studies showing that goat and sheep BSE are more efficient at transmitting to human Tg mice than cattle BSE (Padilla et al., 2011). These findings are of concern with regard to the risk to human health and whereas in cattle BSE infectivity is mainly confined in the central nervous system, in small ruminants BSE has been detected in peripheral tissues and can be transmitted horizontally (Bellworthy et al., 2005; Kao et al., 2002). Thus, the data presented here highlight the requirement to prevent BSE from becoming established in small ruminants.

Overall, our results revealed that while showing some BSE-like properties on transmission in a panel of transgenic mice, V3698 is neither goat BSE nor a classical goat scrapie, and may rather be a BSE-like TSE or an unusual form of scrapie; however, further experiments are required to fully characterize this case. While atypical BSE has been reported in cattle since 2004 (Biacabe et al., 2004; Casalone et al., 2004; Jacobs et al., 2007; Stack et al., 2009b), no cases have been documented in small ruminants. As yet, the origin of atypical BSE is unknown; however, it has been suggested that it may be a spontaneous TSE in cattle, thus it would be interesting to speculate whether this was also possible in goats. We have also considered the possibility that V3698 is a CH1641-like scrapie isolate. These isolates have been described in sheep and goats both experimentally (Foster & Dickinson, 1988; Jeffrey et al., 2006) and naturally (Bellworthy et al., 2005; Bencsik & Baron, 2011; Vulin et al., 2011), and show partial similarities to experimental ovine BSE. Previous studies have used anti-PrP antibody SAF84 to identify an additional lower molecular PrpTSE band which has been shown to be a marker of CH1641 (Baron et al., 2008). Despite our Western blot attempts using SAF84, we were unable to fully investigate the V3698 PrpTSE glycoform due to the extremely low levels of PrpTSE in the Bov6 mice challenged with V3698. Furthermore, without performing transmission studies into Bov6 mice, we cannot fully compare V3698 with CH1641. Presently, the spectrum of TSE agents circulating in the goat population is unknown. Other studies investigating a flock of scrapie-affected goats in Greece have shown the existence of a unique TSE (Bouzalas et al., 2011). These studies showed that a number of clinically healthy goats were found to have a unique C- and N-terminally truncated PrpTSE fragment, which was similar but not identical to atypical scrapie. Similarly to our results, which showed very little PrP deposition in Bov6 mice challenged with V3698, these goats did not show any PrP deposits in the brain (Bouzalas et al., 2011). Therefore, despite the relatively small UK goat population, various TSE agents in goats may exist; however, it is also possible that in certain instances more than one agent may exist in an individual goat. As surveillance of small ruminants continues, the ability to recognize natural goat TSE strains will be of particular importance, not only to improve our knowledge of the variability of goat TSEs, but also to assess any potential risk to human health.

**METHODS**

**Preparation of inocula.** Brain homogenate G8247 from an experimentally BSE-infected goat (2nd goat passage; The Roslin Institute, unpublished), genotype GI0Q/GI0QS (Goldmann et al., 2011) was used as a goat BSE control. The unusual goat TSE case V3698 and the goat scrapie HM control (ref V3981; a 9-year-old Saanen goat) were both supplied by the Animal Health Veterinary Laboratories Agency (AHVLA), Weybridge, UK. All brain tissue supplied was from the thalamic region. All inocula were prepared from brain tissue in sterile saline at a concentration of 10% (w/v).

**Inoculation of transgenic mice.** Gene-targeted Tg mice expressing bovine PrP (Bov6) or human PrP (HuMM, HuMV and HuVV) have been described previously (Bishop et al., 2006). Wild-type 129/Ola mice were used as controls (Bishop et al., 2006). Transgenic mice were grouped (24 mice each) prior to intracerebral inoculation (i.c.) and then injected with 0.02 ml of 10−1 brain homogenate (experimental goat BSE, V3698 or goat scrapie HM) into the right cerebral hemisphere under halothane anaesthesia. All inocula were prepared under licence from the UK Home Office in accordance with UK Animals (Scientific Procedures) Act 1986.

**Vacuolation scoring.** Sections were cut (6 μm) from each mouse brain and stained using haematoxylin and eosin (H&E). TSE-related vacuolation was assessed at nine grey-matter regions (medulla, cerebellum, superior colliculus, hypothalamus, thalamus, hippocampus, septum, retinal cortex, and cingulated and motor cortex) and three regions of white matter (cerebellar white matter, midbrain white matter, and cerebral peduncle). Sections were scored on a scale of 0 (no vacuolation) to 5 (severe vacuolation) for the presence and severity of vacuolation and mean vacuolation scores for each mouse.
group in each experiment were calculated and plotted with standard errors of the mean (SEM) against scoring areas to produce a lesion profile, as previously described (Bruce et al., 1997; Fraser & Dickinson, 1967). While the production of lesion profiles in prion-free clinical mice is not standard practice, all mice which scored positive for vacular pathology were included whether or not clinical signs were present, due to the lack of clinical signs observed in Bov6 mice challenged with V3698.

**IHC analysis of PrP deposition and glial activation in the brain.**

PrPTSE localization in the brain was assessed using immunohistochemistry. Following fixation in 10% formal saline, brains were treated for 1.5 h in 98% formic acid, dissected to expose several brain regions, and embedded in paraffin. Sections (6 μm) were then autoclaved for 15 min at 121 °C and immersed in 95% formic acid for 10 min prior to incubation with 0.44 μg ml⁻¹ anti-PrP monoclonal antibody (mAb) 6H4 (Prionics) at room temperature overnight. Secondary anti-mouse biotinylated antibody (Jackson Immuno Research Laboratories) was added at 2.5 μg ml⁻¹ and incubated for 1 h at room temperature. Immunolabelling was performed using the ABC Elite kit (Vector Laboratories) and the signal was visualized by a reaction with hydrogen peroxidase-activated diaminobenzidine (DAB). The presence of prion disease, was assessed by incubating brain sections (6 μm) with 1.45 μg ml⁻¹ anti-glia fibrillary acidic protein (GFAP; DAKO UK) antibody at room temperature for 1 h. To detect microglial activation, brain sections were pre-treated using hydrated microwaving for 10 min prior to incubation with 0.05 μg anti-IBA1 antibody ml⁻¹ (Wako Chemicals) at room temperature for 1 h. For both GFAP and anti-IBA1 antibodies, 2.6 μg ml⁻¹ biotinylated secondary anti-rabbit antibody (Jackson Immuno Research Laboratories) was added for 1 h at room temperature. Both astrocytes and microglia were visualized by a reaction with hydrogen peroxidase-activated DAB.

**Detection of amyloid plaques by thioflavin fluorescence.**

Amyloid deposits in tissue sections were observed using thioflavin-S. Following haematoxylin staining, sections (6 μm) were then incubated at 37 °C for 1 h. PrPTSE was precipitated with the addition of a final concentration of 0.3 % NaPTA, incubated at 37 °C for 15 min. Brain homogenate supernatant from the transgenic mice and controls was incubated with 20 μg proteinase K ml⁻¹ for 1 h at 37 °C. Due to the lack of PrP deposition, brains from Bov6 mice were treated with NaPTA to precipitate PrP and concentrate the samples. Briefly, 100 mg of brain tissue was incubated for 15 min at 37 °C in PBS containing 1 mM MgCl₂. The samples were adjusted to 2 % (w/v) Sarcosyl and incubated for 5 min at 37 °C. The samples were then altered to a final concentration of 20 μg proteinase K ml⁻¹ and incubated at 37 °C for 1 h. PrPTSE was precipitated with the addition of a final concentration of 0.3 % NaPTA, incubated at 37 °C for 30 min and centrifuged at 14 000 r.p.m. for 20 min. The pellet was dissolved in 15 μl PBS, pH 7.4, containing 0.1 % Sarcosyl. All products were denatured and separated on a 10–20% NovexTris/glycine gel (Invitrogen) before transfer to PVDF membrane by Western blotting. The amount of brain tissue loaded onto the gels varied between 0.3 mg and 50 mg brain equivalent. PrP was identified with monoclonal antibody 6H4 (0.1 μg ml⁻¹) or 12B2 (0.2 μg ml⁻¹) and bands visualized using horseradish peroxidase (HPR)-labelled anti-mouse secondary antibody (Jackson Immuno Research Laboratories) and a chemiluminescence substrate (Roche). Images were captured on radiographic film and with a Kodak 440CF digital imager.

**PCR genotyping of mouse tail DNA.** All mice were analysed by PCR post-mortem to confirm PrP genotype. Mouse tail DNA was extracted and genotyped as described by Bishop et al. (2006) and Wemheuer et al. (2011).

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