Relevance of oral experimental challenge with classical scrapie in sheep

Guillaume Tabouret,1 Caroline Lacroux,1 Séverine Lugan,1 Pierrette Costes,1 Fabien Corbière,1 Jean Louis Weisbecker,2 François Schelcher1 and Olivier Andréoletti1

Correspondence
Olivier Andréoletti
o.andreoletti@envt.fr

1UMR INRA ENVT 1225, Interactions Hôtes-Agent Pathogènes, Ecole Nationale Vétérinaire de Toulouse, 23 Chemin des Capelles, 31076 Toulouse, France
2INRA domaine de Langlade, 31450 Pompertuzat, France

Oral inoculation is currently considered as the best approach to mimic natural TSE contamination in ruminants. In this study, we compared the timing of abnormal prion protein (PrP Sc ) dissemination and accumulation in the organism of susceptible sheep either orally inoculated or naturally infected with classical scrapie. Both animal groups shared a similar PrP Sc dissemination scheme and accumulation dynamics in lymphoid tissues. However, orally challenged animals displayed an earlier neuro-invasion and a dramatically shorter incubation period than naturally exposed sheep. No differences were observed between the groups with regards to the neuro-invasion route. These results unambiguously indicate that oral inoculation can have an impact on both the earliness of neuro-invasion and the incubation period. They also support the statement that oral inoculation is a relevant model for investigating transmissible spongiform encephalopathy pathogenesis. Nevertheless, data obtained under such experimental conditions should be used with some caution.

Dissemination of the classical scrapie agent in the organism of prion protein (PrP) susceptible sheep naturally exposed to infection was extensively described by several authors (Andréoletti et al., 2000; van Keulen et al., 2000). These studies provided crucial elements towards the understanding of transmissible spongiform encephalopathy (TSE) agent pathogenesis and transmission risks.

However, the possibilities for studying TSE using naturally contaminated animals are limited. It requires flocks with sufficient disease incidence and implies numerous constraints. Consequently, in order to study TSE agents like bovine spongiform encephalopathy (BSE) (Bellworthy et al., 2005b; Foster et al., 1996, 2001b) or atypical scrapie for which natural cases cannot prospectively be investigated in flocks, experimental oral route challenge has been used as a proxy for natural contamination.

Recently, an unusually short incubation period was reported in VRQ/VRQ lambs that were orally challenged with classical scrapie (Ryder et al., 2009). Despite several hypotheses being proposed by the authors, it remains unclear if this phenomenon was due to the scrapie agent (strain) or if it was the consequence of the experimental challenge condition (oral dosing). An impact of oral challenge on TSE agent pathogenesis would raise some concern on the relevancy of data generated under such experimental conditions.

In order to address this issue, we compared VRQ/VRQ sheep groups that were either naturally contaminated or orally challenged by the same classical scrapie agent. Romanov VRQ homozygous lambs (n=50) were produced by orientated mating in the INRA Langlade flock, where a high incidence of classical scrapie has occurred since 1993 (Elsen et al., 1999). The first group of lambs (n=18) was separated from their mothers within the first 6 h following birth. These animals were orally dosed by natural suckling using 10% brain homogenate. The inoculum was prepared by pooling brain stem from Langlade ARQ/VRQ sheep (n=70 animals from a single birth cohort) terminally affected with scrapie. Lambs received two doses of infectious homogenate each corresponding to 2.5 g of tissue. The first inoculation was performed at 12 h of life and the second at 20 days old. Challenged animals were housed in a dedicated A2 facility physically separated from the flock. The second group of lambs (n=32) was raised in the flock to be naturally exposed to scrapie. Animals were sequentially killed (Table 1). All animals included in this study were cared for by following the European Union recommendations for animal welfare, and under the supervision of the local INRA ethics committee.

At each time point of the experiment (Table 1) two orally inoculated and four naturally exposed sheep were culled. A large panel of tissues including lympho reticular tissues, digestive tract, peripheral and central nervous system...
Table 1. PrPSc distribution in the organism of naturally exposed (Natural) or orally challenged (Oral) VRQ/VRQ sheep

First clinical signs occurred at 12 months old in orally challenged animals and at 20 months old in naturally exposed sheep. A systematic PrPSc detection was realized using immunohistochemistry (8G8 antibody) in the collected sheep tissues. PrPSc accumulation level was scored according to a semi-quantitative scale: (−) no PrPSc, (+) minimal PrPSc deposits, (++), (+++) moderate PrPSc deposits and (++++) strong PrPSc deposits. LN, Lymph node; PP, Peyer’s patches; ENS, enteric nervous system; MLN, mesenteric lymph node; ND, not done.

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(CNS) were collected. Each sample was divided into two equal parts. One part was fixed for 10 days in a 10% formalin neutral buffered solution, the second was snap frozen and stored at −80°C. Formalin fixed tissues were then systematically processed for abnormal PrP (PrPSc) immunohistochemistry using a mouse monoclonal antibody 8G8 (IgG2a, raised against the human recombinant PrP protein), which specifically recognizes the 95–108 aa sequence (Andréoletti et al., 2002a).

In the organism of naturally exposed VRQ/VRQ sheep, the PrPSc dissemination scheme and accumulation timing were similar to those reported in previous studies that we carried out in the same flock several years ago (Table 1) (Andréoletti et al., 2000, 2002b). PrPSc was first detected in the ileal Peyer’s patches (18 days old), before deposition in gut-associated lymphoid tissue (GALT) draining lymph nodes (1 month old). At 3 months old, PrPSc could be detected in all GALT structures and in some lymphoid formations unrelated to the digestive tract. By 6 months old, all investigated lymphoid tissues were PrPSc positive. Compared to naturally exposed lambs, orally challenged VRQ/VRQ lambs presented a similar PrPSc dissemination scheme in lymphoid tissues.

A PrPSc immunometric assay (TSE detection test, TeSeE sheep and goat; Bio-Rad) was used to quantify PrPSc accumulation in tissues from sheep belonging to the two groups (Andréoletti et al., 2004) (Fig. 1). In this assay, recombinant ovine PrP protein (VRQ variant) was used as an external standard (range 2000–5 pg ml⁻¹) for quantification purposes. According to this assay, timing of PrPSc accumulation in lymphoid tissues was similar in both groups (Fig. 1a, b); PrPSc deposition exponentially increased, before reaching a plateau at 6 months post-infection or old, and remained constant until the onset of clinical signs. Due to the size of the experimental groups (two orally inoculated sheep and four naturally exposed) a proper statistical comparison of the accumulated PrPSc level could not be carried out. However, in clinically affected sheep such comparison (orally inoculated n=8, natural cases n=4; Mann Whitney) indicated that the PrPSc levels measured in the lymphoid tissues of both animal groups were not statistically different.

In the naturally exposed animal group, PrPSc accumulations in the enteric nervous system (ENS) were not recorded before 6 months old (Table 1). In the CNS minimal PrPSc deposits were observed in a 9-month-old animal (Table 1) in both the dorsal and intermedio lateral column of the thoracic medulla and in the nucleus parasympathicus of the vagal nerve. As previously observed in the Langlade flock (Andréoletti et al., 2000), clinical onset in the naturally exposed sheep occurred between the age of 20 and 21 months and the incubation period in the terminally affected group (n=4) was 24±2 months.

![Fig. 1.](http://vir.sgmjournals.org) PrPSc levels were measured using TeSeE sheep and goats ELISA PrPSc detection kit (Bio-Rad) in tissues collected at different time points of incubation in sheep naturally exposed (○) or orally inoculated (△) with classical scrapie. A dilution series of recombinant ovine VRQ PrP protein was used as an external standard. (a) Ileum Peyer’s patch, (b) ileal mesenteric lymph node, (c) obex and (d) thoracic eighth spinal cord segment.
Strikingly, in orally challenged animals, PrPSc accumulation was observed in the ileal ENS at 3 months post-infection, and at 6 months consistent PrPSc deposits were detected in the obex and in several spinal cord segments (Table 1). In the spinal cord, intermedio lateral column neurons were the first structure to accumulate PrPSc. Brains from all sheep older than 3 months were cut in 2 cm thick slices and each slice systemically processed for PrPSc immunohistochemistry detection. In both orally challenged and naturally exposed animals, the PrPSc deposition pattern was similar.

Finally, clinical onset in the challenged group was observed when the animals were 12 months old and the incubation period in terminally affected animals (n=8) was 14 ± 0.5 months. PrPSc immunometric assays indicated that abnormal PrP accumulation levels in the different investigated CNS areas were similar in the terminally affected animals belonging to both inoculated and naturally exposed groups (Fig. 1c, d).

Some terminally affected animals from the natural infection (n=2) and the orally challenged groups (n=2) were randomly selected. Obex homogenates (10%) were prepared and each inoculated by the intracerebral route into six transgenic mice that overexpress the ovine VRQ prepared and each inoculated by the intracerebral route. Sheeps were similar. Consequently, the acceleration of the Bioassay results demonstrated that TSE agents which in some naturally contaminated ARQ/VRQ or ARQ/ARQ sheep, classical scrapie occurrence was observed in the absence of detectable PrPSc accumulation in lymphoid tissues (Jeffrey et al., 2002; Ligos et al., 2006). TSE dissemination pathways in organisms of those sheep remain unexplained. The disjunction of PrPSc deposition kinetics that we observed in our experiment between ENS and lymphoid organs could suggest that ENS contamination might occur independently from lymphoid replication. This contention is also supported by experiments carried out in SCID mice, which are devoid of functional lymphoid organs. In the absence of possible lymphoid replication, intra-peritoneal scrapie challenge of these mice resulted in disease transmission with a reduced attack rate (Lasmezas et al., 1996).

In small ruminants, TSE oral inoculation is usually performed by administrating either one or two massive infectious doses (commonly 5 g of brain material) (Andrèoletti et al., 2004; Bellworthy et al., 2005b; Foster et al., 2001b). Under natural exposure conditions, contamination with classical scrapie mainly occurs around birth (Detwiler & Baylis, 2003). Numerous potential sources of contamination, like fetal annexes (Andrèoletti et al., 2002b), colostrum, milk (Konold et al., 2008; Lacroux et al., 2008) or even the environment were identified. The precise modalities of the contamination itself remain unknown, even if animals are more likely to be exposed to repeated low infectious loads rather than to a single massive dose. The massive exposure to infectious agent resulting from the experimental oral challenge could represent a plausible explanation for the differences in PrPSc dissemination dynamics observed between both groups.

Bioassay results demonstrated that TSE agents which developed in both naturally exposed and orally challenged sheep were similar. Consequently, the acceleration of the PrPSc neuro-invasion phase observed in the orally challenged animals cannot be attributed to the propagation of different TSE agents. Similarly, considering that no difference could be observed in the kinetics or dynamics of PrPSc accumulation in lymphoid tissues between both groups, lympho-invasion was unlikely to explain the differences observed between orally challenged and naturally exposed animals.

The intestinal autonomic nervous system was described in several natural (Hoffmann et al., 2007; van Keulen et al., 1999) or experimental TSE models (Maignien et al., 1999), to be the entry route of prion in brain and spinal cord. As previously described, in both our experimental groups, PrPSc deposition in CNS initially occurred in the structures where neuronal bodies controlling the autonomic nervous system are located. Moreover, the sequence of PrPSc distribution in brain areas was identical in orally challenged and naturally exposed animals. These observations indicated that the different kinetics of central neuro-invasion observed in orally challenged animals did not result from different CNS entry routes, but were a result of an early and/or faster dissemination of PrPSc in the ENS of challenged animals.

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certainly provide crucial elements for deciphering the pathogenesis of these diseases.

It is impossible to assume that the observations we reported here about the impact of oral challenge on classical scrapie pathogenesis can be extended to other TSE agents or other species. However, the discrepancies we observed between naturally exposed and orally inoculated animals should prompt us to use the data produced in these experimental conditions with some caution.

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References


