Behavioural study of sheep infected by bovine spongiform encephalopathy

F. CALATAYUD1, M.L. MAUBLANC**, E. COL1, F. LANTIER2, P. BERNARDET3, P. BERTHON2, I. LANTIER2, C. BARC1, P. SARRADIN1, O. ANDRÉOLETTI1, E. BIDEAU1

1CEFS – INRA B.P. 52627 F31326 Castanet-Tolosan Cedex FRANCE.
2Unité Infectiologie animale et santé publique – INRA F37380 Nouzilly FRANCE.
3Unité expérimentale Plateforme d'infectiologie expérimentale – INRA F37380 Nouzilly FRANCE.
*Corresponding author: maublanc@toulouse.inra.fr

SUMMARY

We describe the behaviour of sheep inoculated with brain tissue from BSE infected cattle, to search for early behavioural signs of BSE in sheep. Two groups of 11 ARR homozygous and 9 ARQ homozygous sheep were compared. Animals were filmed from the 11th to the 17th month post-inoculation using video surveillance cameras. We analysed the duration of resting, standing, feeding, walking and scraping activities, and the frequency of agonistic behaviour. In addition, head up and head down postures were distinguished. Correspondence factor analysis showed the divergence between behavioural profiles of ARR and ARQ sheep. Hierarchical cluster analysis based on the coordinates of the plots in multivariate space separated the two groups, and distinguished observations made at the beginning of the survey from those made later. An interactive decision tree based on the observations made during the three last months showed that animals of the two groups could be distinguished considering scraping activity, before the standing head up posture and feeding activity, but this analysis was affected by a relatively high misclassification rate (13%). The present study failed to find early behavioural signs of BSE in sheep. Nevertheless, our results do confirm the importance of scraping activity in BSE infected sheep. Moreover, our study failed to show clinical signs which would allow to distinguish BSE from scrapie in sheep.

Keywords: Bovine spongiform encephalopathy, sheep, behaviour, clinical signs.

RÉSUMÉ

L'étude décrit l'évolution des comportements de moutons après inoculation intra-cérébrale d'extraits de cerveau de vache atteinte d'ESB. L'objectif était de rechercher des signes comportementaux précoces de l'ESB, qui permettent de diagnostiquer la maladie en élevage. Nous avons inoculé 11 moutons homozygotes ARR et 9 moutons homozygotes ARQ. Nous avons filmé les animaux à l'aide de caméras de vidéo-surveillance, du 11ème au 17ème mois après inoculation. Nous avons analysé les durées des repos, de l'alimentation, des déplacements, de la station debout et des grattages, et les fréquences des comportements agonistiques. Nous avons en outre distingué deux postures de tête (haute ou basse). L'analyse factorielle des correspondances montre la divergence des profils comportementaux des animaux ARR et ARQ. La classification hiérarchique ascendante réalisée sur les coordonnées des points dans l'espace factoriel sépare les deux groupes d'animals et distingue, chez les ARQ, les observations réalisées au début du suivi des suivantes. L'arbre de décision interactif réalisé sur les observations des trois derniers mois montre que le principal facteur permettant de distinguer les animaux des deux groupes est le grattage, suivi de la station debout tête haute, puis de l'alimentation. Le taux d'erreur dans l'activation des animaux est cependant significatif (13 %). Cette étude n'a pas mis en évidence de signes comportementaux précoces permettant de déterminer l'ESB chez le mouton. Cependant, elle confirme l'importance du grattage comme signe clinique. De plus, elle n'a pas permis de mettre en évidence de signes cliniques spécifiques qui permettraient de distinguer l'ESB de la tremblante chez le mouton.

Mots clés : Encéphalopathie spongiforme bovine, mouton, comportement, signes cliniques.

Introduction

Transmissible spongiform encephalopathies (TSE) or prion diseases are a group of fatal neurodegenerative disorders that affect humans and animals. Human TSEs include Creutzfeldt-Jakob, Gerstmann Strausssler Scheinker syndrome, fatal familial insomnia and kuru [12, 15]. Animal TSEs include small ruminant scrapie [14], bovine spongiform encephalopathy and chronic wasting disease of deer [22, 33-35]. TSEs are characterised by the conversion of the host-encoded prion protein (PrPc) into a misfolded, partly protease-resistant form (PrPres), which accumulates in tissues of the nervous and lymphoreticular systems of the infected animals, causing neuronal loss and lesions in the brain [12]. Examinations of sections of infected animals' brains reveal intraneuronal vacuulation, neuronal degeneration and microcaviation of the grey matter. These lesions expand during the incubation period, inducing neuropathological changes and altered behaviour [11, 19, 23].

In 1988, BSE was experimentally transmitted to sheep and goats by intracerebral and oral inoculation [19]. This study confirmed that the transmission of BSE was modulated by the PrP genotype [16]: all the sheep which succumbed to BSE during the observation period were of genotypes known to be scrapie-susceptible.

Faced with the fear that the BSE agent may have infected sheep, and then humans, it appeared important to distinguish scrapie from BSE-infected sheep [7, 10, 25]. Some data suggest that BSE in sheep can be distinguished from most natural scrapie strains by the size of the unglycosylated PrPres [6] and by the lesion profile [32]. We hypothesise that these molecular and lesion differences could induce diffe-
rences in incubation period and in sheep behaviour, as it has been shown in mice infected with BSE and different strains of scrapie [13].

FOSTER et al. [19] actually found that sheep infected by BSE had extremely short clinical courses lasting between one and five days, with no obvious difference between oral or intracerebral inoculation. But BARON et al. [6] and more recently, KONOLD et al. [27] found a progression of the disease over a period of 14-20 weeks after oral or intra-peritoneal inoculation, which is not different from that of scrapie. At the same time, the information published on the clinical signs of sheep infected by BSE is somewhat contradictory: FOSTER et al. [19] described a rapid progression of ataxia resulting in recumbency, rarely pruritus and a loss of weight. By contrast, BARON et al. [6] noted an intense pruritus and ataxia. Recently, KONOLD et al. [27] also found pruritus in 87% of the BSE-positive sheep, and a repeatable stereotypical response (nibbling or head/body movements) elicited by scratching, in 85% of them. As proposed by KONOLD et al. [27], the methods by which clinical signs were assessed or recorded may explain the differences observed. They suggested that continuous video surveillance would be a good method to allow sheep to behave in a ‘normal way’, without the disturbance caused by the presence of an observer.

In the present study, we used video surveillance to compare the behaviour of ARQ homozygous and ARR homozygous infected sheep over a period of seven months, taking the opportunity of an experiment raised in a BSL 3 animal facility and designed to investigate the BSE susceptibility of scrapie resistant sheep with the ARR/ARR PRNP genotype that is selected throughout Europe as a way to control scrapie epidemics. Recent findings actually show that ARR homozygous sheep can develop classical scrapie [21] and become infected when inoculated intracerebrally [24] or orally exposed [3] to BSE agent, but a low transmission rate of BSE agent and a much longer incubation period are found in animals bearing this genotype. We aimed to study the dynamics of the behavioural differentiation between ARQ (highly susceptible) and ARR (used as controls) sheep, from the start to the end of the disease. Another objective was to search for early signs of the disease that could help farmers and veterinarians to diagnose it as early and as easily as possible. Early diagnosis of TSE in live animals continues to be a challenge, even though it is possible to detect PrPres in accessible peripheral lymphoid tissue (tonsil, 3rd eyelid, rectum) in live animals [17, 20], a technique restricted to scrapie susceptible sheep genotypes and TSE strains that does disseminate in lymphoid tissues.

Materials and Methods

Twenty sheep (Polled Dorset breed, 14 months old) kindly provided by Hugh Simmons (VLA, Defra, UK) of New Zealand origin (a country officially free of classical scrapie) were intracerebrally inoculated with cattle BSE infected brain homogenate on 23rd July 2001. Eleven of the sheep were ARR homozygous and nine were ARQ homozygous. Three ARR homozygous and all the ARQ homozygous sheep were castrated males; the eight remaining animals were ARR homozygous females.

In order to evaluate their susceptibility to BSE, sheep were inoculated (under anaesthesia) through the intracerebral route (right lateral median cortex) with 0.5 ml of a 10% brain homogenate (50 mg of brain equivalent) from French BSE case n° 139, a case officially confirmed by the AFSSA veterinary reference laboratory in Lyon. This isolate has been further inoculated into various mouse lines either transgenic for the sheep or bovine PRNP gene [18] or reference inbred mouse lines for strain typing [29-30]. It presents all the features of a classical BSE strain: Western blot profile, susceptibility to proteinase K, mouse susceptibility pattern, lesion profile in reference RIII mice. These BSE characteristics have been conserved after 3 passages in sheep as well as in mice, suggesting this case was not infected by any other TSE agent [29-30].

All animals were kept under strict biosecurity measures in the same paddock (12m x 3m) and bedded on gratings. Water was available ad libitum via automatic drinkers. The sheep were fed twice a day, at 9:00 in the morning and at 16:00 in the afternoon, using pellet with long fibres strictly from cultivated grass mixed with cereals. They were filmed all day long once a week for seven months, from June to December 2002 (month 11 to 17 after inoculation), using three cameras placed at three different corners of the paddock. We chose to begin the study during the second part of the incubation period, which corresponds to the development of the TSEs’ pathological process (neuroinvasion) in the central nervous system [2], in search of the very early signs of the disease. The back of each sheep was marked with a red number to allow individual identification. The behaviour of each sheep in the group was noted at five-minute intervals for 12-hour observation periods, using the instantaneous scan sampling method [1]. We noted the activity of each animal (feeding, drinking, walking, observing the environment (vigilance), scratching or resting), the posture when it was standing or resting (head up or down) and the occurrence of agonistic behaviour:

- Feeding eating food at the manger or drinking water at the drinking trough
- Walking moving
- Observe standing head up
- Scrapping scratching itself with its teeth, hoofs, or a support (manger, drinking trough, salt stone)
- Resting laying head up
- Head-down (near the floor) when standing or moving
- Resting head down (head at the floor or at the flank)
- Agonistic behaviour pushing another sheep with head or leg, mounting another sheep, head to head interaction

ARQ homozygous sheep were culled when they showed the ultimate signs of the disease (14 to 17 months post-inoculation, 477days ± 30). These presented some individual variations. Severe wasting, eating, moving or waking up difficulties, impossibility to resist the aggressive behaviour...
from other sheep were criteria that determined euthanasia. All these scrapie susceptible sheep were found positive for BSE by the three classical techniques of diagnostic: ELISA, Western blot and immunohistochemistry as described by ANDREOLETTI et al. [3]. Among the ARR homozygous animals, one had an accident and was euthanized at 27 month after inoculation and was found PrPBSE negative, seven finally showed signs of the disease and were culled 46 months (12383 days ± 89) post-inoculation (they were all found positive for BSE), the three others being culled 52 months post-inoculation. Among these 3 sheep that remained negative for PrPBSE, only one was found positive through bioassay [29-30], as its oxeb transmitted the infection in Tg110 mice [18], transgenic for the bovine PRNP gene.

According to ALTMANN [1], behaviour patterns do not occur independently but rather form sequences with definite temporal structures. These structures may build up different ‘contexts’ characterised by the preferential association of different behavioural items. In order to study the behavioural context in which each behavioural variable was expressed, we employed multivariate analysis, choosing the day as the basic temporal unit. The table crossed the individual/day observations in rows with the frequency of each activity in columns and all these values were summed per month in order to evaluate the evolution of behavioural profiles. As raw data did not always display a normal distribution, we could not use principal component analysis. We separated the values of each variable into two modes: agonistic behaviour, scraping and resting head down were noted absent (0) or present (1); the values for feeding, walking, resting and observe were below the median (‘less’, noted 1) or above the median (‘more’, noted 2). The posture ‘head down’ was split into 3 modes (‘absence’, noted 0, below the median, noted 1 and above the median (noted 2). This approach is very useful for creating modes having similar sample sizes and not biasing the multivariate analysis [31]. Relationships between the variable modes were analysed using a Correspondence Factor Analysis [31]. This analysis rates the relationships between the different variable modes according to a $\chi^2$ criterion.

As a great variability was observed in our data, and as advised by LEBART et al. [31], the final interpretation was performed in Hierarchical Cluster Analysis based on the coordinates of the plots in multivariate space. This analysis allows a synthetic view of the results by creating a behavioural profile based on statistical relationships between variables, and summarises all the factorial plans of the multivariate analysis (all the variation appears in one graph). The different classes separated by the cluster analysis were characterised by independent variable modes (gender or genotype). The hypergeometric probability of such an extreme sample was computed (default value fixed at 5 individual-periods). The final predictive validity of the model is then evaluated by a misclassification rate that represents the probability of confusing the two groups (see BREIMAN et al. [9] for more details on this method). In order to assess the sensitivity of the distinction between ARR and ARQ sheep and the suitability of using behavioural measures to discriminate them, we performed two discriminant analyses based respectively on the three first and last months of the observation session, firstly from June to August, and secondly when illness was presumably well-declared from October to December (N=107 and 129, respectively).

Finally, we tested the differences between the relative frequencies of the main behaviours in ARR and ARQ sheep, using Mann-Whitney tests for inter-group comparisons and Friedman tests for intra-group comparisons.

Results

CORRESPONDENCE AND HIERARCHICAL CLUSTER ANALYSES

The F1xF2 plan of the factorial analysis explained 36.38% of the total variance of the dot plot (Figure 1). ARQ sheep presented a behavioural trajectory that clearly diverged from that of the ARR sheep during the observation period. Indeed, after July, ARQ sheep seemed to be less active, less reactive and presented greater scraping activity.

A six-class partition of the hierarchical analysis revealed a clear segregation of ARQ individuals as they became ill (Figure 2). It also revealed a greater heterogeneity in ARR than in ARQ sheep, as the main part of the latest were found in the sixth class (individuals observed in August, September and November) while ARR sheep were distributed in the first four classes ($P<0.001$ in all cases).

ARR individuals observed in July ($P<0.001$) characterised the first class: 31.82% of the ARR individuals observed in July appeared in this class and they represented 14.89% of the class. These individuals were never in a head-down posture ($P<0.0001$), rested more than others ($P<0.0001$), were more vigilant ($P<0.005$) and less frequently observed feeding ($P<0.0001$) than others and were sometimes engaged in agonistic behaviour ($P<0.001$).

ARR individuals observed in October, November and December characterised the second class ($P<0.01$): 34.09% of the ARR individuals observed in October appeared in this class and they represented 19.23% of the class (respectively
FIGURE 1: Results of the Correspondence Factor Analysis of the matrix of the 453 ‘individual-periods’ with the 17 behavioural modes. The arithmetic means of the distribution of sheep on the factorial plane spanned by the first two axes, according to their genotype and to increasing months, are represented by their genotypes (ARQ) or (ARR) followed by the three first letters of the corresponding month (June-December). The sizes of the symbols affected to the behavioural modes are proportional to their contribution to the definition of the two axes.

FIGURE 2: Six-class partition of the Hierarchical Cluster Analysis based on the factorial coordinates of the 453 ‘individual-periods’ and the 17 behavioural modes in the multivariate space. The probabilities of the sensitivity of the characterisation of the classes by the ‘individual-periods’ and modes are given in brackets.

39.39% and 33.33% for the ARR individuals observed in November and 50.00% and 14.10% for those observed in December). These individuals were never in a head-down posture ($P<0.0001$), never lay head down ($P<0.01$), never scraped ($P<0.0001$), rested less than others ($P<0.0001$) and were more frequently observed feeding ($P<0.0001$).

The third class was characterised by ARR individuals observed in November ($P<0.01$): 28.79% of the ARR individuals observed in November appeared in this class and they represented 25.00% of the class. These individuals were frequently in a head-down posture ($P<0.0001$), never scraped ($P<0.0001$), rested less than others ($P<0.0001$) and were more frequently observed feeding ($P<0.0001$).

ARR individuals observed in October and November characterised the fourth class ($P<0.01$): 38.64% of the ARR individuals observed in October appeared in this class and they represented 22.37% of the class (respectively 28.79% and 25.00% for the ARR individuals observed in November). These individuals were occasionally in a head-down posture ($P<0.0001$), they never scraped ($P<0.0001$), rested less than others ($P<0.001$) and were more frequently observed feeding ($P<0.0001$).

The first four classes illustrate active and reactive individuals that never scraped, fed regularly and did not spend a large amount of their time resting (except for resting).

ARRQ individuals observed in July and September characterised the fifth class ($P<0.002$): 55.56% of the ARQ individuals observed in July appeared in this class and they represented 12.20% of the class (respectively 37.78% and 20.73% for the ARQ individuals observed in September). These individuals were occasionally in a head-down position ($P<0.0001$) and rested more than others ($P=0.0001$). They were less frequently observed feeding and they observed more than the others ($P<0.01$).
ARQ individuals observed in August, September and November characterised the sixth class ($P<0.0001$). 48.89% of the ARQ individuals observed in August appeared in this class and represented 23.40% of the class (respectively 42.22% and 20.21% for the ARQ individuals observed in September and 61.29% and 20.21% for those observed in November). These individuals were frequently in a head-down position and scraped ($P<0.0001$); they rested more than the others and often with the head down ($P=0.0001$). They were less frequently observed feeding or walking and observed less than the others ($P<0.0001$). These individuals were the least active and reactive of all the observed animals.

**INTERACTIVE DECISION TREES**

The first decision tree appeared quite ramified and complete discrimination required numerous behavioural variables (Figure 3a). The first variables that best discriminated ARR and ARQ individuals were the feeding and the scraping activity (the ARR individuals fed more and scraped less than the ARQ ones). Nearly all the other behavioural variables were necessary to reach a complete discrimination. The high misclassification rate (0.36) illustrated that the discrimination efficiency of the model was very poor and suggested that early behavioural differences should not be used to diagnose illness.

The second decision tree, based on data obtained at the end of the experiment, was rather compact compared with the first, and a few variables were sufficient to distinguish ARQ from ARR animals (Figure 3b). The combination of resting and scraping activity permitted quite a good discrimination. The time spent observing and the head down posture also contributed to clarifying discrimination. However, we must underline the relatively high misclassification rate (0.13). Hence, although the probability of being wrong during the discrimination was far lower than previously, it remained quite high and suggested a careful use of the model.

**BIVARIATE ANALYSES**

Whereas HCA revealed general behavioural profiles, bivariate graphs showed the dynamics of illness month after month, and confirmed both previous analyses (Figures 4a,b,c,d,e,f). Friedman tests detected a very significant increase of the head down posture and of scraping activity in ARQ individuals ($P<0.0001$ and respectively $F=40.12$ and $F=31.79$). From August for scraping activity and from September for head down posture, ARQ sheep always presented significantly higher scores than ARR ones ($U=880$ and $P<0.01$; $U=526$, 145.5, 102, 0 and $P<0.0001$ for scraping behaviour from August to December; $U=794$, 220, 365.5, 0.5 and $P<0.0001$ for head down posture from September to

![Figure 3a](image1)

**Figure 3a:** Interactive Decision Tree based on the observations made from June to August. White bars: ARR sheep; grey bars: ARQ sheep. Numbers of individual-periods are indicated beside the corresponding boxes.

![Figure 3b](image2)

**Figure 3b:** Interactive Decision Tree based on the observations made from October to December. White bars: ARR sheep; grey bars: ARQ sheep. Numbers of individual-periods are indicated beside the corresponding boxes.
December). Conversely, ARR sheep presented an increase in the time spent feeding ($F = 47.29$ and $P<0.0001$). Moreover, behavioural scores for this variable were always significantly higher for ARR than for ARQ individuals ($U = 85.5$ and $P<0.005$ in June; $U = 284$ and $P<0.05$ in July; $U = 1747$, 2111, 924, 1872, 132 and $P<0.0001$ from August to December). Both ARR and ARQ individuals presented a decrease in the time spent resting ($F = 54.43$ and $P<0.0001$ and $F = 17.43$ and $P<0.01$, respectively), but this was more pronounced in ARR sheep. So, the behavioural scores for this variable were significantly higher for ARQ than for ARR individuals from August to December ($U = 702$, 378, 224.5, 67 and 7.5 and $P<0.0002$). The last two behavioural variables presented less significant results. Indeed, Friedman analyses
did no detect any dynamics in ARR nor ARQ sheep for the observing activity, and only a weak one in ARR sheep for the walking activity \( (F = 15.12 \text{ and } P<0.05) \). These sheep had significantly higher scores than ARQ ones for observing activity in September, November and December \( (U=1691, 1678 \text{ and } P<0.0001 \text{ and } U=105.5 \text{ and } P<0.05, \text{ respectively}) \) and for walking activity in September and November \( (U=1531 \text{ and } P<0.05 \text{ and } U=1575 \text{ and } P<0.0001, \text{ respectively}) \).

**Discussion**

The aim of this study was to describe the appearance and the evolution of behavioural modifications in ARQ sheep inoculated with BSE, and to question whether early behavioural symptoms could be proposed to facilitate illness detection. Most studies on scrapie and BSE briefly described the clinical signs of the disease \[6, 19, 36-37\]. HEALY *et al.* \[23\] quantified the behaviours of healthy and scrapie affected sheep, but their short-term study did not describe the dynamics of altered behaviour. CAPPUCHIO *et al.* \[11\] reported the clinical signs of scrapie in sheep and goats, setting apart ‘early’ and ‘mature and late’ clinical stages. Clinical signs of scrapie vary according to the infected species and to the strain of scrapie. Three main types have been described in sheep: a pruritic form, a paralytic form without pruritic behaviour, and an atypical form, which usually includes signs of cerebellar disease \[8, 26\]. The main clinical signs observed for example by CAPPUCHIO *et al.* \[11\] in scrapie infected sheep were pruritus, ataxia, hyperaesthesia and fearfulness/ anxiety. Concerning BSE, KONOLD *et al.* \[27\] recently compared BSE-positive and BSE-negative sheep in terms of the percentages of animals performing target activities such as scratching, nibbling or teeth grinding, and showing weight loss or abnormalities in fleece or movement (tremor, ataxia). The authors showed the progression of these clinical signs over a period of 20 weeks prior to being culled because of the disease. The present study is the first to quantify behaviour of undisturbed sheep during the 26-week progression of the disease.

The correspondence analysis revealed a rapid separation of ARQ and ARR animals, suggesting that both groups began to differentiate (ARQ animals becoming ill) just before or just after observations took place. However, most of the ARQ sheep observed in June \( (5/9) \) appeared in the first four classes of the hierarchical cluster analysis, highly characterised by ARR individuals, while some ARR sheep \( (3/11) \) appeared in the fifth class that was characterised by ARQ individuals and potential early behavioural symptoms. We found no early difference between the two groups for the main behaviours, except for feeding activity. These results show that the two groups still behaved in a similar way at the beginning of our observations, although they were already engaged in different behavioural trajectories.

The hierarchical cluster analysis revealed two different behavioural profiles of ARQ individuals with a temporal coherence. At the beginning of the observations, from June to September, these individuals globally presented more head down posture, and a smaller activity than ARR ones, which has been shown in scrapie infected goats \[11\], and reminds of dullness or drowsiness already reported in sheep and goats infected with the BSE agent \[27-28\]. Immediately after, from August to November, illness clearly broke out and classical symptoms such as scraping and a decrease of feeding activity appeared. The small variability in ARQ sheep should be noticed, distinguishing only two groups of ARQ individuals: those that just became ill and those that were already obviously ill. It seems that the illness acts as a normalising agent, decreasing the natural interindividual variability that characterises ARR (healthy) animals. Different behavioural profiles actually appeared in these sheep, without any temporal coherence, since different months characterised several classes (e.g. ARR individuals observed in November characterised classes two, three and four). It is difficult to interpret this variability in ARR animals, because some of them showed signs of the disease much later and could have displayed early behavioural signs during the observation period. The use of undosed sheep as controls would have been more appropriate, but was not possible, because of the main objective of the experiment and the exiguity of the BSL 3 animal facility. Nevertheless, inter individual variability is the standard in healthy animals, especially when males and females are pooled, as it was the case in our study.

In search of early behavioural symptoms, we performed two different discriminant procedures, one in the three first months of the study and the other in the three last months. We found different combinations of behavioural variables allowing differentiation between ARR and ARQ individuals in the earlier period of observation. However, the greatest caution should be advised with these results considering the high misclassification rate that illustrates the risk of declaring false negatives. This is consistent with the results of the correspondence and the cluster analyses, showing only minor differences between ARR and ARQ animals at the beginning of the study, i.e. before the end of the incubation period. As expected, the discrimination was far better when considering the last three months of observation, and combinations of more classical behavioural variables were able to differentiate ARR (healthy) from ARQ (ill) sheep. This was associated with a lower misclassification rate. Individuals that scraped themselves and rested more than the others were generally ill. This combination of variables was that which produced the best discrimination between ARR and ARQ individuals. However, other behavioural variables must be used to complete the discrimination. Particularly the head-down posture seemed to be quite interesting as it showed a huge increase along months as presented in the bivariate analysis.

The clinical signs observed in this study were similar to those described by other authors, confirming the similarity between the clinical signs of scrapie and of BSE. The progressive increase of resting behaviour (and decrease in walking behaviour) observed here agrees with the progression of ataxia leading to recumbency, observed by FOSTER *et al.* \[19\] and KONOLD *et al.* \[27\] in BSE infected sheep, or by CAPPUCHIO *et al.* \[11\] and HEALY *et al.* \[23\] in scrapie affected sheep. On the contrary, FOSTER *et al.* \[19\] observed pruritus in only one animal out of 19, whereas we observed scraping behaviour in all ARQ animals. Our results are consistent with those of KONOLD *et al.* \[27\], who observed
pruritus in 87% BSE positive sheep. In scrapie affected sheep too, CAPUCCIO et al. [11] frequently observed pruritus. These authors also observed tremors, visual impairment, fearfulness and hyperaesthesia, which were not detectable in our study conditions. The decrease of feeding activity that we observed was also found by HEALY et al. [23] in scrapie-affected sheep, and agrees with the loss of weight that is reported in other studies. This weight loss could also be link to rumination disturbances. In fact, in TSE affected sheep or in wild ungulates affected by Chronic Wasting Disease, rumination disturbances have been described [5, 23, 34]. We were unable to study this behaviour here because of the low definition of the video sequences, but there is no doubt that it would be interesting to focus on rumination in further experiments.

In conclusion, this study provides an accurate description of the behavioural dynamics of BSE-infected sheep from the start to the end of the illness. However, the perspective of using behavioural symptoms to detect the onset of the disease seems to be reconsidered, since the probability of misinterpretation was very high in the early stage, although these animals were maintained very healthy at the BSL3 level of bio-safety with no parasite or any other interfering pathogen. The illness seems to modify animal behaviour quite late, at least with regard to the limited behaviours evaluated. These results are consistent with those of KONOLD et al. [27], who found a rapid increase of the percentages of animals showing clinical signs, eight (for scraping) or four (for ataxia and weight loss) weeks prior to cull. Finally, the present study failed to show clinical signs which would allow to distinguish BSE from scrapie in sheep.

References


