



REPORT FROM THE SEAC SHEEP SUBGROUP

ISSUE

1. To present SEAC with a report from the SEAC Sheep Subgroup.

BACKGROUND

2. The SEAC Sheep Subgroup met on the 4th October 2007 to address a request from the Department for Environment, Food & Rural Affairs for advice on the following issues:
 - the origins of atypical scrapie in a sheep flock established and managed to minimise the risk of infection by transmissible spongiform encephalopathies (TSEs) and the implications for research using animals from the flock.
 - the interpretation of findings from experiments to characterise the strain of infection in two historic sheep TSE cases and the implications for current understanding of the possible presence of bovine spongiform encephalopathy (BSE) in sheep.
 - the implications of early data from a study of bottle feeding milk from classical scrapie infected ewes to TSE-free lambs.
 - the implications of cases of classical scrapie in ARR/ARR sheep for the scientific basis of the National Scrapie Plan (NSP).
3. The Subgroup report is at Annex 1. The membership of the SEAC Sheep Subgroup is at Annex 2.

ADVICE SOUGHT FROM THE COMMITTEE

4. The committee is invited to comment on the report.



SEAC SHEEP SUBGROUP REPORT OF 2007 MEETING

Issue

1. The SEAC Sheep Subgroup was asked by the Department for Environment, Food & Rural Affairs (Defra) for advice on:
 - the origins of atypical scrapie in a sheep flock established and managed to minimise the risk of infection by transmissible spongiform encephalopathies (TSEs) and the implications for research using animals from the flock.
 - the interpretation of findings from experiments to characterise the strain of infection in two historic sheep TSE cases and the implications for current understanding of the possible presence of bovine spongiform encephalopathy (BSE) in sheep.
 - the implications of early data from a study of bottle feeding milk from classical scrapie infected ewes to TSE-free lambs.
 - the implications of cases of classical scrapie in ARR/ARR sheep for the scientific basis of the National Scrapie Plan (NSP).
2. The Subgroup met on the 4th October 2007 to consider all the relevant information available, much of which is unpublished. This report is a consensus opinion of the Subgroup to address the request for advice.

Atypical scrapie in a sheep flock previously considered TSE-free

Background

3. A sheep flock at the Veterinary Laboratories Agency (VLA) Arthur Rickwood Sheep Unit (ARSU) was established in 1997 to provide a source of animals free of TSE infections for research projects. The founder animals from the flock were imported from New Zealand, a country considered free of TSEs. Since the flock was established, it has been managed to minimise the risk of the flock becoming infected with TSEs.

4. In June 2006, a sheep in the flock developed clinical disease that was subsequently confirmed to be due to atypical scrapie post mortem. The animal was born in 2000 to a surrogate dam imported from New Zealand in 1998. The natural parents were also part of this importation. A second case of atypical scrapie associated with the flock has since been identified. This animal was born in 1999 and imported from New Zealand in 2001. In January 2007, it was used as a surrogate dam for the export of embryos to Italy. It was culled after lambing in June 2007 showing no clinical signs of infection. A diagnosis of atypical scrapie infection was made based on TSE testing of post mortem samples in Italy and the UK.
5. Investigations have been conducted to allow an assessment of the possible origins of these cases. The biosecurity measures in place and sample handling and identification procedures used were independently audited by United Kingdom Accreditation Service¹. Comprehensive epidemiological assessments to examine routes of infection that could have given rise to the cases were conducted by VLA^{2,3}.
6. In addition, TSE testing of all the relevant samples that had been retained from sheep imported into, or produced from, the flock was conducted by VLA to determine whether other sheep associated with the flock may have been infected with atypical scrapie⁴.

Scale of infection

7. The results from testing of retained tissues from animals from the flock, suggests that a large-scale outbreak of atypical scrapie infections in the ARSU sheep flock has not occurred.
8. The life histories of both cases show they were not related and that there may have been some limited contact between them during their lives. Thus, although transmission between the sheep is possible, the cases may represent independent occurrences.

Origins of infection

9. There are four hypotheses for the origins of the atypical scrapie infections:

¹ United Kingdom Accreditation Service. (2007) Unexpected atypical scrapie case: audit of sample handling and biosecurity.

http://defraweb/animalh/bse/othertses/scrapie/nsp/pdf/assessment_report.pdf

² VLA (2007) Atypical scrapie Arthur Rickwood Sheep Unit (ARSU) epidemiology report.

³ VLA (2007) Report on the Grass nut production for ARSU.

⁴ Paper to SEAC 97/2 Annex 1. <http://www.seac.gov.uk/papers/97-2.pdf>

- the ARSU environment is contaminated with the atypical scrapie agent.
 - a breach in biosecurity led to entry of atypical scrapie to ARSU.
 - the founder animals imported from New Zealand were infected with atypical scrapie.
 - the atypical scrapie cases arose spontaneously.
10. The investigations undertaken suggest that, although all these hypotheses are unlikely, none can be excluded. As no livestock grazed on the ARSU site in the 50 years prior to establishment of the Unit, the likelihood that the land was sufficiently contaminated with the atypical scrapie agent to give rise to infections appears low.
11. There are comprehensive and robust biosecurity measures taken to minimise the possibility of transfer of any TSE agent into the Unit. The low density of the sheep population in the area around ARSU, together with the lack of reported atypical scrapie cases from surveillance over recent years, suggests that the risk of transfer of the atypical scrapie agent from the surrounding environment is low. In addition, the risk of exposure of the ARSU sheep to atypical scrapie via contaminated feed is minimised by careful sourcing of feed ingredients.
12. Although New Zealand is classified as a country free of TSEs, the surveillance programme in place there was not, until very recently, capable of detecting atypical scrapie. Thus, the possibility that a small number of the founder sheep for the flock imported from New Zealand may have been infected with atypical scrapie cannot be ruled out. Surveillance that includes testing a very large number of sheep using tests capable of detecting atypical scrapie would inform an assessment of the prevalence of atypical scrapie, if it is present in that country.
13. It is possible that atypical scrapie arose spontaneously in one or both sheep. The nature of the infectious agent, a misfolded protein, allows for this possibility through misfolding of the endogenous protein. Unpublished epidemiological analyses suggest that the prevalence of atypical scrapie is relatively uniform across Europe, the prevalence of atypical scrapie is not rapidly increasing and clusters of atypical scrapie infections have not been found. In addition, unpublished research using transgenic mice expressing an ovine form of the prion protein gene suggests that atypical scrapie may not be readily transmissible between sheep

by natural routes of infection. Taken together, all these data are consistent with, but do not prove, the hypothesis that atypical scrapie may arise spontaneously.

Implications for research

14. As atypical scrapie has been, and may still be, present in the ARSU flock, at least at a low level, it is important that analysis of results from research using sheep from the Unit be conducted in the knowledge that the animals could potentially be infected with atypical scrapie. However, tests to discriminate between atypical scrapie and other sheep TSEs can be applied retrospectively to verify whether atypical scrapie is present in sheep from the Unit that have been used in research studies. Such examinations cannot, however, rule out the possibility that disease has arisen spontaneously in such sheep, or, in some circumstances arisen due to infection after departure from the breeding flock.
15. Recognising that the flock provides an important source of animals for research, it is recommended that consideration be given to further measures that could be introduced to minimise the risk of spread of atypical scrapie at ARSU.

Characterisation of the infection in historic sheep TSE cases

Background

16. TSE strains can be characterised and differentiated through intracerebral inoculation and serial sub-passage in different strains of inbred mice (strain typing bioassays). Strains can be distinguished on the basis of characteristic incubation periods and the severity, form and distribution of lesions (lesion profiles) and the distribution of abnormal prion protein (PrP^{Sc}) in the brain. Normally on primary passage, the incubation period is relatively long and the number of animals that develop disease (the attack rate) is low. However, following secondary and subsequent passages, the incubation period normally shortens and the attack rate increases. A number of sub-passages are required before TSE agents adapt fully and the incubation period and lesion and PrP^{Sc} profiles stabilise.
17. VLA is using strain typing bioassays to characterise the properties of TSE infections in 204 historic sheep TSE cases originally diagnosed as classical scrapie. Isolates from the cases have been characterised through serial sub-passage in the C57Bl and VM strains of mice, which because of their differing prion protein

genotypes, modulate the infection phenotype, allowing differentiation of strains with similar properties. In addition, inoculations have been performed using the RIII strain of mouse as a distinctive phenotype is produced in this mouse strain on primary passage of BSE, thus indicating the presence of BSE. The data from this study are unpublished.

Data

18. None of the isolates inoculated have given rise to infections in RIII mice characteristic of BSE, suggesting that the clinically observed disease in these sheep was not BSE. However, two isolates when passaged in C57Bl and VM mice produced a PrP^{Sc} distribution pattern similar to mouse adapted BSE.
19. The attack rate from inoculations of one isolate into C57Bl and VM mice was low but all the transmissions produced a PrP^{Sc} distribution similar to mouse adapted BSE. On serial sub-passage, the incubation period, lesion profile and PrP^{Sc} distribution remained similar to mouse adapted BSE.
20. The attack rate in C57Bl and VM mice of the other isolate was higher, however only one VM mouse produced a PrP^{Sc} distribution pattern similar to mouse adapted BSE. Serial sub-passage of an isolate from this mouse gave an incubation period, lesion profile and PrP^{Sc} distribution similar to mouse adapted BSE. In all the other VM and C57Bl mice inoculated with the sheep isolate, the PrP^{Sc} distribution pattern was similar to classical scrapie passaged in mice.
21. Comparison of PrP^{Sc} profiles by western blotting of samples from the first and second passages of the two isolates and from well characterised TSE strains proved inconclusive and did not allow the TSE strains transmitted to the mice to be identified.
22. The two isolates were from an ARQ/ARQ Swaledale sheep born in 1992 with a diagnosis of classical scrapie in 1996 and an ARQ/ARQ Suffolk Cross sheep born in 1998 with a diagnosis of classical scrapie in 2000. The original diagnosis has been confirmed by application of tests that discriminate BSE, classical scrapie and atypical scrapie in sheep to the isolates.

Interpretation

23. There are a number of possible interpretations of the data for each of the two isolates:

- an experimental error occurred leading to accidental contamination of inocula.
 - the features observed on passage in mice are characteristic of classical scrapie infection in sheep of the genotype and/or breed of sheep from which the isolates were derived.
 - a conversion occurred of the classical scrapie strain that infected the sheep giving rise to a strain with features similar to BSE passaged in mice.
 - a mixed infection of BSE and classical scrapie was present in the sheep.
24. An internal VLA audit of the experimental procedures used provided no evidence to suggest that an error may have occurred that would give rise to these findings. Plans for an independent audit are welcomed.
25. The possibility that the features observed may be a normal consequence of passage of classical scrapie in sheep of these breeds and genotype may be informed by a retrospective investigation to determine whether cases of classical scrapie in sheep of the Swaledale or Suffolk Cross breeds and the ARQ/ARQ genotype have been studied by strain typing bioassay and comparison of these data with those from the VLA study. However, as such analysis of classical scrapie in sheep breeds and genotypes is incomplete, this comparison may not be possible.
26. The possibility that a TSE strain may be converted to a different strain is supported by experimental evidence that passage of an isolate assumed to be infected with a single TSE strain can give rise to strains with differing properties in mice^{5,6}. This phenomenon was most recently described in a study of secondary passage of L-type BSE in mice, which produced an infection phenotype identical to classical BSE⁷. The mechanisms by which conversion of a single TSE strain to two or more strains could occur are not understood.

⁵ For example Bruce & Dickinson (1987) Biological evidence that scrapie agent has an independent genome. *J. Gen. Virol.* 68, 79-89.

⁶ Lloyd SE *et al.* (2004) Characterization of two distinct prion strains derived from bovine spongiform encephalopathy transmissions to inbred mice. *J. Gen. Virol.* 85, 2471-2478.

⁷ Capobianco *et al.* (2007) Conversion of the BASE prion strain into the BSE strain: the origin of BSE? *PLoS Pathol.* 3, e31.

27. It is possible that the findings represent a mixed infection of BSE and classical scrapie. Although the original diagnosis of classical scrapie was confirmed using discriminatory tests, the biochemical properties on which these tests are based may limit the sensitivity of the tests to detect small amounts of BSE-derived PrP^{Sc} in the presence of larger amounts of classical scrapie derived PrP^{Sc}. A published study showed that only the PrP^{Sc} profile of mouse adapted classical scrapie could be observed by western blot in mice co-infected with equivalent doses of mouse adapted strains of classical scrapie and BSE of similar incubation periods⁸. The lack of BSE transmission on inoculation of the two sheep isolates to RIII mice suggests that, if BSE is present, it is likely to be a relatively minor component of the infection load in both isolates.
28. Data from transmissions of experimentally produced mixed infections do not closely resemble the findings from the strain typing experiments on the two sheep isolates, however the data are too few to allow definitive conclusions to be reached about the possibility of the presence of a mixed infection in these isolates. Preliminary unpublished data suggest that when mice are inoculated with a mixture of equivalent doses of BSE and classical scrapie inocula, classical scrapie is the predominant infection as determined by lesion profile and PrP^{Sc} distribution pattern⁹. Other unpublished experiments suggest that when mice are inoculated with a mixture of equivalent doses of BSE and classical scrapie, a new phenotype is observed with incubation periods in primary transmissions to RIII and C57Bl mice not differing significantly, unlike the characteristic ~100 day difference seen with BSE infection. Attack rates, however resemble those seen with BSE in that most mice developed disease, whereas few mice were affected with classical scrapie. This phenotype was also observed after infection of mice with a 1:7 mixture of BSE and classical scrapie inocula. Sub-passage from both mixtures (1:1 and 1:7) in mice did not produce features of BSE-like strains but instead resembled classical scrapie-like strains. Data on the properties of a mixed BSE and classical scrapie infection in sheep when then passaged in mice are limited to one unpublished experimental result from co-infection of ARQ/ARQ sheep with equivalent doses of BSE and classical scrapie. Passage of an isolate in mice appears to indicate that BSE had replicated in the donor sheep¹⁰.

⁸ Baron & Biacabe (2001) Molecular analysis of the abnormal prion protein during co-infection of mice by bovine spongiform encephalopathy and a scrapie agent. *J. Virol.* 75, 107-114.

⁹ Unpublished VLA studies.

¹⁰ Unpublished Roslin Institute studies.

Further research

29. Further transmission experiments of the original isolates from the two cases in parallel with previously characterised classical scrapie, ovine BSE and BSE isolates using wild-type and transgenic mice expressing the ovine, bovine and human forms of prion protein would provide additional data that may aid interpretation of the present findings. Comparisons of PrP^{Sc} distribution patterns obtained for the sheep isolates on first passage in mice with profiles of ovine BSE on first passage in mice may also be informative. Further western blotting experiments to examine the samples from further serial sub-passages and to enhance the resolution of PrP^{Sc} profiles may allow better comparisons with those of well characterised TSE strains.

Implications for current understanding of possible BSE in sheep

30. The possibility that BSE could be present in sheep but remain undetected if classical scrapie is also present could imply that there may have been sheep TSE cases diagnosed as classical scrapie when BSE may have also been present. Such mixed infections potentially could have arisen initially from the ingestion of BSE contaminated feed by sheep infected with classical scrapie or by sheep ingesting feed contaminated by both BSE and classical scrapie. However, for mixed infections to arise following the introduction of the reinforced meat and bone meal feed ban in 1996, sheep would need to be exposed independently to classical scrapie *and* ovine BSE infections *or* be exposed to ovine BSE and classical scrapie as mixed infections. In the former scenario, the probability of mixed BSE and classical scrapie infections arising currently in the national sheep flock is likely to be very low given the low prevalence of classical scrapie and the very low prevalence of BSE in sheep as estimated from discriminatory testing of confirmed sheep TSE cases¹¹. In the latter scenario, both BSE and classical scrapie would need to be transmitted together sufficiently efficiently for mixed infections to persist, with the BSE component remaining undetected, within the national sheep flock. However, there are insufficient data with which to evaluate the plausibility or likelihood of this theoretical scenario.

¹¹ An estimate of the prevalence of mixed infections of classical scrapie and BSE suggests that the prevalence is likely to be less than 0.000003% in the national sheep flock based on data from abattoir surveys and discriminatory testing of confirmed sheep TSE cases, assuming that the occurrence of BSE in sheep is independent of that of classical scrapie. Unpublished calculations provided by Dr Simon Gubbins, Head of Mathematical Biology Group, Institute for Animal Health.

31. Given the low prevalence of classical scrapie and assuming that BSE in sheep would occur independently from that of classical scrapie, if BSE is or was present in the national sheep flock, it would have been expected to arise more frequently as a single infection rather than mixed with classical scrapie. However, since BSE as a single infection in sheep has never been found, it is highly unlikely that an appreciable number of mixed infections of classical scrapie and BSE are present currently in sheep unless BSE and classical scrapie are more efficiently transmitted together between sheep compared with BSE in isolation but there are no data to suggest this may or may not be the case. However, unpublished results from inoculations into mice of a mixed inoculum compared with individual inocula indicate an increased attack rate suggesting higher transmission efficiencies of mixed strains is a possibility¹².
32. Analyses of the prevalence of BSE in the national sheep flock based on the application of discriminatory tests to isolates from sheep TSE cases identified from 1998 that were reviewed by the SEAC Sheep Subgroup previously estimated that the most likely prevalence of BSE in the UK sheep flock is zero and in the worse case no more than 10 flocks would be affected¹³. A recent analysis by the European Food Safety Authority based on data from TSE surveillance of sheep slaughtered for human consumption suggests that there are between zero (the most likely prevalence) and, depending on assumptions made about the sensitivity of discriminatory testing, two to four BSE infections per 10 000 sheep slaughtered for human consumption in the UK¹⁴. A low probability of mixed infections of BSE and classical scrapie occurring would not significantly influence these estimates of the prevalence of BSE in the UK sheep flock.

Summary

33. The data from the strain typing study, while intriguing and not fully explained, provide no evidence for the presence of BSE in sheep as a single infection. Whilst these data may indicate the presence of mixed BSE and classical scrapie infections, this is one of several possible interpretations. These data should not give rise to concern that there is an appreciable number of mixed BSE-

¹² Unpublished Roslin Institute studies.

¹³ SEAC Sheep Subgroup statement (2006) <http://www.seac.gov.uk/statements/sheepsubgrp-statement131006.pdf>

¹⁴ Opinion on the quantitative risk assessment on the residual BSE risk in sheep meat and meat products. *The EFSA Journal*. (2007) 442, 1-44. [http://www.efsa.europa.eu/EFSA/Scientific Opinion/biohaz_op_ej442_qra_sheep_en.3.pdf](http://www.efsa.europa.eu/EFSA/Scientific%20Opinion/biohaz_op_ej442_qra_sheep_en.3.pdf)

classical scrapie infections that would significantly influence estimates of the prevalence of BSE in the UK sheep flock.

Maternal transfer of classical scrapie prion protein via sheep milk

Background

34. The mechanisms for transmission of classical scrapie between sheep are not fully understood, however there is evidence to suggest that the risk of transmission is high during the neonatal period¹⁵. A study by VLA is examining whether milk may be a significant route of transmission by bottle feeding milk collected from ewes genetically susceptible to, and infected with classical scrapie, to genetically susceptible TSE-free lambs.

Data

35. Early unpublished findings from the study suggest that milk may be a route of transmission. Post mortem examination of three lambs that were bottle-fed milk from classical scrapie infected ewes which died early in the study from intercurrent disease revealed the presence of PrP^{Sc} in gut lymphoid tissue in two lambs. The milk fed to these two lambs was from two ewes that developed clinical signs of classical scrapie during lactation. The milk fed to the third lamb was from a ewe with clinical signs of classical scrapie at the beginning of lactation from which only a relatively small volume of milk was produced. Somatic cell counts were high in at least a proportion of the milk collected from the ewes. PrP^{Sc} was not found in a control lamb, which also died from intercurrent disease, that was fed milk from an uninfected ewe.

Implications

36. These data suggest that PrP^{Sc} may be transmitted from ewe to lamb via milk or colostrum. As a full lactation was fed to the lambs it is not possible to determine whether transmission occurred via colostrum and/or the subsequent milk. The study is at too early a stage to assess whether classical scrapie develops as a result of this exposure, although this should be considered likely.

¹⁵ For example, Foster *et al.* (1996) Observations on the transmission of scrapie in experiments using embryo transfer. *Vet. Rec.* 8, 138, 559-562 and Ryder *et al.* (2004) Demonstration of lateral transmission of scrapie between sheep kept under natural conditions using lymphoid tissue biopsy. *Res. Vet. Sci.* 76, 211-217.

37. Other research has suggested that concomitant infections of the mammary gland can increase levels of PrP^{Sc} at this site¹⁶ and therefore could potentially raise PrP^{Sc} concentrations in milk. In view of the high somatic cell counts found in at least a proportion of the milk samples collected from the ewes, which can suggest the presence of a mammary gland infection, the findings may not be applicable to ewes with no such infection. It is recommended that the morphology of the mammary gland and udder of the ewes be examined to establish whether a co-infection could have been a factor in the transmission of PrP^{Sc}. In addition, as the study continues, it would be important to assess whether such transmission is observed when lambs are fed milk with a lower somatic cell content.
38. Although these data are preliminary, they suggest that classical scrapie may be naturally transmitted from ewe to lamb via milk. They provide evidence in support of established farm practices to reduce the spread of disease by weaning lambs separately from ewes with classical scrapie.
39. These data have no direct implications for human health as there is no evidence that classical scrapie is transmissible to humans. Although it is possible that PrP^{Sc} could accumulate in milk from ewes infected with BSE, estimates of the prevalence of BSE in the UK sheep flock suggest the prevalence of BSE is likely to be zero or low if present at all (see paragraph 32).

Classical scrapie in ARR/ARR sheep

Background

40. Two cases of classical scrapie in sheep of the ARR/ARR genotype have been identified from retrospective genotyping of cases of classical scrapie in France and Germany¹⁷.

Interpretation

41. These data indicate that sheep of the ARR/ARR genotype are not completely resistant to classical scrapie. However, the lack of other cases of classical scrapie in sheep of the ARR/ARR genotype from surveillance in the UK and elsewhere and from

¹⁶ Ligios *et al.* (2005) PrP^{Sc} in mammary glands of sheep affected by scrapie and mastitis. *Nat. Med.* 11, 1137-1138.

¹⁷ Groschup *et al.* (2007) Classical scrapie in sheep with ARR/ARR prion genotype in Germany and France. *Emerg. Infect. Dis.* Epub ahead of print.

experimental studies, suggests that sheep of this genotype are highly, although not completely, resistant to classical scrapie.

42. The high resistance of ARR/ARR sheep to classical scrapie suggests that the scientific basis of the NSP, aimed at increasing the resistance of the national flock to classical scrapie by selective breeding using rams of relatively resistant genotypes, remains valid.

SEAC Sheep Subgroup November 2007

DRAFT

Membership of the SEAC Sheep Subgroup
Members

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Professor C. Bostock
Mr P. Jinman
Dr. H. Laude
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Professor A. Williams

Professors J. Manson and J Wilesmith and Drs N. Hunter and W. Goldman could not attend the meeting.

Presented data to the Subgroup

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