



CONTINGENCY PLANNING FOR THE CONFIRMATION OF BOVINE SPONGIFORM ENCEPHALOPATHY (BSE) IN SMALL RUMINANTS

ISSUE

All Member States are required to produce a contingency plan in the event BSE were found in sheep. The UK is currently finalising its detailed plan, for submission to the Commission this summer.

The European Commission has recently produced guidelines for Member States on the points which should be considered in drawing up their contingency plans. ([Annex 1](#)). The guidelines suggest that Member States should consider a worst case scenario where sheep meat is excluded from the food chain. This is based on an opinion, adopted by the Scientific Steering Committee (SSC) in April 2002, entitled "Safe sourcing of small ruminant materials" ([Annex 2](#)). The SSC Opinion states that if BSE were found in sheep, then only the following animals should be allowed into the food chain:

- ARR homozygous sheep under the age of 18 months
- ARR heterozygous sheep under the age of 6 months.
- Sheep (and goat) milk, colostrum and milk products from suspect BSE cases should be excluded from the food chain.

The EC guidelines (SANCO/19/2003 Rev.2) state a worst case scenario where:

- small ruminant meat is excluded from the food chain unless derived from:
 - homozygous ARR sheep under the age of 18 months
 - heterozygous sheep under the age of 6 months
- sheep and goat milk is excluded from the food and feed chain:
 - completely, or
 - unless derived from sheep carrying at least one ARR allele, or
 - unless derived from holdings certified TSE resistant or TSE free on the basis of solid criteria. They should also make an inventory of their capacity in terms of genotyping, individual identification and registration of animals, TSE testing and in terms of flock certification on the basis of history, monitoring etc.

In March 2002, a SEAC Sub-Group considered the risks associated with certain genotypes entering the food chain if BSE were ever isolated from sheep. In contrast to the SSC opinion, SEAC concluded that:

- In line with previous SEAC advice, only animals carrying the ARR allele should enter the food chain
- On a precautionary basis, the *12 month* cut off previously advised by SEAC remained appropriate for *ARR heterozygotes*. However, in view of existing SRM regulations *there was no justification for any age cut off in ARR homozygotes*
- In line with SEAC advice in 2001, only milk from ARR homozygous sheep could be considered as highly unlikely to contain the infectious agent. Further experimental work was required before potential risks from small ruminant milk from goats and semi-resistant or susceptible sheep could be excluded.

There is therefore a disparity of opinion between the SSC and SEAC on this issue. Whilst recognising the uncertainties relating to the science in this area, it is important that contingency planning is based on the most up to date scientific developments and assessments of risk that are available. SEAC will be presented with an update on the ongoing BSE in sheep studies, funded by Defra (Annex 3¹). This covering paper also provides a history of previous SEAC advice on this issue.

BACKGROUND

Chronology of previous SEAC advice

SEAC has advised on the scientific basis of this contingency plan on a number of occasions. The summary below details SEAC's previous opinions on the safety of ARR homozygous and heterozygous sheep entering the food chain *if* BSE were confirmed in sheep.

November 2000 – SEAC was first asked for comment on this issue as part of the work towards developing the NSP (meeting 64/5); the issue was not discussed at the meeting but was devolved to a SEAC Sub-Group

¹*Annexes 3 of Paper 78/9 has not been circulated outside the committee as this annex contain new scientific data that have not yet been published in a scientific or medical journal. As premature release of unpublished data may prejudice publication the annexe is not released prior to publication. This is in accordance with SEAC's code of practice*

December 2000 – The SEAC Sub-Group met and issued an opinion on a range of issues relating to the National Scrapie Plan (NSP) and contingency planning.

In terms of contingency planning, the Sub Group stated that the range of uncertainties about BSE in sheep made it likely that estimates of the level of disease in national flock would be difficult to determine if one or only a few cases were found. It was agreed that a risk reduction strategy that would provide some degree of reassurance [in terms of public health] would be to:

- only permit animals carrying at least one ARR allele to enter the food chain
- couple this to an age cut off, perhaps 1 year of age and
- include additional SRM controls to remove tissues such as intestine from the food chain

At that time (December 2000) ARR homozygotes intracerebrally (ic) challenged with bovine infected brain homogenate, showed no evidence of clinical disease during their life span. In addition, oral dosing studies of ARR homozygotes and heterozygotes were consistently negative in all tissues examined up to 22 months post exposure using IHC (SE1929, Annex 3). Evidence at that time was heavily dependent upon the sensitivity of immunohistochemistry (IHC) techniques. The Sub-Group noted that tissues from ARR homozygotes and heterozygotes were also being tested (by mouse bioassay) for the presence of infectivity in parallel with the IHC studies. However the Sub-Group noted that the mouse bioassays would take time.

The Sub-Group agreed that there remained some question about the sensitivity of the techniques employed to detect infectivity. In order to gain greater confidence in the negative results emerging from the heterozygous and homozygous ARR animals in the sheep pathogenesis studies, the Sub-Group recommended that further research be conducted using more sensitive techniques to test peripheral tissues from:

- a) Ongoing BSE in sheep pathogenesis experiment;
- b) Flocks naturally exposed to scrapie

Experiments should include the use of:

- a) Analysis by western blot using concentrated samples to increase sensitivity of detection.

- b) Intra-cerebral challenge of New Zealand-sourced sheep of susceptible genotypes with tissues taken from ARR heterozygous and homozygous animals.
- c) The use of transgenic mice if available

Milk

In terms of the risk associated with milk, it was considered that dairy sheep, which are kept to an older age, are most likely to have received feed containing MBM before the ban², and at greater levels compared with sheep reared for meat. It was also noted that infectivity had been demonstrated in lymphoid tissue in animals with susceptible genotypes. Therefore the Group concluded that it was likely that recirculating lymphocytes and other white cells are present in milk, and could potentially represent a source of infectivity.

The Group agreed that as a precautionary measure, and until it can be established that milk does not carry detectable infectivity, the potential risk associated with the use of sheep other than ARR homozygous was greater than for ARR homozygous dairy animals. As goats do not appear to carry the ARR allele, and have been shown to be susceptible to BSE, the Group agreed that it would not be appropriate for goats to be considered safe for dairy production if BSE were found in small ruminants.

The Sub-Group's opinion was presented to SEAC, and accepted at the **February 2001** meeting.

March 2002 – A SEAC NSP Working Group was asked to consider (by the FSA) if their opinion of Feb 2001 required revision in the light of updated results from the sheep pathogenesis study

The Working Group noted that the previously suggested 1 year cut-off had been based on the results of ongoing research in the sheep pathogenesis study (where sheep were orally dosed with 5g bovine infected brain homogenate – SE1929, Annex 3). At March 2002, an update on the sheep pathogenesis study reported that no evidence of infection had been detected in ARR heterozygote and homozygotes animals up to 34 months post oral inoculation. The Working Group considered the latest research update, but did not consider that it changed their advice and endorsed their previous opinion that 12 months was appropriate for ARR heterozygote animals, given the limits of sensitivity of the diagnostic tests to detect infectivity.

² The feeding of ruminant proteins to ruminants (e.g. sheep and cattle) was prohibited in 1988.

However, with regard to ARR homozygote animals, the Working Group considered that, based on experimental data, an age cut off was not warranted.

The Working Group's opinion was accepted by SEAC in April 2002. The main difference between the April 2002 opinion and the previous SEAC advice (February 2001) was that there should be no age cut off for ARR homozygotes.

Milk

In terms of the risk assessment of milk, the Group agreed with the SEAC advice of February 2001 that if BSE were found in sheep, both sheep and goats should not be used for dairy production, with the exception of ARR homozygous animals. It was noted that research from experimental sheep TSE models did not suggest that lymphocytes were infectious. However, they noted that there is often widespread infectivity throughout the lymphoreticular system. The Group therefore considered it possible that circulating lymphocytes within milk may carry infectivity.

Update on scientific research since the last SEAC opinion on this issue

Previous SEAC opinions were based on research updates on pathogenesis studies investigating experimental BSE in sheep, as well as a summary of the current state of knowledge (at that time) on the impact of genotype on the distribution of infectivity and markers of infectivity in sheep with TSEs. An update on the ongoing Defra funded BSE in sheep studies is provided at Annex 3. Dr Danny Matthews will also provide an oral update on one particular study (SE1929, Annex 3), which is investigating the pathogenesis of experimental BSE in sheep following oral dosing. This work has found that PrP^{Sc} can be detected in a number of tissues from as early as 4 months post challenge in ARQ/ARQ sheep. However analysis³ of tissues from ARQ/ARR and ARR/ARR animals has failed to detect PrP^{Sc} up to 48 months post challenge, and the remaining sheep are healthy up to 5.5 years post challenge.

More recently, in December 2002, a SEAC Sheep Sub-Group considered an interim result from a project conducted at the Institute for Animal Health. The study is determining the susceptibility of TSE free sheep from New Zealand (SE1432, Annex 3). The new research

³ Tissues analysed by IHC. Tissues also tested by mouse bioassay, results awaited.

reported the experimental transmission of BSE to ARR homozygous sheep following intracerebral challenge with 0.5ml 10% BSE-infected bovine brain homogenate. The incubation period of disease was approximately twice the average incubation period of 556 days reported in BSE susceptible genotypes (ARQ/ARQ). Members agreed that the transmission of BSE following intracerebral inoculation shows that the resistance of ARR/ARR sheep to TSE infection cannot be regarded as absolute. They noted however that intracerebral inoculation is not a natural route of transmission and had BSE been introduced into the sheep population, the most likely route of exposure was oral.

An update on the ongoing scrapie studies will be provided at a subsequent SEAC meeting.

Confirmation of sheep age by dentition

SEAC agreed (April 2002) that an age cut off of 12 months for ARR heterozygotes could be used as a precautionary threshold. This was based on practical considerations, as dentition checks can confirm that a sheep is less than 12 months of age, by establishing that no permanent incisors have erupted. There is some variability between animals in terms of when the first two incisors erupt. However, this is essentially a risk management consideration.

Sensitivity of diagnostic tests

In many of the studies reported to SEAC, which are investigating the pathogenesis of sheep TSEs, a combination of IHC and mouse bioassays have been used to detect the presence or absence of PrP^{Sc} or infectivity respectively. The SEAC Sub-Group which met in December 2000, recommended that further research, aimed at improving the sensitivity of diagnostics tests, be undertaken to verify negative results obtained by these methods.

Advice sought from the Committee

The Committee is asked to advise on whether or not recent scientific developments indicate that there has been a change in the risk associated with the consumption of the following if BSE in small ruminants becomes probable:

- ARR heterozygous sheep (a) under 12 months and (b) over 12 months
- ARR homozygous sheep of all ages
- Sheep milk from ARR homozygous sheep of all ages



CONTINGENCY PLANNING FOR THE CONFIRMATION OF BOVINE SPONGIFORM ENCEPHALOPATHY (BSE) IN SMALL RUMINANTS: SSC OPINION ON SAFE SOURCING OF SMALL RUMINANT MATERIALS (SHOULD BSE IN SMALL RUMINANTS BECOME PROBABLE)

ISSUE

All EU Member States are required to produce a contingency plan for use if BSE were to be found in sheep. The UK is finalising its plan for submission to the European Commission (EC) this summer.

The EC has recently produced guidelines for Member States on the points which should be considered in drawing up the contingency plans. The guidelines were based on an Opinion adopted by the Scientific Steering Committee (SSC) on 4/5 April 2002, entitled "Safe sourcing of small ruminant materials (should BSE in small ruminants become probable)".

In March 2002 a SEAC Sub-Group considered the risks associated with certain sheep genotypes entering the food chain should BSE be isolated from sheep. SEAC's opinion differed from that of the SSC regarding:

1. Age limit for ARR homozygous sheep entering the food chain
 - **SEAC:** In view of existing SRM regulations there was **no justification for any age cut off**
 - **SSC:** only ARR/ARR sheep under **18 months** of age allowed
2. Age limit for ARR heterozygous sheep entering the food chain
 - **SEAC:** only ARR heterozygous sheep under **12 months** of age allowed
 - **SSC:** only ARR heterozygous sheep under **6 months** of age allowed
3. Sheep (and goat) milk
 - **SEAC:** Only milk from ARR homozygous sheep could be considered as highly unlikely to contain the infectious agent. Further experimental work was required before potential risks from milk from goats and semi-resistant or susceptible sheep could be excluded.

- **SSC:** Regarding the safety of milk in small ruminants, its Statement of 30 March 2001 remains valid, namely that the evidence available (to the date of the Statement) does not point to milk or colostrum representing a possible risk but for precautionary reasons milk, colostrum or milk products from suspect BSE cases should not be offered for consumption.

BACKGROUND INFORMATION

The SSC Opinion is based on a report of the TSE/BSE *ad hoc* Group's Report of 21 March 2002 on Safe Sourcing of Small Ruminant Materials.

The SEAC Secretariat have summarised the supporting evidence from the paper documentation that appears to underpin the SSC Opinion and our interpretation is given below. References to pages in the TSE/BSE *ad hoc* Group's Report are given where appropriate.

Concerning the SSC 18 month age limit for ARR homozygous sheep entering the food chain

From the SSC Opinion:

The available evidence indicates that sheep which are homozygous for the arginine (R) allele at codon 171 are the most resistant to development of disease upon challenge with BSE-infected material. The likelihood of ARR/ARR animals becoming infected with BSE post exposure is very small or, if it occurred, very slow.

Therefore, based on available but relatively limited data indicating no evidence of scrapie nor BSE infectivity in resistant animals of any age, it is a reasonable worst case assumption that the tissues from sheep which are homozygous for the arginine (R) allele at codon 171, including those tissues listed in Section 1 p. 2 of the Opinion, do not pose any significant BSE risk below **18 months** of age.

Supporting evidence from the TSE/BSE *ad hoc* Group's Report

- The 18 months of age cut off point could relate to a dental aging point where molar 3 erupts through at 18 months of age (*see report page 26 Section VII.3c*)
- From *page 31 Section VIIIc* of the report it is stated that for ARR/ARR animals, tissues listed on *pages 24 and 25 of the report (Section VII.2e)* pose a risk for animals above approximately **18 months (a suggested expert judgement)**. For resistant genotypes the probability of becoming TSE infected is very low, disease pathogenesis is likely to be slow and would not result in significant

infectivity levels in young animals. The threshold of 18 months also corresponds approximately to the lower quintile of the incubation period distribution (SN: *it is not clear which study this relates to*).

- *Page 44,45 Annex 1, Section II.2b: Studies by Jeffrey et al., 2001 upon oral dosing of ARR/ARR sheep with BSE indicated no infectivity had been detected by bioassay up to 24 months post inoculation (SN: the Jeffrey et al.,2001 paper states 'no clinical disease, CNS vacuolation or disease-specific PrP accumulation in brain, viscera or peripheral nervous system was found within 24 months of inoculation in sheep of the resistant genotype PrP^{ARR/ARR} or sheep of genotype PrP^{ARQ/ARR} (of low susceptibility). The final results will be reported in due course.'*) This point is also discussed in the report on *pages 48 and 49 in Annex 2*, where it is also stated that in cases of natural scrapie, PrPres has not been detected in ARR/ARR ovines of any age.

Concerning the SSC 6 month age limit for ARR heterozygous sheep entering the food chain

From the SSC Opinion:

Sheep which are heterozygous with one arginine (R) at codon 171 show an intermediate degree of resistance to BSE infection and a distinct pathogenesis and a much longer incubation period compared to the susceptible genotypes. During the pre-clinical phase, PrP^{sc} does not appear to be detectable in the digestive autonomic nervous system of ARR/ARQ or ARR/VRQ sheep (*see report p. 49 in Annex 2*).

For these reasons, sheep tissues from heterozygous ARR/ARQ or ARR/VRQ ovines, including those tissues listed in Section 1 p. 2 of the Opinion, can be considered free from BSE infectivity only **below 6 months** of age.

Supporting evidence from the TSE/BSE *ad hoc* Group's Report

- *page 49 Annex 2: In heterozygotes with one copy of the ARR allele, observations to date in natural scrapie (van Keulen et al., 1999 and Andreoletti et al., 2000) and in experimental BSE (Jeffrey et al., 2001) show that ARR/VRQ and ARR/ARQ are not carriers of PrPres before the age of one year at least in all the tissues studied; the CNS, lymph tissues and the autonomous digestive nervous system.*
- *From page 31 Section VIIIc of the report it is stated that for semi-resistant animals, tissues listed on pages 24 and 25 of the report (Section VII.2e) pose a risk for animals above approximately 6 months (a suggested expert judgement). (SN: it is not clear on which studies the 6 month cut off is based)*

- From dental information 6 months of age would correspond approximately to an animal that has one molar but not yet 2 molars (see report page 26 Section VII.3c)

SSC Opinion on Sheep (and goat) milk entering the food chain

The TSE/BSE *ad hoc* Group in its report of 21 March 2002 (page 23) considered that the Statement of the SSC of 30 March 2001 on the safety of milk remained valid, namely that the evidence available (at the time of the report) did not point to milk or colostrum representing a possible risk but for precautionary reasons milk, colostrum or milk products from suspect BSE cases should not be offered for consumption. The recommendations for research made in the SSC Statement of 30 March 2001 remained valid. The TSE/BSE *ad hoc* Group took note of the risk assessment of milk products presented in AFSSA (Agence Française de Sécurité Sanitaire des Aliments) 2002. This assessment included a theoretical analysis of the exposure to consumers of milk products and cheese produced from milk from a flock with BSE.

References

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