



QUANTITATIVE RISK ASSESSMENT ON THE RESIDUAL BSE RISK IN SHEEP MEAT AND MEAT PRODUCTS

Opinion of the Scientific Panel on Biological Hazards

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Opinion of the Scientific Panel on Biological Hazards on a request from the European Commission on the Quantitative risk assessment on the residual BSE risk in sheep meat and meat products¹

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SUMMARY

The European Commission (DG SANCO) requested the Scientific Panel on Biological Hazards (BIOHAZ) of the European Food Safety Authority (EFSA) for an update on the risks posed by tissues of sheep to human health in the case where Bovine Spongiform Encephalopathy (BSE) is confirmed in sheep and, more particularly, on the feasibility of carrying out a Quantitative Risk Assessment (QRA).

In replying to this mandate, the experts considered all necessary elements needed to carry out a quantitative risk assessment of BSE transmission from sheep to humans: (1) prevalence estimates of BSE infection in EU sheep (2) the amount and distribution of infectivity in sheep tissues and body fluids (3) the apparent barrier *i.e.* “species” barrier for transmission of BSE from sheep to humans and (4) human consumption of sheep products. The experts concluded that there are currently insufficient data for a QRA of the risks posed by tissues of sheep to human health in the case where BSE is confirmed in sheep.

However, they recognised that the prevalence of infection would have most significant impact on any QRA. Using the latest Transmissible Spongiform Encephalopathy (TSE) prevalence figures for Member States (MS) and the results of BSE/scrapie discriminatory testing, the experts were able, with certain assumptions, to estimate BSE prevalence in sheep. For example, they calculated by one method that there is a 95% confidence that in the high risk sub-group of countries there is less than 0.3-0.5 cases of BSE per 10,000 healthy-slaughtered animals.

In sheep experimentally infected with BSE, the distribution of the infectious agent (prion) in tissues is wide-spread as the prion can be found in secondary lymphoid tissue, skeletal muscle and blood. In considering more recent attempts at quantifying the risk specifically from this experimental ovine BSE, and in reviewing biochemical approaches to quantify titres in affected animals, a major stumbling block to quantification was identified to be the fact that the influence of age and genotype on the distribution of BSE infectivity in sheep is only defined qualitatively. The BIOHAZ panel agreed that absolute quantification of prions by biochemical methods was difficult. However, in the absence of comprehensive infectivity data to facilitate a QRA, it was concluded that Specified Risk Materials (SRM) removal alone was unlikely to be sufficient to eliminate the residual BSE risk to the consumer from a BSE-infected sheep carcass.

The experts further assumed that there is no intrinsic species barrier for sheep BSE transmission to humans, which is in line with past opinions of the Scientific Steering Committee.

Human consumption levels of sheep meat and other sheep products (domestic produced or imported) are high within the EU. Data from two EU countries were used (Italy and GB) and gave intake estimates that were broadly in agreement with the overall trade data for sheep carcasses for these countries provided by Eurostat. This gave some confidence in using Eurostat data as basis to estimate an average daily intake of sheep meat or meat products in the EU of 8.4-9.3 g per person.

KEY WORDS: BSE, sheep, prevalence, QRA, human exposure

Table of Contents

SUMMARY	2
1. BACKGROUND	5
2. TERMS OF REFERENCE	6
3. THE SCOPE OF THE MANDATE, CURRENT LEGISLATION AND RELEVANT PAST OPINIONS	6
3.1 <i>THE MANDATE</i>	6
3.2 <i>EXISTING COMMUNITY LEGISLATION ON BSE IN SMALL RUMINANTS</i>	6
3.3 <i>THE EFSA BSE IN GOATS OPINION AND REPORT: WHAT ADDITIONAL DATA ARE AVAILABLE FOR A QRA OF BSE IN SHEEP?</i>	7
3.4 <i>THE OPINIONS OF THE SCIENTIFIC STEERING COMMITTEE (SSC) ON SAFE SOURCING OF SMALL RUMINANT MATERIALS OF APRIL 2002 AND ITS COMPLEMENT OF SEPTEMBER 2002.</i>	7
4. RISK ASSESSMENT	8
4.1 <i>APPROACH TO THE MANDATE</i>	8
4.2 <i>PREVALENCE OF INFECTION</i>	8
4.2.1 <i>PREVALENCE ESTIMATES IN THE HEALTHY-SLAUGHTER EXIT STREAM</i>	8
4.2.2 <i>PREVALENCE ESTIMATES IN EU25 AND NORWAY USING DATA FROM ALL EXIT STREAMS</i>	12
4.2.3 <i>CONCLUSIONS</i>	14
4.3 <i>DISTRIBUTION OF INFECTIVITY IN TISSUES AND INFECTIOUS LOAD</i>	14
4.3.1 <i>INFECTIVITY OF TISSUES OF SHEEP INFECTED WITH BSE AND SCRAPIE AGENTS</i>	14
4.3.2 <i>BLOOD</i>	15
4.3.3 <i>INTESTINE</i>	16
4.3.4 <i>MILK</i>	17
4.3.5 <i>BIOCHEMICAL APPROACHES TO “PRION” QUANTIFICATION IN TISSUES</i>	17
4.3.6 <i>CONCLUSION</i>	18
4.4 <i>SPECIES BARRIER (SB)</i>	19
4.4.1 <i>DEFINITION</i>	19
4.4.2 <i>QUANTITATIVE ESTIMATES OF ANIMAL-TO-HUMAN BSE TRANSMISSION BARRIERS FROM EPIDEMIOLOGICAL STUDIES</i>	19
4.4.3 <i>INFERENCES FROM EXPERIMENTAL STUDIES</i>	20
4.4.4 <i>NEW TYPES OF BSE IN CATTLE AND THE VARIABLE PATHOGENICITY OF SCRAPIE STRAINS</i>	20
4.4.5 <i>CONCLUSION</i>	21
4.5 <i>HUMAN CONSUMPTION</i>	21
4.5.1 <i>THE ITALIAN SURVEY</i>	21

<i>4.5.2 THE BRITISH SURVEY</i>	21
<i>4.5.3 CALCULATION OF SHEEP MEAT CONSUMPTION FROM EUROSTAT FIGURES AND COMPARISON WITH SURVEY DATA</i>	22
<i>4.5.4 CALCULATION OF SHEEP MEAT CONSUMPTION IN THE EU15 FROM EUROSTAT FIGURES</i>	23
<i>4.5.5 CAVEATS TO THE INTERPRETATION OF ESTIMATES OF SHEEP MEAT CONSUMPTION</i>	23
5. AFSSA OPINION	24
6. CONCLUSIONS	25
<i>6.1 GENERAL</i>	25
<i>6.2 THE KEY ELEMENTS OF A SHEEP QRA</i>	26
<i>6.2.1 PREVALENCE OF INFECTION</i>	26
<i>6.2.2 INFECTIOUS LOAD AND DISTRIBUTION IN TISSUES</i>	26
<i>6.2.3 SPECIES BARRIER</i>	27
<i>6.2.4 HUMAN CONSUMPTION LEVELS</i>	27
7. RECOMMENDATIONS	28
8. REFERENCES	29
ANNEX 1: APPLICATION OF PRP ^{Sc} QUANTIFICATION TO EXPERIMENTAL BSE SHEEP TISSUES	35
ANNEX 2: INFECTIVITY OF TISSUES IN SHEEP AND GOATS WITH NATURAL SCRAPIE	37
ANNEX 3: CALCULATION OF SHEEP MEAT CONSUMPTION IN THE EU15 FROM EUROSTAT FIGURES	39
ANNEX 4: IMPORTS OF SHEEP OR GOAT MEAT (FRESH, CHILLED OR FROZEN) PRODUCTS FROM AUSTRALIA AND NEW ZEALAND, 1995-2004	40
ANNEX 5: EU SURVEILLANCE DATA	41
ANNEX 6: POOLED DATA	44

1. BACKGROUND

a) Confirmation of Bovine Spongiform Encephalopathy in a goat.

Diagnosis of BSE in a goat in France on 28 January 2005 (Eloit *et al.*, 2005) led to EU legislation requiring the differentiation of scrapie and BSE for all confirmed positive scrapie cases in both sheep and goats (EC regulation 36/2005) and an increase in surveillance for TSEs in goats for both healthy and fallen stock (EC regulation 214/2005). The European Commission (EC) also invited (EC letter, 2005) the European Food Safety Authority to carry out “A quantitative assessment of risk posed to humans by tissues of small ruminants (SMRU) in case BSE is present in these animal populations”.

b) EFSA Opinion on a quantitative assessment of risk posed to humans by tissues of small ruminants in case BSE is present in these animal populations (EFSA 2005a).

The original mandate requested a quantitative assessment of the risk posed by human consumption of meat derived from goats and sheep of different ages and genotypes in the case where BSE is confirmed. The BIOHAZ Panel and its Working Group considered scientific data on the epidemiology and the pathogenesis of BSE in sheep and goats under experimental conditions and gathered additional surveillance and technical data from the EC, individual Member States and EFSA and its Advisory Forum (AF). Since the index case of BSE in small ruminants was in goats not sheep, the BIOHAZ panel set aside questions relating to sheep and focused on the risks, if any, of consuming goat meat products. They concluded that:

1. there were not enough data to allow a quantitative assessment of risk to humans associated with consumption of meat and meat products derived from goats infected with BSE;
2. the qualitative assessment of risk to humans associated with consumption of meat and meat products derived from goats infected with BSE was small for goats born after the EU-wide feed ban of 2001;
3. previous generalisation of goat-related risks derived from sheep data was no longer appropriate;
4. a comprehensive and reliable, quantitative risk assessment of sheep and goat-related risks to the consumer may become possible in the future, and new data should be reviewed for this purpose when available. In considering these new data for a QRA of sheep and goat meat, accurate prevalence information of BSE in these species was emphasised as a key input factor.

2. TERMS OF REFERENCE

Following the EFSA opinion on the QRA for goat meat and goat meat products (EFSA, 2005a), EFSA received a formal mandate from DG SANCO of the European Commission (EC) requesting EFSA's Scientific Panel on Biological Hazards for an update on the risks posed by tissues of sheep to human health in the case where BSE is confirmed in sheep and, more particularly, on the feasibility of carrying out a Quantitative Risk Assessment (QRA). If a QRA was not feasible the request was to carry out a Qualitative RA and, in the light of its conclusions, update the opinions of the Scientific Steering Committee (SSC) on safe sourcing of small ruminant materials of April 2002 and its complement of September 2002.

In a response to the EC, EFSA indicated that, while a QRA-sheep would be possible in the future when more data become available, at present it would be possible only to conduct a Qualitative RA (EFSA Letter, 2005).

During the course of this mandate the French Food Safety Authority (AFSSA) finalised an opinion (AFSSA, 2006) on a similar subject related to risk of BSE in small ruminants. Their mandate covered both the risk assessment and science of the TSE in sheep tissue and management of that risk. As such, the AFSSA mandate was much broader than the remit of this EFSA mandate. EFSA was asked by the EC (EC Letter, 2006) to take this AFSSA opinion into account in the on-going evaluations. This undertaking was confirmed by EFSA (Letter EFSA, 2006). Account is given in this report of the consideration of the AFSSA opinion, where applicable and when appropriate.

3. THE SCOPE OF THE MANDATE, CURRENT LEGISLATION AND RELEVANT PAST OPINIONS

3.1 *The Mandate*

In the absence of essential data the current mandate required the Panel and its Working Group (WG) to carry out a qualitative risk assessment, including new data, on the risks posed by tissues of sheep to human health if BSE is confirmed in the sheep flock. The mandate then requires this assessment to be utilised to update opinions of the Scientific Steering Committee (SSC) on safe sourcing of small ruminant materials of April 2002 and its complement of September 2002 (EC 2002a).

3.2 *Existing community legislation on BSE in small ruminants*

Existing community legislation requires Member States (MS) to draw up guidelines specifying the national measures to be implemented in relation to culling, removal of SRM and testing of animals where cases of TSE are confirmed². The Commission requested each member state (MS) to draft a contingency plan in case BSE is detected in small ruminants based on the opinion on safe sourcing of small ruminant materials adopted by the Scientific Steering Committee (SSC) (EC, 2002a).

² This is in accordance with Article 14 of Regulation (EC) No 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain TSEs, as last amended by Commission Regulation (EC) No 1993/2004.

EU Law (Regulation 999/2001) also lays down rules for eradication measures to be applied to TSE infected flocks, for monitoring, and for the removal of SRM. The current SRM list consists of skull, brain, eyes, tonsils and spinal cord of sheep and goats over 12 months of age and the spleen and ileum of sheep and goats of all ages. Commission Decision 2003/100/EC introduced breeding programmes for resistance to TSEs in sheep as part of the overall strategy for reducing risks from TSEs in small ruminants. The EFSA Opinion and Report “Breeding for resistance to TSEs” (EFSA 2006) has recently re-affirmed the value of these voluntary breeding programmes as part of a risk reduction strategy.

3.3 *The EFSA BSE in Goats Opinion and Report: what additional data are available for a QRA of BSE in sheep?*

The EFSA Opinion and Report on BSE in Goats (EFSA 2005a) identified the factors that would be required in order to conduct both a sheep and a goat QRA and also highlighted the differences in regard to a species-specific assessment of the QRA of BSE in sheep compared to that in goats. The Opinion stated:

“A QRA-sheep was thought more feasible than a QRA-goat because more information was available about the phenotype and case definition, genotype effects, infectious load³ and prevalence of BSE in sheep than BSE in goat. However, even in this more favorable case, the paucity of quantitative data on infectivity titers of BSE agent in sheep tissues and the lack of a methodology for the geographical BSE risk assessment for sheep (GBR-S) precludes a worthwhile QRA-sheep”.

The BIOHAZ panel recognized the relative importance of accurate prevalence estimates for BSE in small ruminants in this risk assessment. In this context, more information on surveillance for BSE in sheep (at least in Great Britain and France) was available than in goats. Additionally, experimental strain typing in wild-type mice and use of discriminatory PrP^{Sc} diagnostic testing in active and passive surveillance programmes have been conducted on national populations, thus providing a basis for prevalence estimation of BSE in sheep (Gravenor *et al.*, 2003; Stack *et al.*, 2006).

3.4 *The opinions of the Scientific Steering Committee (SSC) on safe sourcing of small ruminant materials of April 2002 and its complement of September 2002.*

The SSC opinion on safe sourcing of small ruminant materials (EC, 2002a) indicated that, in case BSE should become probable in small ruminants, safety of sourcing of small ruminant materials could be achieved by combining different approaches. These would include the removal of tissues known to pose a risk of infectivity as from a given age, testing for BSE, genotyping and breeding for BSE resistance, flock certification and individual animal and flock tracing. The opinion recognized the qualitative risk for humans and provided an approach to reducing that risk but made no estimation of the risk for humans or a quantitative calculation.

Essentially, this SSC Opinion (EC 2002a) and its complement comprehensively assessed the potential risk to human health and indicated how those risks could be minimized by a range of risk management procedures. However, the logistics and cost of these measures inhibit their practical enforcement and part of the rationale behind this current mandate is to review

³ This information is available for experimental BSE in sheep.

the potential for the simplification of the control measures (to, for example, only removal of SRM) based on a more quantitative approach to the risk issues.

Some issues of the opinion have already been reconsidered and were confirmed by EFSA in its Opinion of the Scientific Panel on Biological Hazards of EFSA on the interpretation of results of EU surveillance of TSEs in ovine and caprine animals, culling strategies for TSEs in small ruminants and the TSE-related safety of certain small ruminant products, adopted on 26 November 2003 (EFSA, 2003).

4. RISK ASSESSMENT

4.1 Approach to the mandate

The BIOHAZ panel considers that the key inputs used in the previous assessment of risk posed to humans by consumption of meat and meat products derived from goats infected with BSE, were also indispensable for conducting the present assessment.

The key inputs considered necessary are data related to:

- a. Prevalence of infection.
- b. Infectious load and distribution in tissues.
- c. The species barrier.
- d. Human consumption levels.

If the surveillance data support the perception that BSE in sheep is improbable then, as for goats, estimations of the prevalence of BSE in sheep becomes the most important of these inputs.

4.2 Prevalence of infection

Since the publication of the SSC 2002 Opinion, the European Commission had sponsored large-scale surveillance for TSEs in small ruminants and, since 2005, each positive index case detected in a TSE-affected flock was required to be further analysed using newly-developed tests capable of *in vitro* discrimination of BSE and scrapie in sheep⁴. The raw data from this surveillance are tabulated in Annex 5 and used in the calculations of prevalence in the healthy slaughter exit stream in section 4.2.1. However, this treatment excludes other informative data and a second approach is presented in section 4.2.2 using discriminatory testing data obtained from all exit streams, some obtained by retrospective testing of passive surveillance cases identified between 1998-2004 (Stack *et al.*, 2006).

4.2.1 Prevalence estimates in the healthy-slaughter exit stream

A model has been developed to work out the uncertainty distribution of the BSE cases in the healthy-slaughter sheep population; this uses data collected in the EU between 2005 to December 5, 2006 (see Annex 5). The model is used to assess the probability of BSE in this exit stream rather than estimating simple prevalence in the sheep population because the healthy-slaughter population is most relevant to human consumption. Therefore, TSE

⁴ Stack *et al.*, in preparation

suspects, fallen stock and sheep culled for destruction and tested from TSE-affected flocks are excluded from the analysis as they are not considered for human consumption.

No discriminatory test results were available per exit stream and so the number of discriminatory tests on healthy slaughter animals was estimated as the total number in this exit stream multiplied by the fraction of all samples subject to discriminatory tests. The distribution of BSE positives in the healthy-slaughtered TSE-positive population is estimated by working out the hyper-geometric likelihood and from this the distribution of BSE positives in the healthy slaughtered population can be estimated using the assumption of a binomial distribution. The specificity of the screening tests were set at 100% (EFSA scientific report 2005a; EFSA scientific report 2005b) and the effects of their sensitivity on these outputs were calculated: the mean and upper 95th percentile of the estimated prevalence for sensitivities 90%, 70% and 50% for the UK and BSE high risk II group are shown in Table 2; the sensitivity and specificity of the discriminatory tests were both set at 100% although the BIOHAZ panel noted only a very limited evaluation of these tests had been executed (Stack *et al.*, in preparation).

Data and calculation results for healthy-slaughtered sheep for a) high risk BSE I, UK; b) high risk BSE II, UK, Portugal, Ireland, and France; c) the EU15 and d) the new EU10 (EU25-EU15) are tabulated (Table 1).

Table 1: **Post-mortem TSE surveillance in 2005 and 2006.**

Member state	TSE positives in slaughtered for human consumption			Estimated number tested with discriminatory test	BSE positives in tested TSE positives slaughtered for human consumption: discriminatory test results	Prevalence**			Figure
	2005	2006	Combined [rate per 10,000]			Mean	Mode	Upper 95 th percentile	
UK	30/11,816	7/7,774	37/19,590 [18.9]	29	0	0.70	0	2.20	Fig 1
UK+Ireland+Portugal+France	75/86,036	110/158,072	185/244,108 [7.6]	83	0	0.10	0	0.29	Fig 2
EU15* minus the above four	62/57,591	32/69,229	94/126,820 [7.4]	31	0	0.27	0	0.80	
EU15*	137/143,627	142/227,301	279/370,928 [7.5]	114	0	0.08	0	0.23	
Norway, EU25 minus EU15	109/22,366	9/18,273	118/40,639 [29.0]	11	0	2.32	0	6.67	

* Counted as EU15 = Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Luxembourg, Netherlands, Portugal, Spain, Sweden, UK.

** Mean (per 10,000)
Most likely (mode)
Upper 95th percentile (per 10,000)

From Table 1, it is clear that the most likely (mode) prevalence is zero irrespective of our assumptions and input data streams. However, the confidence intervals widen considerably compared to the approach using pooled data because only part of the data set is considered (see section 4.2.2). Intuitively, this approach could penalise countries with good passive surveillance and comprehensive testing schemes.

If inferences are based solely on the UK data, a mean BSE prevalence of 0.70 per 10 000 (upper 95th percentile, 2.20 per 10,000) is estimated in the healthy slaughtered population. However, for the high risk BSE II group as a whole, the absence of BSE in 83 tested from 185 TSE positives in the healthy-slaughter population leads to a BSE prevalence estimate of 0.10 per 10,000 on average with an upper 95th percentile of 0.29 per 10,000.

Surveillance data from the remaining EU15 member states are less powerful despite the similar TSE positivity rates in healthy adult sheep, and surveillance data from the newer member states are both highly heterogeneous between countries and surveillance years and insufficiently powerful. They were not considered further for the assessment of the prevalence in the healthy slaughter exit stream.

Figure 1: Cumulative uncertainty distribution of the BSE prevalence in the healthy slaughter exit stream of High Risk group I (UK)

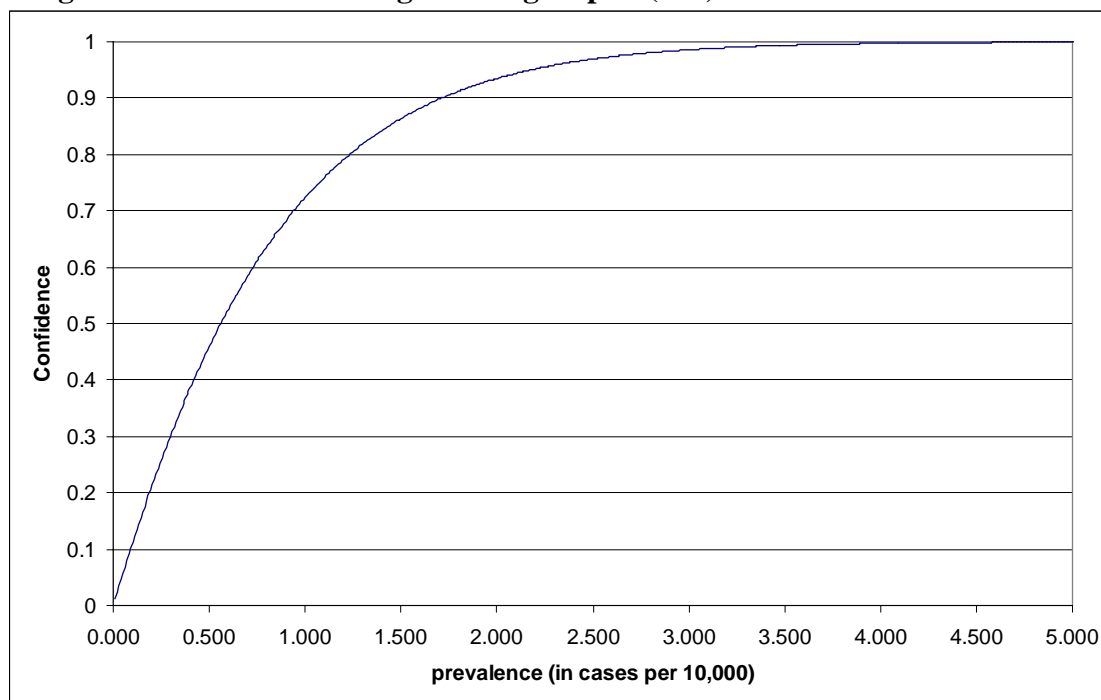


Figure 2: **Cumulative uncertainty distribution of the BSE prevalence in the healthy slaughter exit stream of High Risk group II (UK, Ireland, Portugal and France)**

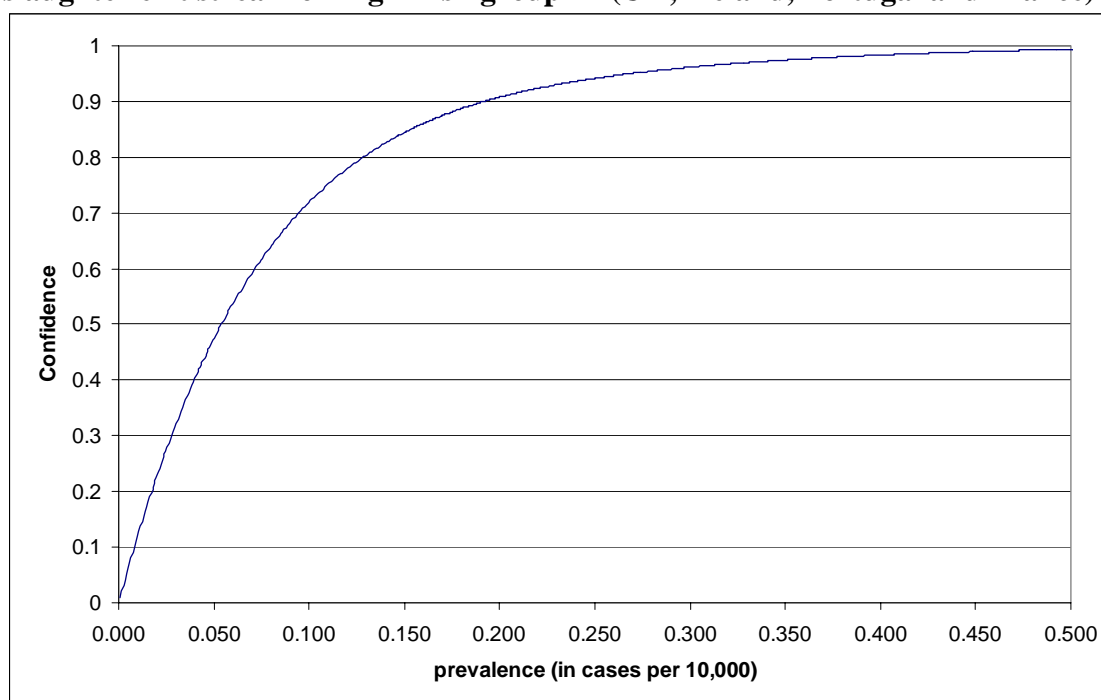


Table 2: **Prevalence estimates (in cases per 10,000) for different sensitivities of the TSE screening test**

	Sensitivity					
	90%		70%		50%	
	mean	P95	mean	P95	mean	P95
High Risk I (UK)	0.70	2.20	0.89	2.66	1.26	3.77
High Risk II (UK, Ireland, Portugal, France)	0.10	0.29	0.13	0.38	0.17	0.50

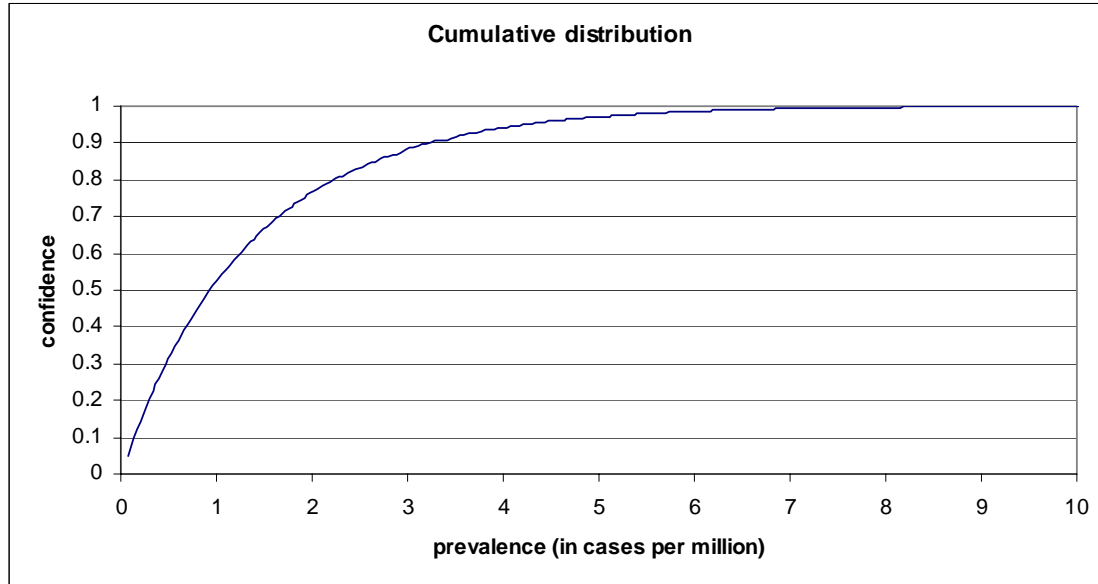
4.2.2 Prevalence estimates in EU25 and Norway using data from all exit streams

No case of BSE has yet been confirmed in the EU25 and so pooled data extending back to 1998 was used to calculate the cumulative uncertainty distribution of BSE prevalence in EU sheep (Annex 6, Table 1)⁵. This was done in two stages: i) the distribution of BSE positive cases in the TSE positive groups was estimated by working out the hyper-geometric likelihood, and ii) from this uncertainty distribution, the distribution of the number of cases in the population was simulated by Monte Carlo methods, assuming a binomial process and taking into account the sensitivity of the TSE screening test⁶. This cumulative uncertainty distribution is plotted in Figure 3.

⁵ The BIOHAZ Panel recognised that if a case was confirmed in a MS then re-calculations based on data from each individual MS would be more appropriate.

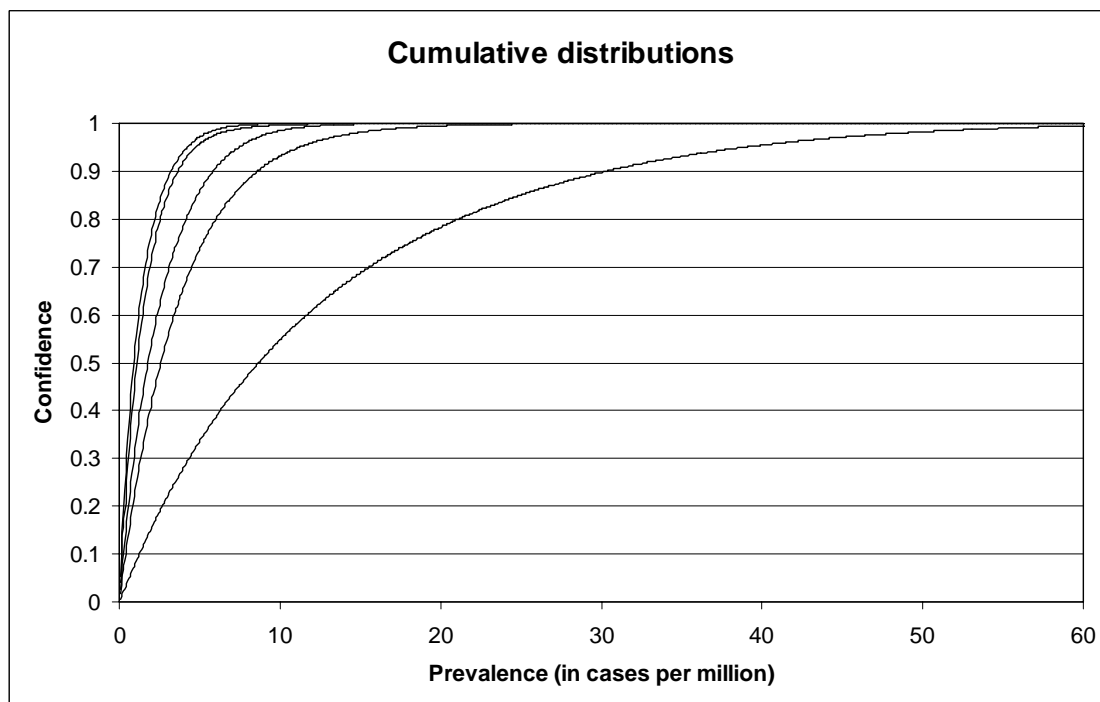
⁶ Software used is @Risk© 4.5.5 (Palisade) and Splus© 6.2 (Insightful); test sensitivity, 0.9; specificity, 1.0.

Figure 3: Cumulative uncertainty distribution of the BSE prevalence in the EU sheep population



From Figure 3, it was seen that there is a 95% confidence that the number of cases is equal to or below 4 BSE cases per million sheep; at a 99% confidence level, the number becomes equal to or below 6 cases per million. Since no BSE case has yet to be confirmed in sheep, the most likely prevalence is zero.

Figure 4: Cumulative uncertainty distribution of the BSE prevalence in the EU sheep population. Influence of the sensitivity of the screening test.



The different cumulative uncertainty distributions in the graph illustrate from the left to the right the increase in uncertainty on the prevalence given different sensitivities of the screening test (90%, 70%, 50%, 30% and 10%).

Figure 4 illustrates how the cumulative uncertainty distribution of BSE prevalence in sheep varies with the operational sensitivity of the primary screening tests used to detect suspects. For passive surveillance, this sensitivity will depend on the veracity of reporting notifiable disease and the vigilance of inspection officers at slaughter; for cases detected by rapid testing of healthy-slaughter animals then the numbers will depend of the proportion of the whole population tested and the operational sensitivity of the primary screening tests; all tests approved for screening for TSEs in small ruminants in the EU have diagnostic specificities and sensitivities of ~100% (with 95% confidence, 98-100%) although they vary in their analytical sensitivities and hence their potential to detect an infected, pre- or sub-clinical animal (EFSA scientific report 2005a; EFSA scientific report 2005b).

4.2.3 Conclusions

In 2005-2006, in Norway and the EU25, 397 cases of TSE were confirmed following screening of 411 567 healthy-slaughter sheep; 125 of these cases were tested using the EU CRL-approved discriminatory tests and no BSE was found (section 4.2.1). Broadening the analysis to include retrospective testing and all exit streams, 7898 cases of TSE have been confirmed following 1 508 410 tests; 4122 of these cases were tested using EU CRL-approved discriminatory tests and no BSE was found (section 4.2.2). However, this can not be interpreted to imply that there are no BSE-infected sheep in the European flock because not all animals (even those slaughtered for human consumption) are tested and the screening tests themselves have variable and, largely, undetermined sensitivities for detecting an infected but pre-clinical animal.

Depending on the statistical model and the sub-set of input surveillance data, it was calculated that there is 95% confidence that in the UK there is less than 2-4 BSE cases per 10,000 healthy-slaughter animals and, combining data from other countries with a substantial BSE history (Ireland, France, and Portugal), there is a 95% confidence that in this sub-group of high risk countries there is less than 0.3-0.5 cases of BSE per 10,000 healthy-slaughter animals.

The BIOHAZ panel also noted that, axiomatically, assuming lower sensitivities for the TSE screening and discriminatory tests will give higher prevalence estimates and further experimental evaluation of these parameters should be considered.

4.3 Distribution of infectivity in tissues and infectious load

4.3.1 Infectivity of tissues of sheep infected with BSE and scrapie agents

Historical findings on mouse bioassay of infectivity in sheep and goat tissues from naturally occurring pre-clinical and clinical cases of scrapie are given in Annex 2. These are reproduced here as they have previously provided the principal data on infectivity of tissues in sheep and goats infected with the scrapie agent (see EC 2002b). Clearly, the data have the limitations of the methods' sensitivity, the lack of PrP genotype data on the subjects and do not reflect our knowledge of the pathogenesis of experimental infection of sheep with cattle BSE.

The SSC Updated Opinion on TSE infectivity distribution in ruminant tissues (EC, 2002b) considered that, pending more experimental data becoming available, the historical data presented in Annex 2 for scrapie could be used to infer risk of BSE. An important exception was stated, namely that lympho-reticular tissues in sheep with BSE should, provisionally at least, be considered comparable in their level of infectivity with central nervous system tissues. Since then, several pathogenesis studies have been initiated to test this assumption but although qualitative data have been reported on the extra neural distribution of PrP^{Sc} and infectivity in ARQ/ARQ sheep experimentally infected with BSE by the oral route (up to 16 months p.i.) (Bellworthy *et al.*, 2005a), there is no information on relative titres compared to the CNS.

In the same series of experiments, there was no indication of clinical disease nor evidence of PrP^{Sc} accumulation in the brain, lymphoid tissue, viscera or peripheral nervous system of ARQ/ARR heterozygote and ARR/ARR homozygous sheep up to 46 months post-inoculation.

Of interest, but less relevant because of its more abnormal route of challenge, was the observation of abnormal prion protein (by Western blotting and immunohistochemistry) (Ronzon *et al.*, 2006) and infectivity (by transmission to transgenic mice overexpressing the ovine PrP gene) (Cordier *et al.*, 2006) in the CNS of a healthy ARR/ARR sheep six years after intra-splenic injection of BSE-affected cattle brain homogenate (0.5 g). The peripheral nervous system and lymphoid tissues (lymph nodes, tonsils, spleen and digestive tract) of this animal were negative when examined by a sensitive IHC method (Bencsik and Baron, J. Infect. Dis., in press).

In sheep, meat has not previously been considered a risk tissue, but in cases of natural scrapie, PrP^{Sc} has been recovered from muscles using IHC, ELISA and WB in clinically affected animals (Andreoletti *et al.*, 2004) and similar data have been found in sheep orally infected with BSE-affected cattle brain homogenate (Andreoletti *et al.*, personal communication).

4.3.2 Blood

One of the critical unknowns (for any naturally occurring TSE in any species) is that related to the possibility of infectivity in blood. Information on the incubation stage(s) wherein this happens is meagre and it is not yet known if this is an inconsistent chance event or an important way in which the pathogen spreads within the body. The data published by Houston *et al.* (2000) and Hunter *et al.* (2002) showed that a high volume blood transfusion from sheep to sheep can transmit BSE as well as scrapie within the same species. With both diseases, infectivity could also be transmitted using blood taken during the asymptomatic incubation period of the disease in the donor sheep.

More recent studies have removed the doubt expressed in the SSC 2002 Opinion that these transmissions were authentically caused by transfusion, as it has been conclusively shown that the BSE phenotype of the donor is conserved in the infected recipient (Siso *et al.*, 2006). Little in terms of infectivity, data relative to the incubation period can be drawn directly from these results, although some preliminary modeling has estimated that infusion of a unit of

sheep blood (400 ml) is equivalent to or greater than a dose of 200 mg of BSE-affected cattle brain⁷.

4.3.3 Intestine

Processing of intestines for use in the preparation of sausages was specifically considered in the SSC 2002 Opinion supplementary report. The infectious load in intestine and ancillary tissues relating to its “desliming” during the preparation of sausage casings are covered below.

PrP^{Sc} in the digestive tract has been described in sheep exposed to natural scrapie (Van Keulen *et al.*, 1999); (Andreoletti *et al.*, 2000). Most of the data available were obtained in natural scrapie and cannot be directly extrapolated to BSE infection in sheep although, in agreement with previous SSC Opinions, it was acknowledged that the spread of PrP^{Sc} and the structures where it accumulates appear similar in both sheep naturally exposed to scrapie or orally challenged with BSE. The prion protein genotype of the sheep is a critical factor in the uptake and dissemination of the agents of BSE and scrapie in the gut of the sheep.

In VRQ/VRQ sheep exposed to natural scrapie infection, PrP^{Sc} can be detected in ileal Peyer’s patches (PP) from 21 days post-partum and in other PP’s of the alimentary canal and in the tonsil of the lamb by 60 days of age. In similar conditions, PrP^{Sc} is detectable in the enteric nervous system (ENS) at 7 months old, almost three months prior to its first detection in the obex (Andreoletti *et al.*, 2000). Hence, during surveillance, screening the obex using rapid testing for PrP^{Sc} is a poor indicator for the absence of TSE infection in the digestive tract of the lamb.

Sheep with at least one ARR PrP allele have relatively lower susceptibility to clinical TSE disease than sheep homozygous or heterozygous for the other common alleles (e.g. VRQ, ARQ) and, in infected ARR/xxx⁸ animals, the levels and rate of accumulation of PrP^{Sc} are lower in both CNS and non-CNS tissues than in ARQ/ARQ sheep (Bellworthy *et al.*, 2005a). In ARR/ARR sheep⁹, PrP^{Sc} has been reported in spleen following oral dosing with BSE-infected brain homogenate (Andreoletti *et al.*, 2006) but in other BSE oral-challenge studies, to date, no PrP^{Sc} or infectivity has been found in gut-associated lymphoid tissue or the CNS in ARR/ARR sheep (see above, (Bellworthy *et al.*, 2005b)).

In France, estimations have been made of the impact of the “desliming” industrial process on level of PrP^{Sc} in intestine from scrapie-affected sheep. Detailed results of these investigations were presented in two AFSSA reports. These investigations concluded that (i) there was a mean 5-fold reduction in the amount of PrP^{Sc} during processing but that (ii) structures (ENS and lymphoid tissues) associated with the accumulation of PrP^{Sc} were only partially removed.

⁷ This amount of BSE-affected cattle brain produces disease when given intravenously into Cheviot sheep in 702 days (SD, 61 days; n =8/8); the codon 136/154/171 genotypes of these animals were either ARQ/ARQ (1) or ARQ/AHQ (7) but all were homozygous for P (not L) at codon 168. Transfusion of a unit of blood from BSE-affected sheep donors into the same breed and codon 168 genotype of sheep produced disease in 560 days (SD, 32; n =3/3) (Goldmann *et al.*, 2006).

⁸ xxx = any PrP allele except ARR.

⁹ ARR/ARR sheep are highly resistant to developing experimental BSE (and natural scrapie) and breeding such animals is considered a strong deterrent against the introduction and spread of BSE and scrapie within a flock (EFSA, 2006). However, robust traceability linking tissues such as intestine with the donor sheep, especially the young sheep providing sausage casing precursors, and its genotype are a pre-requisite of good safe sourcing incorporating this approach (EC, 2002a). In the absence of this traceability, AFSSA advised that all intestine from duodenum to rectum, not simply ileum, be treated as SRM (AFSSA report 2006, Annex). EFSA recognized this as a management rather than science-based factor.

These analyses indicate processing may be less efficient in reducing a TSE risk than had previously been estimated in the SSC 2002 opinion on safe sourcing of small ruminant materials.

4.3.4 Milk

Several qualitative risk assessments of the safety of bovine milk and milk from sheep and goats (SR) have been made in the past.¹⁰ It was concluded that the risk from milk from cattle, sheep and goats to humans was considered negligible but needed to be kept under review. AFSSA considered a QRA for milk in the event of BSE being present in small ruminants (2005) and came to a similar conclusion, although they emphasized that the safety of milk products from small ruminants could not be considered as equivalent to that of comparable products made using milk from cattle.

Some recent SSC and EFSA opinions have considered a QRA for milk from small ruminants¹¹. These opinions recognized the qualitative risk for humans and provided an approach to reducing that risk but made no estimation of the risk for humans or a quantitative calculation.

One of the main difficulties is that there is no published information on infectivity of sheep milk although important studies involving milking BSE-affected ewes and feeding this milk to neonatal lambs are underway (UK, Defra project SE1855). Biochemical analysis for PrP^{Sc} (Everest *et al.*, 2006) or intra-cerebral inoculation into bovinised or ovinised transgenic mice (Buschmann and Groschup, 2005) may allow detection of any infectivity in sheep milk; similar approaches applied to bovine milk and colostrums have given negative results.

The BIOHAZ panel noted that recent information suggesting that inflammatory changes occurring in various tissues, notably kidney and mammary gland (see below), at any stage of incubation may facilitate the potential for spread of TSE infectivity in that tissue or organ (Andreoletti *et al.*, 2004); (Heikenwalder *et al.*, 2005) or modify susceptibility of that tissue or organ to such spread (Gruner *et al.*, 2004). This ectopic replication requires careful consideration with respect to the safety of sheep milk. Indeed, this observation appears to be supported by the recent results obtained by Ligios *et al.* (2005) where sheep naturally infected with scrapie, and having co-incident lympho-follicular mastitis accumulated PrP^{Sc} in the mammary gland, in cases of lenti-viral and TSE co-infected sheep.

4.3.5 Biochemical approaches to “prion” quantification in tissues

According to the prion hypothesis, PrP^{Sc} is an infectious protein and the causative agent of TSEs (Prusiner, 1982). In TSEs, the accumulation of PrP^{Sc} in tissues of infected individuals is correlated with the presence of infectivity (McKinley *et al.*, 1983, and *inter alia*, Race *et al.*, 2001). While titration of infectivity through bioassay remains the only effective tool for quantifying natural TSE agent, the development of sensitive PrP measurement tools, combined with the use of recombinant PrP as external standard, has allowed a robust quantification of PrP^{Sc} in various tissues (Gatti *et al.* 2002; ti *et al.* 2004). In recent studies,

¹⁰ In a number of former opinions of the Scientific Veterinary Committee (1996), the Multidisciplinary Scientific Committee (1997) and the Scientific Steering Committee (SSC) (i.e. SSC Opinion on vertical transmission of 1999 as well as in a number of SSC opinions on TSE in small ruminants such as the SSC Opinion of 10-11 January 2002 “TSE infectivity distribution in ruminant tissues” and the document, “Safety of milk with regards to TSE: State of affairs”, Adopted by SSC on 28-29 June 2001).

¹¹ EC 2002a, EFSA 2003, EFSA 2005a, EFSA 2005c.

the PrP^{Sc} quantities (after PK digestion) were compared to infectious titre as assessed in a transgenic (VRQ PrP protein) ovine mouse model (Andreoletti *et al.* 2004) and an apparent linear relationship was established over a limited range of PrP concentrations. In this experiment infectious titre could still be detected in the absence of a PrP^{Sc} positive signal ($\sim 10^2$ LD₅₀ per g).

Not surprisingly the biochemistry of PrP varies with the type of TSE disease as illustrated recently by atypical scrapie in sheep. Le Dur and colleagues titrated a “discordant” case of sheep TSE in tg338 mice over-expressing the VRQ allele of ovine prion protein (Le Dur *et al.*, 2005). They found high levels of infectivity ($> 10^8$ LD₅₀ per g) in brain with a very low content of protease-resistant prion protein. In a recent field trial, no PrP^{Sc} signal could be detected below 1/500 dilution by any rapid tests in cerebral cortex of Nor98 atypical cases (EFSA, 2005a).

4.3.6 Conclusion

Taken together these data appear to indicate:

- (i) The correlation between the amount of PrP and infectivity depends on the type of TSE agent
- (ii) Sensitivity of abnormal PrP biochemical detection is still lower than most efficient bioassays: failure to detect abnormal PrP does not guarantee absence of infection in a tissue

New methods of detection of abnormal PrP are promising (*e.g.* PMCA, Castilla *et al.* 2005) but will require further refinements in terms of robustness and repeatability before they can be used to quantify prion protein in sheep tissues and body fluids.

Bioassay in rodents can, in itself, be insensitive to infectivity compared to transmission studies of scrapie and BSE within the same species (*e.g.* cattle and sheep). Transgenic mice over-expressing ovine or bovine prion protein have been used to improve sensitivity and the efficiency of transmission from cattle or sheep tissues. However the quantitative significance of these transmissions and their interpretation in terms of human or native species risk as a basis for defining or updating SRM lists is still under discussion.

While absolute quantification of prions by biochemical methods is difficult, and the experiments needed to correlate their outputs to bioassay titres costly and time-consuming, measurements of abnormal PrP in two tissues of the same animal may be compared as a first approach to an assessment of the ratio of infectivity in each tissue, and their intrinsic relative risk following exposure to humans. Some data illustrating this approach are presented in Annex 1, where ratios of 1:50-100 for lymphoid tissue: CNS and 1:1000 for muscle¹² : CNS were obtained in a comparison of tissues from two ARQ/ARQ sheep orally infected with BSE-affected sheep brain homogenate. Encouragingly, these ratios are similar for those derived for the relative risks of scrapie from the mouse bioassay data in Annex 2.

¹² This is the oculomotor muscle of the eye and this 1:1000 estimate is based on the highest abnormal PrP measurement of 1 pg/mg on 10 samples from the same animal; therefore it should be regarded as “worst case”.

4.4 Species barrier (SB)

4.4.1 Definition

The concept of a barrier to transmission of TSEs between species is critical in assessing the risk for humans of developing variant CJD after consumption of BSE-infected ruminant tissues or by-products (Cousens *et al.*, 1997). Experimental infection of one species by exposure to infected material from another species is usually an inefficient, stochastic process that results in prolonged survival times that shorten on re-cycling and re-infection within the new host. This prolongation of incubation times, and the apparent resistance of some animals to infection acquired from another species, is termed ‘the TSE species barrier’ (Dickinson, 1976). By definition, for cattle-to-cattle (or human-to-human) transmission, there is no species barrier although in other species, notably sheep, the effects of agent strain and host PrP genetics may combine to create an apparent within-species barrier to transmission of disease (see SSC Opinion “The policy of breeding and genotyping sheep for resistance to scrapie” Adopted 22-23 July 1999) and may do so in man to an extent that is highly uncertain for secondary vCJD.

Quantitatively, the relative species barrier for oral transmission of TSE from species A to species B ($SB^{A \rightarrow B \text{ oral}}$) can be defined as:

$$SB^{A \rightarrow B \text{ oral}} = \text{Titre of [IA]}^{\text{oral}} \text{ in species A} / \text{Titre of [IA]}^{\text{oral}} \text{ in species B}$$

where [IA] is the infectious inoculum derived from species A. In practice, very few experimental estimates of the relative species barrier have been made, and these have almost invariably been estimated using the intracerebral route of infection. For example, the cattle-mouse barrier to intracerebral infection has been calculated at 1,000-100,000 but estimates of $SB^{\text{cattle} \rightarrow \text{mouse oral}}$ have been confounded by the poor oral transmission rates of cattle BSE to mice (Middleton and Barlow, 1993). For obvious reasons, no experimental estimate of the sheep-to-human species barrier has been made from any sheep PrP genotype infected with any of the three categories of small ruminant TSE: classical, atypical or “BSE in sheep” (EFSA opinion, 2005a).

4.4.2 Quantitative estimates of animal-to-human BSE transmission barriers from epidemiological studies

In previous SSC and EFSA risk assessments, it was recommended that, in the absence of data, the cattle to human species barrier should be set at 1. Revision of this conservative figure has been proposed in the light of these new estimates of exposure and of the size of the UK vCJD epidemic. To date there have been a total of 165 probable or confirmed cases (deceased or alive) of vCJD in the United Kingdom, and it now seems that there has been a slowing down in the rise of the numbers of vCJD cases (CJDSU website: 2nd March 2007, <http://www.cjd.ed.ac.uk>). Current estimates of the total size of the vCJD epidemic have reduced significantly from the high numbers thought possible a few years ago to an upper limit of 550 in a recent report (Clarke & Ghani, 2004). In fact Clarke and Ghani give a best estimate of 70 future deaths in methionine homozygous (MM) individuals, and stated that “*even in the worst case scenario, when non-MM homozygous individuals are equally susceptible but have longer mean incubation period than MM homozygous individuals, the best estimate of the potential scale of the epidemic is unlikely to exceed 400 future cases.*” (Clarke and Ghani, 2005). From this upper estimate, the species barrier for MM individuals may be of the order of 4000.

Furthermore, in a simulation to estimate the number of future vCJD cases in France, Chadeau-Hyam and Alperovich (2005) worked out (assuming an age-dependant susceptibility) the number of infectious units (CoID₅₀) to cause one human infection to be 280 (95% CI, 167-1382) units for the youngest cohort and 420* (95% CI, 106-972) units for the older cohort.

4.4.3 Inferences from experimental studies

An empirical estimation of the cattle to human species barrier by the oral route has been proposed as 7-20 fold, but this is based on an extremely small amount of data from oral challenges of macaques with infectious cattle brain (Lasmézas *et al.* 2005). The BIOHAZ panel considered that these data were not relevant to estimation of a sheep-to-human or cattle-to-human species barrier, particularly as there are many precedents for modification of TSE agents after passage through another species (Bartz *et al.*, 1998; Nonno *et al.*, 2006). Furthermore, the statistical value of data based on only two animals is questionable and calls into doubt the significance of the paper for public health.

Many historical (Collinge *et al.*, 1995; Asante *et al.*, 2002) (Telling *et al.*, 1994; Hsiao K *et al.*, 1994) and recent studies (Taguchi *et al.*, 2003a; Espinosa *et al.*, 2007; Buschmann *et al.*, 2006; Asano *et al.*, 2006a; Beringue *et al.*, 2006a; Baron *et al.*, 2006a; Bishop *et al.*, 2006) (Taguchi *et al.*, 2003b; Asano *et al.*, 2006b) using transgenic mice expressing human or other heterologous prion proteins in the presence or absence of mouse prion proteins have attempted to characterise the mechanisms underlying the species barrier effect but their interpretation remains controversial. Similarly, *in vitro* conversion studies have replicated aspects of the host range, strain specificity and efficiency of the change from normal prion protein to its abnormal isoform following incubation with PrP^{Sc} (Kocisko D A *et al.*, 1995b; Bessen R A *et al.*, 1995b; Kocisko D A *et al.*, 1994b; Bessen R A *et al.*, 1995a; Kocisko D A *et al.*, 1994a; Kocisko D A *et al.*, 1995a; Raymond *et al.*, 1997; Kirby *et al.*, 2003). These studies have re-inforced the rationale that homologous protein interactions influence the efficiency of conversion (and by implication, replication of infectivity and transmissions to other species) but that, as with transmissions to transgenic mice, other factors are also important. Therefore the species barrier cannot be quantified *a priori* using this empirical approach.

4.4.4 New types of BSE in cattle and the variable pathogenicity of scrapie strains

Discriminatory testing for BSE in sheep is based on immuno-histochemistry (IHC), ELISA and/or western blotting of brain (or lymphoid tissue) for abnormal prion protein in animals experimentally challenged with infectious material taken from cases of cattle BSE defined as “typical” or consistent with the original clinical and histopathological case definition of the disease (Wells *et al.*, 1987). The methodologies have been evaluated, and shown to be fit-for-purpose, in an EU-wide ring trial using a variety of test materials covering oral and intracerebral (ic) challenged sheep of a limited range of PrP genotypes, and include cases of secondary ic transmission of sheep BSE to sheep (Stack *et al.*, in preparation). However, the sample-set was necessarily restricted to what was available and no meaningful estimate of the sensitivity and specificity of these tests has been made. Nor were these tests evaluated for their ability to discriminate types of BSE (cattle TSE) now regarded, on molecular and histopathological criteria, to lie outside the original case definition of BSE (Buschmann *et al.*, 2006; Beringue *et al.*, 2006a; Baron *et al.*, 2006a; Casalone *et al.*, 2004; Beringue *et al.*, 2006b; Baron *et al.*, 2006b). These forms of cattle TSE have been dubbed H-type BSE and L-type (or BASE) BSE. The significance, origin and transmissibility of these H- and L-types of BSE to sheep are only speculative at present and the BIOHAZ panel recommended a

watching brief on this area to monitor its relevance to the TSE risk of human consumption of ovine products. Similarly, the potential variable pathogenicity of scrapie strains (such as atypical scrapie) and their modification on transmission through a third species which may affect barriers to transmission to humans remain speculative and need continual review.

4.4.5 Conclusion

The BIOHAZ panel considered that these data were not directly informative in quantifying a sheep-to-human species barrier particularly, as referenced in section 4.4.4, since there are many precedents for modification of TSE agents after passage through another species. For the purposes of a QRA for sheep products, the BIOHAZ panel confirmed the use of the previous assumption of a species barrier of 1 for putative sheep-to-human transmissions of BSE.

4.5 Human consumption

There are few estimates of the level of consumption of sheep meat and meat products in the European Union and most surveys focusing on nutrition and diet (e.g. the EPIC survey, www.iarc.fr/epic) include these foods under the cover of more general food categories. Exceptions have included two surveys in Italy and Great Britain and these have been used to check if Eurostat data on the production of ovine carcasses, import and export in the EU15 countries since 1995 could be used to validate EU-wide per capita annual intake data.

4.5.1 The Italian Survey

The Italian INN-CA survey covers the 1994-1996 period (Turrini *et al.*, 2001). It randomly sampled 1,147 households and involved 1,978 individuals of all ages. A self-compiled 7-day food diary was used to gather data for analysis by sex and age categories. Among the food items “sheep meat” was specified. The percentage of (sheep meat) consumers and daily food intake by the total sample of individuals or by “consumers only” were calculated. An overall mean daily intake was calculated of 3.2 g (median 0.0g). Understandably, consumption was higher in the “consumers only” group (which represented 7.9% of the sample population) with a mean and median intake of 40 and 25 g respectively.

4.5.2 The British Survey

The National Diet and Nutrition Survey of Great Britain is based on a representative multistage random sample of 2,251 households in GB. One individual per household, 19 to 64 years of age, was requested to compile a 7-day food diary which included the category “lamb and dishes”. As in the Italian survey the consumption figures were expressed by the total sample of individuals or by “consumers only” (22% of total). The mean 7-day intakes were 51 g for the total sample and 226 g (median, 150 g) for “consumers only”.

The Italian and GB weekly intake were therefore comparable (22.4 g (Italy) vs. 51 g (GB) in the whole sample population, and 280 g (Italy) vs 226 (GB) in the “consumers only” group). (<http://www.food.gov.uk/multimedia/pdfs/ndnsprintedreport.pdf>, accessed on 7th March 2006).

4.5.3 *Calculation of sheep meat consumption from Eurostat figures and comparison with survey data*

Consumptions inferred from Eurostat data were compared with the intakes calculated in the GB and Italian surveys. Figures on “sheep carcass production” (A), “import of all type of sheep meat” (B) and “export of all type of sheep meat” (C) were used to extrapolate the “sheep meat gross apparent consumption” (A+B-C). These figures are tabulated for Italy in Table 3, 4 and for GB in Table 5, 6.

Table 3: Eurostat (Italy) data relating to sheep products (1994-1996).

Period	Carcass production (1,000 tons)	Import	Export	Extrapolated gross apparent consumption
Jan.-Dec. 1995	72.0	20.8	0.7	92.1
Jan.-Dec. 1996	73.6	20.5	1.2	93.0

To estimate the total Italian annual consumption of meat sheep based on the INN-CA survey data, the mean daily intake during the 1994-1996 period was attributed to the overall Italian population and these figures are given in Table 4.

Table 4: Total Italian annual consumption of meat sheep based on the INN-CA survey data (1994-1996).

IT population (Millions)	Daily Intake (g)	Annual Intake (Kg)	Total IT Annual Consumption (1,000 tons)
56.8	3.2	1.2	66.4

The figures obtained in the last column are about 70% of those calculated on the base of Eurostat figures. The discrepancy may be because Eurostat’s carcass¹³ production is a wider category than the “sheep meat” category of the INN-CA survey.

¹³ Carcass weight is the weight of the slaughtered animal’s cold body after having been bled, skinned and eviscerated, and after removal of the head (severed at the atlanto-occipital joint), of the feet (severed at the carpo-metacarpal or tarso-metatarsal joints), of the tail (severed between the sixth and seventh caudal vertebrae) and of the genital organs (including udder). Kidneys and kidney fats are included in the carcass.

Table 5: Eurostat (UK) data relating to sheep products (x1000 tons) for 2000-2001.

Period	Carcass production (1000 tons)	Import	Export	Extrapolated gross apparent consumption
Jan.-Dec. 2000	360.7	108.6	98.2	371.2
Jan.-Dec. 2001	259.3	92.9	30.2	322.0

The UK survey data represents the weekly “lamb and dishes” consumption of one 19 to 64 years old individual per household. This item is consumed by 22% of the interviewed people.

Table 6: Total GB annual consumption of meat sheep based on 2001 survey data.

Year	19-64 years UK pop. (millions)	Weekly Intake (g)	Annual Intake (Kg)	Total UK Annual Consumption (1,000 tons)
2001	35.4	51	2.7	93.9

The 19-64 age class accounts for 60.2% of the total 2001 UK population. However even in the case in which the 100% of the population were available instead of the subset 19-64 years, the Eurostat data would suggest higher consumptions. Thus, the UK survey data represents an underestimate of the true consumption. Using the survey data, only “lamb and dishes” are considered: it is likely that part of the sheep meat is also included in the consumption of items such as “burger & kebab”, “meat pies and pastries”, “other meat and meat products”.

4.5.4 Calculation of sheep meat consumption in the EU15 from Eurostat figures

The sheep meat consumption in the EU15 for the period 1995-2004 calculated as above from Eurostat figures is tabulated in Annex 3. In applying the methodology of sections 4.5.2 and 4.5.3, adjustments needed to be made to take into account imports from BSE free-countries such as Australia and New Zealand. Figures for imports of sheep or goat meat (fresh, chilled or frozen) are tabulated in Annex 4; live imports are negligible.

4.5.5 Caveats to the interpretation of estimates of sheep meat consumption

The following pitfalls, potentially affecting both validity and usefulness of the estimates obtained, must be taken in account:

- a) The “EU daily individual intake in g” in the Table in Annex 3 refers to a European average and only a proportion of the general population actually consumes sheep products. This leads to a large underestimate of the real intake among the exposed and vice versa: for example, in Italy, 3.2 g daily per person among the Italian population is the estimate but 40 g daily per person among the “consumers”.
- b) Large differences exist in the patterns of sheep meat consumption and this is shown clearly by comparing the mean and median values for a consumer category. For example, with Italian consumers in the INN-CA survey, the daily sheep meat intakes

have a mean of 40 g and a median value of 25 g; in other words, there may be a few individuals consuming a large amount of sheep meat – with the associated higher risk - when compared with the rest of consumers. This needs to be allowed for by factoring consumption into a quantitative risk assessment.

- c) Eurostat data (at the national or European level) do not allow discrimination between direct consumption (when sheep meat is consumed directly by the consumer) and indirect consumption (when the meat is transformed and blended into a general “meat” product). This was also acknowledged in the previous SSC Opinion on Human Exposure Risk (EC, 1999) and this “global” methodology for estimating sheep meat intakes does not take into account:
- how different risk tissues are used in the food chain,
 - the other routes (apart from ingestion) by which humans could be exposed,
 - which parts of the carcass is most used,
 - the age of the infected animal that is slaughtered and “normally” processed,
 - ethnic differences in consumption patterns,
 - cross-contamination of sheep meat and meat products with specified risk materials.

5. AFSSA OPINION

During the course of this mandate (25 August, 2006), the French Food Safety Authority (AFSSA) provided their experts’ opinion on “the risk assessment regarding the potential presence of BSE in small ruminants”, and their TSE SSC Epidemiological Workgroup Report on “TSE in the French sheep population: current situation – prioritisation of measures for improving surveillance with a view to protecting public health” for consideration by EFSA. Their mandate covered both the risk assessment and science of TSEs in sheep and management of that risk, and the report contains a sound review of the current TSE situation in France. Its arguments and logic are based on qualitative risk assessment because, like the EFSA WG, the AFSSA experts recognised that attempts at a quantitative assessment were premature. The list of questions the AFSSA experts were asked to address (see footnote¹⁴) included the focus of this present EFSA BIOHAZ Panel Opinion – assessment of the potential human exposure risk to the sheep BSE agent - although the key related issue of estimating the potential prevalence of BSE in sheep was deferred until their next mandate (Section 4, page 6, AFSSA Opinion). Amongst the other questions, several have been addressed, in the general context of all small ruminant TSEs, by recent EFSA BIOHAZ Opinions and will not be reiterated here (EFSA 2006 breeding for resistance, EFSA 2005c, EFSA scientific report 2005a; EFSA scientific report 2005b), while the remaining questions are on risk management

¹⁴ The AFSSA were asked to: a) assess the classification of the entire intestine as specified risk material (SRM) for animals born before 2002, b) update the risk assessment relating to milk produced by sheep, and c) evaluate the different screening programmes in abattoirs for small ruminants under 18 months of age. Additionally, they were asked to classify the expected benefits of various measures relating to SRM (intestine) removal, screening programmes, milk collection in affected livestock, genetic selection and flock register schemes; propose a sampling scheme for the surveillance of TSEs in sheep under 18 months of age; propose recommendations on the rapid TSE test to use for this and the sample to be analysed; and estimate the potential human exposure risk to the sheep BSE agent.

issues (e.g. traceability, flock registration, etc.) which, although important parts of safe sourcing of small ruminant materials, are beyond the Panel's risk assessment and communication remit.

The AFSSA report did, however, highlight the one issue that overlaps with our mandate and has not yet been dealt with elsewhere – namely, the importance of classifying all intestines of small ruminants as specified risk material (SRM), regardless of animal age and genotype. This extends beyond current requirements where the ileum is the only part of the intestine specified for removal as SRM, and those of the SSC Opinion “Safety with regard to BSE risks of sheep intestines and casings” (SSC, 12-13 September, 2002). The scientific basis for this conclusion by AFSSA was not clear, especially in the case of sheep carrying the ARR allele of the prion protein gene (and perhaps other genotypes with apparent specific resistance to development of clinical experimental BSE (ARP¹⁶⁸Q allele carriers, (Goldmann *et al.*, 2006)). While the study of Jeffrey and colleagues (Jeffrey *et al.*, 2006) quoted in its support that did observe transient (within 2h) uptake of PrP^{Sc} in the isolated gut loop epithelium after introduction of a large amount of infectious brain homogenate into the loop (in an ARR/ARR homozygous sheep), it failed to demonstrate either PrP^{Sc} or infectivity at subsequent time points. Furthermore no infectivity or clinical disease has been observed in the intestine or elsewhere in ARR/ARR homozygous sheep orally challenged with BSE (Bellworthy *et al.*, 2005b). In this respect, our current qualitative assessment remains in line with the past SSC Opinion (September, 2002), namely, that the residual BSE risk associated with intestines (excluding the ileum) is “likely to be small (negligible) if they are sourced according to the scientific principles regarding genotype, age, rapid TSE testing, early TSE testing, flock certification and geographical origin presented in the SSC Opinion of 4-5th April 2002.”

However, this assessment will require re-consideration if PrP^{Sc}/infectivity is ever found in the gut of a TSE-affected ARR/ARR sheep as the AFSSA report found the previous estimate of the reduction in risk due to processing of intestine in the manufacture of sausage casings to be inaccurate and overly optimistic.

6. CONCLUSIONS

6.1 General

The BIOHAZ panel retains the view that there are insufficient data for a quantitative assessment of the risks posed by tissues of sheep to human health in the case where BSE is confirmed in sheep. Moreover, even in the absence of quantitative information, recent data on the preclinical distribution of PrP^{Sc} and infectivity in skeletal muscle tissues and blood following experimental BSE challenge support their previous view that risk reduction strategies relying on SRM removal in sheep or goats will not be fully effective.

The continuing lack of comprehensive quantitative data on species barrier and infectious load with respect to BSE in small ruminants prevents any meaningful quantitative risk assessment and precludes revision of the recommendations in the opinions of the Scientific Steering Committee (EC 2002a) on safe sourcing of small ruminant materials of April 2002 and its complement of September 2002.

6.2 The key elements of a sheep QRA

Each element of a quantitative risk assessment of BSE transmission from sheep to humans was considered in the Report and these considerations are summarized below:

6.2.1 Prevalence of infection

Since the publication of the SSC, 2002 Opinion, the European Commission (EC 2002a) had sponsored large-scale surveillance for TSEs in small ruminants and, since 2005, each positive index case detected in a TSE-affected flock was required to be further analysed using newly-developed tests capable of *in vitro* discrimination of BSE and scrapie in sheep. The BIOHAZ panel reviewed the latest TSE prevalence figures for member states and the results of BSE/scrapie discriminatory testing. From these data, with certain assumptions, they were able to estimate the BSE prevalence in sheep.

The BIOHAZ panel recognised that this parameter would have the largest impact on any quantitative risk assessment. In 2005-2006, in Norway and the EU25, 397 cases of TSE were confirmed following screening of 411,567 healthy-slaughter sheep; 125 of these cases were tested using the EU CRL-approved discriminatory tests and no BSE was found. Broadening the analysis to include retrospective testing and all exit streams, 7,898 cases of TSE have been confirmed following 1,508,410 tests; 4,122 of these cases were tested using EU CRL-approved discriminatory tests and no BSE was found (section 4.2.2). However, this can't be interpreted to imply that there are no BSE-infected sheep in the European flock because not all animals, even those slaughtered for human consumption, are tested and the screening tests themselves have variable and, largely, undetermined sensitivities for detecting an infected but pre-clinical animal.

Depending on the statistical model and the sub-set of input surveillance data, it was calculated that there is a 95% confidence that in the high risk sub-group of countries (UK, France, Ireland and Portugal) there is less than 0.3-0.5 cases of BSE per 10,000 healthy-slaughter animals.

The scrapie/BSE discriminatory tests are robust judging by their performance against a small number of samples in a blinded ring trial organised by the EU TSE Community Reference Laboratory (Stack *et al.*, in preparation) and their application as part of small ruminant surveillance was continuing to improve the accuracy of these prevalence estimates. However, balanced against this optimistic scenario, the BIOHAZ panel accepted that the sensitivity and specificity of the discriminatory tests had, for logistical reasons, not been experimentally evaluated and potential confounding factors, such as concomitant infection of the same animal with scrapie and BSE, remained to be investigated.

Evaluation of these test parameters was therefore required, in tandem with continued surveillance, in order to improve the accuracy of these prevalence estimates. In the interim, other control measures - especially SRM removal, and breeding for resistance - needed to continue in order to further reduce human exposure to BSE should there be a sub-detectable level of infection in the sheep flocks of Europe.

6.2.2 Infectious load and distribution in tissues

Many factors affect the infectious load and tissue distribution in a TSE-affected sheep. Previous SSC opinions on the safe sourcing of tissues from small ruminants (EC 2002a) integrated modelling studies and limited data of titre in scrapie-affected sheep to estimate risk

in mouse ic ID₅₀ units per tissue throughout the incubation period of a susceptible sheep exposed to BSE. Much of the experimental data on tissue distribution and infectious load accruing from current BSE challenge studies was found to be qualitative. Spreading of the agent in BSE affected sheep is large and may affect secondary lymphoid tissue, skeletal muscle and blood which implies that a risk reducing strategy based on tissue removal (according to the currently defined list) would be insufficient. The BIOHAZ panel considered more recent attempts at quantifying the risk specifically from ovine BSE and reviewed biochemical approaches to quantifying titres in affected animals. However, the influence of age and genotype on the distribution of BSE infectivity in sheep in quantitative terms is poorly documented.

The BIOHAZ panel agreed that absolute quantification of prions by biochemical methods was difficult, and the experiments needed to correlate their outputs to bioassay titres costly and time-consuming, but that measurements of abnormal PrP in two tissues of the same animal might be compared as a first approach to an assessment of the ratio of infectivity in each tissue, and their intrinsic relative risk following exposure to humans. However, to date, comprehensive data of this nature were not available to facilitate a QRA. Even in the absence of this data, the BIOHAZ panel concluded that SRM removal alone was unlikely to be sufficient to eliminate the residual BSE risk to the consumer from a BSE-infected sheep carcass.

6.2.3 Species barrier

We cannot quantify a species barrier effect for specific TSEs such as BSE transmitting from sheep to humans from the available epidemiological data. Estimates for the barrier to transmission of BSE from cattle to human vary from 70:1 to more than 4,000:1 but accepting these figures in a QRA for the sheep to human barrier assumes no lowering of the effect on passage through the bridging species (sheep) – a phenomenon documented recently in laboratory facsimiles of cross-species transmission in transgenic mice. In alignment with past SSC opinions, the BIOHAZ Panel assumed that there is no intrinsic species barrier for sheep BSE transmission to humans.

6.2.4 Human consumption levels

Large amounts of sheep meat and other products are consumed daily within the EU, some produced within member states and some imported from other countries. Surveys of sheep meat and meat product consumption in Italy and GB gave intake estimates broadly in agreement with the overall trade data for sheep carcasses for these countries from Eurostat. This gave some confidence in using Eurostat data for the EU15 to estimate an average daily per person intake of sheep meat or meat products of 8.4-9.3 g, although large differences in the patterns of consumption by individuals were apparent judging by gaps between mean and median consumption values seen in surveys in Italy and GB. Amongst several other caveats in any future attempt to quantify the BSE risk associated with this consumption, the BIOHAZ panel recognized that imports from countries such as New Zealand and Australia, categorized as negligible BSE risk countries, would also need to be taken into account.

7. RECOMMENDATIONS

1. More detailed evaluation of the screening and discriminatory TSE test parameters are recommended, in tandem with continued surveillance, in order to improve the accuracy of the BSE in sheep prevalence estimates. To facilitate this, all TSE positive cases including secondary cases diagnosed in infected flocks should be systematically submitted for discriminatory testing.
2. Emerging data on the early accumulation of abnormal prion protein and infectivity in peripheral tissues of sheep exposed to natural scrapie and BSE before their detection in the CNS re-inforce the value of maintaining current measures – especially SRM removal, and breeding for resistance¹⁵ - previously recommended to reduce human exposure to TSEs.
3. Further surveillance and multi-disciplinary investigation of all animal TSEs, including atypical types of scrapie and BSE, are recommended to evaluate their implications for human health.

¹⁵ Breeding for resistance is recommended to reduce the infectious load in the sheep population of TSE (including BSE). (EFSA, 2006).

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Annex 1: Application of PrP^{Sc} quantification to experimental BSE sheep tissues¹⁶

Tissues from sheep fed with ovine BSE-affected sheep brain homogenate were used in this experiment to quantify abnormal prion protein. PrP^{Sc} detection was carried out using immunohistochemistry (IHC), ELISA and Western-Blot (WB). PrP^{Sc} IHC detection was performed using BAR221 antibody (raised against recombinant ovine protein: amino-acids encoded by codons, 141-152). ELISA measurements were performed using the TeSeE® sheep/goat kit (Bio-Rad). Western-blot (preceded by immuno-concentration) was performed using SHa-31 which binds the 145-152 sequence of PrP [YEDRYYYRE].

In ARQ/ARQ animals, PrP^{Sc} was detected by IHC in various lymphoid tissues as early as 4 months post-challenge (pc) and in the CNS (obex and medulla) from 10 months pc. At 10 and 19 months pc., the PrP^{Sc} accumulation in secondary lymphoid organs was substantial, consistent with previous data (Bellworthy *et al.*, 2005). PrPres was measured by ELISA kits in a panel of 80 tissue including various lymphoid tissue and CNS.

In that experiment the number of included animals was low and additional BSE infected sheep tissue should be considered before drawing any definitive conclusion on relative infectivity. However data collected in other experiments with scrapie, under natural infection conditions or experimental oral infection, led to similar ratio of PrPres accumulated in lymphoid or muscle tissue / CNS.

¹⁶ Tissues from Suffolk lambs (ARR/ARR, ARR/ARQ and ARQ/ARQ) orally challenged with two doses of 2.5g of BSE-infected sheep brain before 24 hours of age and at two weeks of age. Sequential autopsies of ARR/ARR (=3), ARR/ARQ (n=3) and ARQ/ARQ (n=2) lambs at 4 month, 10 months of age and at time of clinical signs were executed. Clinical signs in ARQ/ARQ animals were observed at 19 months old. To date no clinical signs have been observed in the remaining ARR/ARR (n=4) and ARQ/ARR (n=3) animals (42 months post challenge).

Table 1: PrP^{Sc} quantification in experimental BSE sheep tissues

		4 months	10 months	19 months
Tonsil	Animal 1	-	85 pg/mg	209 pg/mg*
	Animal 2	-	1 pg mg	256 pg/mg
Ileal PP	Animal 1	19 pg/mg	259 pg/mg	231 pg/mg
	Animal 2	105 pg/mg	56 pg/mg	56 pg/mg
Spleen	Animal 1	57 pg/mg	33 pg/mg	62 pg/mg
	Animal 2	42 pg/mg	114pg/mg	31pg/mg
Obex	Animal 1	-	-	1,124 pg/mg
	Animal 2	-	17 pg/mg	1,011 pg/mg
Th6	Animal 1	-	10 pg/ml	474 pg/mg
	Animal 2	-	3 pg/ml	530 pg/ml
Mesenteric LN	Animal 1	1 pg/mg	118 pg/mg	109 pg/mg
	Animal 2	2 pg/mg	51 pg/mg	70 pg/mg
Oculomotor muscle	Animal 1	ND	ND	1 pg/mg**
	Animal 2	ND	ND	1 pg/mg**

* ELISA signal rates (pg of ovine VRQ recombinant protein/mg of fresh tissue)

** Highest observed values in ten individual samples from same animal.

According to the realized measurements,

- PrPres lymphoid tissue (terminally affected animals) / CNS ratio ranged between 1/50 to 1/100
- PrPres muscle(when the sample is positive) / CNS was over 1/1000

Annex 2: Infectivity of tissues in sheep and goats with natural scrapie

Table 1: **Natural scrapie in sheep and goats: classification of tissues by agent titre in Swiss mice and by age, in pre-clinical and clinical cases of Scrapie in Suffolk sheep and in goats**¹⁷

(Unamended from Annex: Opinion on The Policy of Breeding and Genotyping of Sheep, 22-23 July 1999) (EC 1999)

Group	Infectivity Titre (approx.range)	PRE-CLINICAL				CLINICAL	
		SHEEP				SHEEP	GOATS
		≤8 months.(0/16)	10-14 months (8/15) ¹⁸	25 months (1/13)	> 25 months (1/6)	34-57 months (9/9)	38-49 months (3/3)
A	HIGH ≥ 4.0					Brain Spinal cord	Brain Spinal cord
B	MEDIUM 3.2 – 4.0		Colon-proximal, Ileum-distal, LN (RP/MP), Spleen	Colon-proximal, Ileum-distal, LN (RP/MP), Tonsil		Colon-proximal, Ileum-distal, Spleen, Tonsil LN (BM), LN (PF, 1/9 negative), LN (PS, 2/9 negative), LN (PR/MP), (rectum-distal+),	Colon-proximal, Ileum-proximal, LN (BM), LN (RP/MP), LN (s. mammary), Pituitary, (Rectum-distal +), Spleen
C	LOW ≤ 3.2 or titre unknown		LN (PS/PF) Tonsil	Brain (medulla/diencephalon), LN (BM), LN (PS/PF), Spleen		Adrenal, Bone marrow**, Colon-distal, CSF, Liver**, LN (s.mammary x2), Nasal mucosa, Pancreas **, Pituitary, Sciatic nerve, Thymus**, Placenta **	Adrenal, Colon-distal, CSF Nasal mucosa, Sciatic nerve, Thymus

¹⁷ After Hadlow *et al.* (1979, 1980, 1982), Pattison *et al.* (1964, 1972), Groschup *et al.* (1996). Regarding DRG: see text.

¹⁸ Techniques for the determination of infectivity become more and more sensitive. The age range may go below 10 months. In individual cases, tonsil infectivity has been detected in lambs of 16 weeks. Placenta has been placed in Group C, but titres are unknown.

Group	Infectivity Titre	PRE-CLINICAL				CLINICAL	
		SHEEP				SHEEP	GOATS
D	Undetectable	Ileum, LN (PS/PF) LN (RP/MP), Thymus, Tonsil Spleen	Blood clot, brain (medulla), Colon-distal, Faeces, LN (BM), Serum	Adrenal, Brain (cortex mid-brain), Colon-distal, LN (s. mammary), Nasal mucosa, Salivary glands, Spinal cord, Thymus	Colostrum	Blood clot, Foetus, Heart, Kidney, Lung, Mammary gland, Muscle-skeletal, Ovary, Saliva, Salivary gland, Sem. Vesicle, Testis, Thyroid, Uterus	Blood clot, Bone marrow, Faeces, Kidney, Mammary gland, Milk, Muscle-skeletal, Ovary, Salivary gland, Serum, Uterus

(-/-) = (Number positive / number examined)

* = Log₁₀ mouse intracerebral LD/50 per 30 mg tissues

+ = Not assayed but high content of lymphoreticular tissue

° = Negative in other studies

** = Trace or exceptional

PF = Prefemoral

PS = Prescapular

RP = Retropharyngeal

MP = Mesenteric/portal

CSF = Cerebro-spinal fluid

LN = Lymph node

Annex 3: Calculation of sheep meat consumption in the EU15 from Eurostat figures

Year	Carcass production (1000 tons)	Import (1000 tons)	Export (1000 tons)	Ext. Gross consumption (1000 tons)	EU15 pop (millions)	EU15 daily individual intake (g)
1995	1,057.6	206.8	5	1,259.4	371.2	9.3
1996	1,050.4	217.9	4.2	1,264	372.1	9.3
1997	1,006.9	218.6	2.6	1,222.9	373	9
1998	1,049.9	215	2.6	1,262.3	373.9	9.3
1999	1,043.4	214.7	2.5	1,255.6	374.9	9.2
2000	1,048.2	221.8	3	1,267	376.2	9.2
2001	945	221	2.8	1,163.2	377.9	8.4
2002	961.4	222	3	1,180.3	379.8	8.5
2003	946.3	223	2.9	1,166.4	381.9	8.4
2004	975	215.5	4.1	1,186.4	384.2	8.5

Annex 4: Imports of sheep or goat meat (fresh, chilled or frozen) products from Australia and New Zealand, 1995-2004

Year	Total imports (1000 tons)	NZ	AUS	% import from NZ- AUS	% imported meat consumed
1995	206,9	184,1	14,8	96,1%	16,4%
1996	218,0	189,8	15,8	94,3%	17,2%
1997	219,9	191,5	17,3	95,0%	18,0%
1998	215,8	188,0	17,2	95,1%	17,1%
1999	215,5	185,9	17,5	94,4%	17,2%
2000	222,3	189,6	16,9	92,9%	17,5%
2001	222,0	185,8	17,2	91,4%	19,1%
2002	223,2	187,7	17,7	92,0%	18,9%
2003	224,2	184,8	16,4	89,7%	19,2%
2004	216,5	174,4	17,2	88,5%	18,2%

Annex 5: EU Surveillance data

TSE positive tests in different exit streams 2005-2006

2005	Culled for destruction		Not slaughtered for human consumption		Slaughtered for human consumption		TSE suspects	
	Animals tested	Positive	Animals tested	Positive	Animals tested	Positive	Animals tested	Positive
Austria	0	0	4,180	0	116	0	1	0
Belgium	8	0	1,451	2	10	0	8	0
Bulgaria			2,918	0	4,016	0		
Cyprus			46	11	1,881	105	1410	599
Czech Republic	0	0	360	1	35	0	53	0
Denmark			4,295	0	97	0	2	0
Estonia			283	0	968	0		
Finland	43	0	899	1	394	0	1	0
France	9,823	232	22,411	42	12,246	11	44	26
Germany	3,743	18	29,550	18	14,894	10	51	0
Greece	55	0	1,597	100	4,484	13	397	142
Hungary			5,877	0	3,133	0	34	0
Ireland	1,670	24	10,374	20	10,689	2	6	5
Italy	5,158	280	8,398	17	14,173	10	35	31
Latvia			43	0	0	0		
Lithuania			82	0	946	0		
Luxemburg			428	0	238	0		
Malta			219	0	24	0	13	0
Netherlands	1,018	27	10,085	23	8,910	14	2	0
Norway	248	0	3,615	1	10,889	2	8	1
Poland	0	0	0	0	0	0	0	0
Portugal	0	0	21,230	25	51,285	32	1	0
Slovakia	8	0	2,365	7	250	2		
Slovenia	307	97	1,648	4	224	0	6	0
Spain	4,636	73	14,881	19	14,274	15	38	8
Sweden	33	0	3,239	1	1	0	0	0
United Kingdom	104	4	25,019	137	11,816	30	322	179
Sum:	26,854	755	175,493	429	165,993	246	2,432	991

Opinion on the Quantitative risk assessment on the residual BSE risk in sheep meat and meat products

2006	Culled for destruction		Not slaughtered for human consumption		Slaughtered for human consumption		TSE suspects	
	Animals tested	Positive	Animals tested	Positive	Animals tested	Positive	Animals tested	Positive
Austria			4,989	0	97	0		
Belgium	81	0	1,814	2	3,324	1	29	0
Czech Republic			332	0	535	0	3	0
Denmark	3,065	0	142	1	296	0	3	0
Estonia			343	0	1,561	0		
Finland	134	0	822	1	1,981	1		
France	14,316	53	178,042	189	87,510	49	23	12
Germany	2,050	0	20,753	17	12,344	2	41	0
Hungary			2,914	0	2,522	0	8	0
Ireland	1,433	56	10,846	31	30,906	6	14	8
Italy	1,558	75	5,517	50	17,165	13	14	13
Latvia			33	0	227	0		
Lithuania			164	0	1,235	0		
Luxemburg			145	0	284	0		
Malta			314	0	26	0		
Netherlands	419	18	7,051	14	6,211	5	9	5
Norway	301	6	4,126	3	9,223	3	22	0
Portugal			7,551	12	31,882	48	1	0
Slovakia	320	0	3,099	3	2,806	6		
Slovenia	283	27	1,446	12	138	0	2	0
Spain	1,636	23	23,771	10	27,527	10	26	3
Sweden	5	0	3,316	2	0	0	7	0
United Kingdom	3,952	52	19,228	54	7,774	7	150	77
Sum:	29,553	310	296,758	401	245,574	151	352	118

Discriminatory test results 2005-2006

	2005					2006		
	Sheep	Goats	Total			Sheep	Goats	Total
Belgium	1	0	1		0	0	0	
Czech Republic	1	0	1		0	0	0	
Denmark	0	0	0		0	0	0	
Germany	24	0	24		19	0	19	
Estonia	0	0	0		0	0	0	
Greece	30	9	39		73	4	77	
Spain	n/a	n/a	n/a		n/a	n/a	n/a	
France*	70	12	82		343	6	349	
Ireland	10	1	11		23	0	23	
Italy	114	3	117		72	4	76	
Cyprus	72	104	176		55	6	61	
Latvia	0	0	0		0	0	0	
Lithuania	0	0	0		0	0	0	
Luxembourg	0	0	0		0	0	0	
Hungary	1	0	1		3	0	3	
Malta	0	0	0		0	0	0	
Netherlands	37	0	37		29	0	29	
Austria	0	0	0		0	0	0	
Poland	0	0	0		0	0	0	
Portugal	12	0	12		6	0	6	
Slovenia	28	5	33		79	0	79	
Slovakia	0	0	0		0	0	0	
Finland	1	3	4		1	0	1	
Sweden	0	0	0		0	0	0	
United Kingdom	242	3	245		185	8	193	
Total	643	140	783		888	28	916	

Annex 6: Pooled data

Table 1: TSE test monitoring in sheep (EU 25, total EU25 sheep population: 65,000,000)

	2002-2004	2005	Cumulative total
Number of TSE tests	1,239,565	268,845	1,508,410
Number of TSE positives	6,060	1,838	7,898
Number of discriminatory tests	3,506	616	4,122
Number of discriminatory tests excluding BSE	3,506	616	4,122

EU Law (Regulation 999/2001) lays down rules for eradication measures to be applied to TSE infected flocks, for monitoring, and for the removal of SRM. Inclusion of discriminatory testing, became mandatory from January 2005 on by Commission Regulation (EC) No 36/2005. Approximately two-thirds of the discriminatory tests enumerated here were applied retrospectively to confirmed passive surveillance cases identified in Great Britain between 1998-2001, and prospectively to passive and active surveillance cases identified between 2001-2004. All discriminatory tests were negative (D. Matthews, VLA, pers. comm.) and epidemiological interpretation of the 450 flocks sampled in this GB study indicated that the maximum proportion of sheep TSE cases that could be BSE was 0.66% (Stack *et al.*, 2006).