

TSE risk assessment from carcasses of ovine and caprine animals below 6 months of age from TSE infected flocks intended for human consumption¹

Scientific Opinion of the Panel on Biological Hazards

(Question No EFSA-Q-2007-202)

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SUMMARY

Following a request from the European Commission, the Panel on Biological Hazards (BIOHAZ) was asked to deliver a scientific Opinion on a TSE risk assessment from carcasses of ovine and caprine animals below 6 months of age from TSE infected flocks intended for human consumption. Its terms of reference were as follows: to provide an assessment on the existence of a significant additional risk to human health compared with the actual situation, founded on the scientific evidences, from the consumption of carcasses from ovine or caprine animals below 6 months of age from TSE affected flocks (without been subjected to a TSE rapid test and irrespectively of the genotype) provided that the entire head and the viscera of the thoracic and abdominal cavities are removed and excluded from human consumption and provided that BSE is excluded (in the outbreak) according to the procedure laid down in 3.2 (c), Chapter C of Annex X to the Regulation (EC) 999/2001.

After clarification from the Commission, the BIOHAZ Panel was able to refine the ToR to focus on the change of human exposure that might result from the proposed change of risk management procedure and that it specifically required an estimate of the relative levels of TSE infectivity in the carcass of a lamb or kid less than 3 months of age from which spleen and ileum have been removed, compared to the carcass of a lamb or kid less than 6 months of age from which the spleen, the ileum, the head and the viscera of the abdominal and thoracic cavity have been removed.

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In answer to these ToR, the BIOHAZ Panel concluded:

- A quantitative comparison of infectivity load in both scenarios is not possible, because there are no data available on the amount of infectious tissues that would be still present on the carcasses of 3 months and 6 months of age of lambs and kids, prepared according to the terms of reference (*i.e.* 3 months with head and viscera from the thoracic and abdominal cavity remaining for human consumption, but excluding the spleen and the ileum which is currently removed as Specified Risk Material; 6 months of age without head and all the viscera from the thoracic and abdominal cavities).
- There is an increase, between 3 and 6 months of age, of the number of PrP^{res} accumulating lymphoid formations. A part of these newly involved lymphoid formations would remain on dressed carcasses.
- In the worst case scenario, there would be an increase in infectivity level in lymphoid tissue between ages of 3 and 6 months (approximately 10 fold) on a per unit weight basis.
- The level of infectivity in secondary lymphoid tissues that may remain on the dressed carcasses, can reach by 6 months of age a level of infectivity per gram equivalent to 1/50 of that found in the same amount of brain tissue from a terminally affected sheep.
- Removal of the head and the thoracic and abdominal viscera will result in incomplete removal of the infectivity load at both 3 and 6 months of age.
- In the absence of new quantitative data on the tissue infectivity load in kids and lambs, the risk assessment and procedures for safe sourcing of small ruminant materials proposed in 2002 by the SSC, including the use of the combination of genotype and age as sourcing criteria, remain valid.

The BIOHAZ Panel further recommends that to facilitate future attempts at quantitative risk assessments in this field, more experimental work is needed to define the variability and uncertainty of both the estimates of relative infectivity titre at different ages in young lambs and kids and of the weights of lymphoid tissue entering the food chain.

Key words: TSE, Scrapie, ovine, caprine, carcasses, human exposure.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Community legislation on measures following the confirmation of a TSE case in ovine and caprine animals

Regulation 999/2001 lays down rules for eradication measures to be applied following the confirmation of a TSE case in ovine and caprine animals. In particular, point 3.3 Chapter A of Annex VII to the Regulation lays down the conditions to which the movement of the animals from a TSE affected holding is subject. According to these conditions, a derogation is granted to Member States in order to allow lambs and kids less than 3 months old, irrespective of the genotype, to be moved from the holding to go directly for slaughter for human consumption², provided that Bovine Spongiform Encephalopathy (BSE) is excluded³ according to the procedure laid down in 3.2 (c), Chapter C of Annex X to the Regulation (discriminatory tests) and certain tissues are removed⁴.

Scientific advice on TSE infectivity in ruminant tissues

The distribution of infectivity in TSE-infected small ruminants has been addressed in several scientific Opinions from the former Scientific Steering Committee and was last updated with the Opinion from EFSA of 25th January 2007 on a quantitative risk assessment on the residual BSE risk in sheep meat and meat products. The main conclusion from these scientific Opinions is that the spread of the TSE agent in TSE-affected ovine or caprine animals is large and may affect a high number of tissues. The age and the genotype (in sheep) have an influence on that distribution.

According to the Opinions, the amount of infectivity (infectivity titre) in young (less than 8 months old) pre-clinical animals infected by natural scrapie agents is very low or undetectable (table 1 in Annex 2 to the EFSA Opinion of 25th January 2007).

In its Opinion of 8 March 2007 on certain aspects related to the risk of TSEs in ovine and caprine animals, EFSA concluded that there is no evidence for an epidemiological or molecular link between classical and/or atypical scrapie and TSEs in humans. The BSE agent is the only TSE agent identified as zoonotic. However, in view of their diversity, it is currently not possible to exclude transmissibility to humans of other animal TSE agents.

Current situation

The consumption patterns of sheep and goat meat, in particular regarding to the age of lambs and kids slaughtered for human consumption, are different across the EU. In some Member States, the consumption of heavy and older lambs is common. Further amendments to the eradication measures might be considered to resolve problems encountered in some Member States in relation to those young animals. The current restrictions in EU legislation regarding the movement from TSE affected holdings of lambs and kids intended for slaughter for human consumption may be revised following a favourable risk assessment in order to allow the placing on the market of lambs or kids below 6 months of age. It seems necessary to request an assessment from the EFSA on this subject.

² Without been tested for TSE.

³ In the TSE outbreak.

⁴ The referred tissues are spleen and ileum, in line with Regulation (EC) 999/2001.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Food Safety Authority (EFSA) is invited to provide an assessment on the existence of a significant additional risk to human health compared with the actual situation, founded on the scientific evidences, from the consumption of carcasses from ovine or caprine animals below 6 months of age from TSE affected flocks provided that the entire head and the viscera of the thoracic and abdominal cavities are removed and excluded from human consumption and provided that BSE is excluded according to the procedure laid down in 3.2 (c), Chapter C of Annex X to the Regulation (EC) 999/2001.

Clarifications on the Terms of Reference

Two issues were addressed to the European Commission (EC) for clarification.

Firstly, the EC clarified that the term “significant” inserted in the Terms of Reference of the mandate was not to be considered in its statistical meaning.

Secondly, it was agreed with the EC that the risk that would be the subject of this risk assessment will be that of the existence of an additional human exposure risk to TSE agents due to the new proposed scenario, rather than the assessment of the existence of an additional human health risk. The reason for this is that any potential zoonotic aspects of the TSE agents present in ovine and/or caprine animals has already been specifically covered by a recent EFSA Scientific Opinion and a Scientific and Technical Report (EFSA, 2007a; EFSA, 2008). There is neither new scientific data nor research findings that would indicate that those recent documents need to be reviewed.

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ASSESSMENT

1. Introduction

1.1. Regulatory Background

Regulation (EC) 999/2001 lays down rules for eradication measures to be applied following the confirmation of a TSE case in ovine and caprine animals. In particular, point 3.3 Chapter A of Annex VII to the Regulation lays down the conditions to which the movement of the animals from a TSE affected holding is subject. According to these conditions, a derogation is granted to Member States in order to allow lambs and kids less than 3 months old, irrespective of the genotype, to be moved from the holding to go directly for slaughter for human consumption without been tested for TSE, provided that Bovine Spongiform Encephalopathy (BSE) is excluded in the TSE outbreak according to the procedure laid down in 3.2 (c), Chapter C of Annex X to the Regulation (discriminatory tests) and certain tissues are removed.

These tissues are those referred to as Specified Risk Material in point 1 (b) (ii) of Annex V to Regulation (EC) 999/2001; they are the spleen and ileum. Thus, in carcasses from lambs or kids less than 3 months of age, the head including brain and eyes, tonsils, and spinal cord as well as the viscera from the thoracic and the abdominal cavity are allowed for human consumption.

The Mandate therefore requires an estimate of the relative levels of TSE infectivity in the carcass of a lamb or kid less than 3 months of age (including head and viscera) from which spleen and ileum have been removed compared to the carcass of a lamb or kid less than 6 months of age from which the ileum, the spleen, the head and the viscera of the abdominal and thoracic cavity have been removed.

1.2. Previous SSC and EFSA Opinions

The opinion of the Scientific Steering Committee (SSC) on safe sourcing of small ruminant materials and its complement (SSC, 2002a; SSC, 2002b) 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 recognised the qualitative risk for humans and provided an approach to reduce that risk but made no estimation of the risk for humans or a quantitative calculation.

These approaches are relevant in the light of the current uncertainty on the zoonotic potential of small ruminant TSE agents other than BSE, as highlighted in a recent EFSA report (EFSA, 2008).

The SSC Opinion (SSC, 2002a) and its complement (SSC, 2002b) comprehensively assessed the potential risk to human health and indicated how those risks could be minimized by a range of risk management procedures. A qualitative and quantitative approach to the risk related to TSEs in small ruminants was dealt by with subsequent EFSA Opinions (EFSA 2005; EFSA 2007b).

While these later Opinions re-affirmed the view that there was insufficient data for a quantitative assessment of the TSE risk posed by tissues of sheep and goats to human health,

they did identify information gaps and outlined the key data inputs considered necessary for such an assessment. These included information on:

- a. Prevalence of infection in sheep and goats.
- b. Infectious load and distribution in tissues.
- c. The species barrier (small ruminants to humans).
- d. Human consumption levels.

1.3. Approach to the current mandate.

In the current opinion, the BIOHAZ Panel has addressed the mandate by reviewing aspects of (a) prevalence of infection and (b) infectious load and distribution in tissues in order to assess the relative change in human exposure to TSE Agents (excluding BSE) due to raising the age limit of lambs and kids entering the human food chain from TSE affected flocks and taken into account the specific tissues or organs that would be removed in both scenarios.

Since this assessment has to be made irrespective of the genotype of the animal or knowledge of the likely dose of exposure to TSE agents (excluding BSE), this EFSA opinion considers the worst case scenario where lambs and kids of susceptible genotypes are exposed to high natural infection pressure. This is considered to be the exposure of a VRQ/VRQ lamb from a scrapie affected VRQ/VRQ dam *in utero* or at and after birth to the blood, placental annexes and fluids, and milk/colostrum of its mother.

There are practical difficulties in verifying the exact age of kids and lambs, and hence the implementation of any age-based TSE controls. Some elements related to this risk management issue have been discussed in previous SSC Opinions (SSC, 2002a; SSC, 2002b) but are beyond the scope of this risk assessment.

Due to the lack of more specific data in goat species this risk assessment was performed employing data from TSE in sheep. However, this was considered valid also for TSE in goats. The limited amount of data available for goats is presented in section 3.1 of this report.

2. Risk Assessment

2.1. Prevalence of infection in flocks of small ruminants where a TSE is identified.

The prevalence of clinically and subclinically affected animals in a classical scrapie-affected flock has been investigated by different research groups for several flocks and in different European countries.

Genotype distribution in the flock has a major influence on this prevalence. Flocks with a high proportion of VRQ carriers can show much higher prevalence. In this type of flocks prevalence can reach 76% in animals bearing VRQ allele (Elsen *et al.*, 1999). In flocks with mostly ARQ carriers, the prevalence have been analysed for naturally-affected flocks in Belgium (Roels *et al.*, 1999), Germany (Reckzeh *et al.*, 2007), Iceland (Thorgeirsdottir *et al.*, 2002; Georgsson *et al.*, 2008), Italy (Vascellari *et al.*, 2005; Ligios *et al.*, 2006), Norway (Ersdal *et al.*, 2003),

Shetland (UK) (Jeffrey *et al.*, 2002), and the United States (Caplazi *et al.*, 2004), and in The Netherlands (Langeveld *et al.*, 2006). For these flocks, the prevalence varied between 3% (Jeffrey *et al.*, 2002) and 41% (Ligos *et al.*, 2006). Detailed references to these studies are presented in Appendix I.

The reported prevalence in TSE affected sheep flocks vary from 3% to 41%. Similar prevalence differences were evidenced in affected goat herds. In the general EU sheep population TSE prevalence is currently estimated to be 0.1% at the level of the animal (0.06% in healthy slaughter, 0.17% in at risk) on the basis of the EU monitoring in 2006 (EC, 2006).

More specifically, the prevalence of the disease in flock where a classical scrapie case has already been identified cannot be compared directly to the prevalence observed at the abattoir (test at the time of slaughter) or at the fallen stock (at death). Fediaevsky *et al.* (2008) report the prevalence of classical scrapie at animal level at the abattoir and fallen stock in 20 European countries. They state that in countries with positive cases, the prevalence of classical scrapie varied between 0.03% and 3% at the slaughter house and 0.2 % and 21 % (country with very special situation) in the fallen stock depending on countries and years. Even if the annual incidence cannot be compared easily with prevalence at the abattoir, it appears that the prevalence of positive animals in affected flocks might be much higher than the prevalence at the abattoir (average 0.3% for this set of countries). Furthermore, it is even higher if we focus on genetically non-resistant animals in affected flocks.

The detectability of PrP^{res} deposition in lymphoid tissues (which is used as a surrogate of infection) of sub-clinically affected animals depends on both the animal inter-individual variability and the biodiversity of the TSE Agents. The earliest detection of abnormal PrP (and infectivity) of any TSE occurs naturally in the gut-associated lymphoid tissue of VRQ/VRQ lambs born in flocks affected by classical scrapie (*inter alia*, Elsen *et al.*, 1999).

Less is known about the annual within-flock incidence of atypical scrapie. However, in a recent review, Hopp (2007) reports that concerning prevalence in culled affected flocks, a single case (the index case) has been detected in most of the flocks while only one or two additional cases have been reported in a few flocks. The incidence of the disease seems rare in affected flocks. In the overall population, Fediaevsky *et al.* (2008) also report that the prevalence of atypical scrapie are (a) at the abattoir, between 0,06% and 3,4% and (b) in fallen stock, between 0.28% and 7% in the EU countries.

In conclusion, it is not possible to predict the scrapie prevalence in a given flock but observations carried out in naturally infected flocks with classical scrapie indicate that prevalence can range from 3% to more than 40%. In the context of a “worst case”, a VRQ/VRQ lamb born into a TSE-affected flock from a scrapie-affected VRQ/VRQ dam is at high risk to be infected at or around birth and develop clinical scrapie within 2-3 years.

2.2. Infectivity distribution of tissues of small ruminants infected with TSE Agents.

2.2.1. General Background.

Under natural exposure conditions, transmission of scrapie is considered to preferentially occur at or around birth (Hourrigan *et al.*, 1979; Andreoletti *et al.*, 2000; Heggebo *et al.*, 2000; Andreoletti *et al.*, 2002). Placentae and foetal- maternal tissues from scrapie-infected ewes have long been recognised as sources of infection for lambs (Pattison *et al.*, 1972). More recently

evidence supporting the role of colostrum and/or milk from scrapie-infected ewes in maternal scrapie transmission has been reported (Konold *et al.*, 2008). Therefore lambs or kids in an infected herd or flock will be exposed to the disease and, at a very young age, are likely to be infected. Further data relevant to the timing and spread of infection in VRQ/VRQ lambs is presented in Appendix II.

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 Appendix III. 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 (SSC, 2002a). Clearly, the data have the limitations of (a) the methods' sensitivity, (b) the lack of PrP^{res} genotype data on the subjects and (c) not reflecting our current knowledge of the pathogenesis of experimental infection of sheep with TSEs.

In lambs of susceptible PrP genotypes exposed to natural scrapie infection, the earliest evidence of infection is found in the first month of life in the alimentary canal and its associated lymphoid structures. PrP^{res} can be later detected in most secondary lymphoid formations⁵ and in the enteric nervous system. PrP^{res} deposition in the CNS becomes detectable at about mid-incubation (Andreoletti *et al.*, 2000; Jeffrey *et al.*, 2001; Van Keulen *et al.*, 2002). Hence, screening the obex using rapid testing for PrP^{res} is a poor indicator for the absence of TSE infection in small ruminants peripheral tissues (EFSA, 2007a).

Illustrative examples of the kinetics and distribution of infectivity in VRQ/VRQ sheep in French and Dutch sheep are presented below.

2.2.2. Infection of VRQ/VRQ lambs in French and Dutch naturally infected flocks.

In two individual flocks affected with natural scrapie (one in the Netherlands – one in France: Langlade flock) investigations carried out in VRQ/VRQ, using PrP^{res} as a marker for infectious agent dissemination indicated that:

PrP^{res} is detectable in secondary lymphoid formation annexed to ileum as early as 21 days old (Andreoletti *et al.*, 2002).

PrP^{res} dissemination and accumulation in other Gut Associated Lymphoid Tissues (GALT) (including cephalic lymphoid structures) can occur within the two first months of age (Andreoletti *et al.*, 2000; Andreoletti *et al.*, 2002, Van Keulen *et al.*, 2002, Heggebø *et al.*, 2000).

After 2 months, a progressive dissemination of PrP^{res}, to all secondary lymphoid structures (including those remaining on commercial carcasses) is observed (Andreoletti *et al.*, 2000; Andreoletti *et al.*, 2002; Van Keulen *et al.*, 2002).

PrP^{res} accumulation in secondary lymphoid organs increases rapidly and reaches a plateau in animals older than 6 months (Andreoletti *et al.*, 2000).

In very young animals (harbouring identical genotype), a certain degree of inter-individual heterogeneity in the PrP^{res} dissemination stage (extent of lymphoid dissemination) and accumulation level was reported (Andreoletti *et al.*, 2000; Andreoletti *et al.*, 2002; Van Keulen *et al.*, 2002). For instance, at 3 months old, only a proportion of the infected individuals

⁵ Lymphoid formations can be divided in (a) primary, which includes thymus and bone marrow and (2) secondary, which includes lymph nodes, spleen, tonsils and mucosa associated lymphoid tissue.

accumulated PrP^{res} in other than GALT lymphoid formations, while by 4 months old all individuals were found positive by that criterion.

Other studies carried out in sheep harbouring other susceptible genotypes (e.g., ARQ/ARQ) and potentially exposed to other TSE agents, found a similar but slower dissemination scheme (Jeffrey *et al.*, 2001; Van Keulen, 2008), indicating the strong influence of PrP host genotype and the nature of TSE agent, on agent distribution dynamics in the organism.

3. Difficulties of quantitative TSE risk assessment of small ruminants

Quantitative assessment of infectivity that would enter into the food chain associated with carcasses (and potentially offal) of scrapie-infected lamb or kid according to the age is not feasible because:

- The inter-individual variability observed in prion (PrP^{res}) dissemination and level of accumulation in young animals (PrP genotype, level of exposure and TSE Agents).
- The limited data available related to infectivity load (assessed by bioassay) in scrapie affected lambs or kids tissues.
- The absence of quantitative information regarding the mass of secondary lymphoid formations that would remain on dressed carcasses.

A more comprehensive report of the difficulties of small ruminant TSE quantitative risk assessment was prepared earlier by EFSA (EFSA, 2007b) in which a semi-quantitative approach to the problems was also outlined.

In Appendix IV, a development of a small ruminant TSE semi-quantitative risk assessment (sQRA) is included.

3.1. Review of related knowledge in goats.

Current understanding about the kinetic and distribution of PrP^{res} / infectivity in the tissues of goat affected by scrapie are based solely on few observations from naturally-infected animals and by inferences drawn from studies of scrapie in sheep.

As illustrated above, in sheep after crossing the mucosal barrier, infectivity and PrP^{res} first accumulates in the gut-associated lymphoid tissues (GALT) of the tonsil and the Peyer's patches of the ileum at 2-3 month post infection (Andreoletti *et al.*, 2000; Heggebø *et al.*, 2000). This may also be the case in goats although the effects of PrP genotype and agent strain have not been so well investigated in this species.

The few observations on PrP^{res} distribution from naturally infected goats have been done in recent studies which were aimed to establish potential association between certain PrP gene polymorphisms and resistance/susceptibility to scrapie. In a study of Billinis *et al.* (2002) a total of 44 clinically healthy goats of 3-7 years age range from a scrapie affected herd were selected and their brains examined by histological and PrP immunohistochemistry. Eight animals (18%) were found with PrP^{res} deposition in the brain.

In an Italian survey the brain and several lymphoid districts of 89 clinically healthy goats, from scrapie affected, herds were examined by western blotting analysis showing that a total number of 28 (31%) goats were found scrapie affected. Nine (10%) of them had PrP^{res} in the brain and in one of the examined lymphoid tissue, while 19 (21%) had PrP^{res} deposition only in the lymphoid tissues (Vaccari *et al.*, 2006). In a very similar study, which was again carried out in

Italy by other authors (Acutis *et al.* 2006) 25 out of 177 (14%) examined goats, all from herds confirmed to have clinical occurrence of scrapie, were found to be pre-clinically affected by the disease. However, these last two studies should be taken carefully into consideration since a iatrogenic origin of scrapie were suspected in some Italian outbreaks (Agrimi *et al.*, 1999).

Finally, in Sardinia (Italy), of 93 goats coming from a scrapie affected herd, 5 pre-clinically affected goats were found having scrapie by the immunohistochemical examination of the nervous and lymphoid tissues (Leoni *et al.*, 2004).

These limited data demonstrated that a number, even high, of goats could be found pre-clinically scrapie affected in herds within which the clinical cases of the disease were occurring. As in sheep, in goats the TSE agent replication occurs in the lymphoid districts before the onset of the clinical signs, although a precise mapping of the lymphoid districts that are involved in the scrapie pathogenesis is not available for this species so far. Moreover, in all these previously mentioned works, only adult sheep were examined, so that no data have been published on early distribution and progression of PrP^{res} / infectivity in organs of less than 1 year old kids.

CONCLUSIONS

1. General conclusions:

- Lambs and kids from TSE affected flocks in the form of classical scrapie have a clearly higher probability of infection than those from the general population. In the general EU sheep population TSE prevalence at animal level is estimated to be about 0.1 %. In classical scrapie affected sheep flocks the reported prevalence vary from 3% to 41%.
- In susceptible lambs (VRQ/VRQ), infectivity will replicate in lymphoid tissues from birth and involve most secondary lymphoid formations before 4 months old.
- In worst cases, infectivity in lymphoid organs reaches its maximal level before 6 months old. This maximal level infectivity in lymphoid organs (per mass unit) can be estimated to be about 1/50 of the infectivity found in obex from a terminally affected ewe.
- According to currently available data, carcasses of animals of less than two months of age, providing that the head and the thoracic and abdominal viscera are removed, do not contain detectable PrP^{res} but this does not exclude the possibility of infectivity.

2. Answer to the terms of reference

When specifically addressing the ToR of this mandate, and in the context of Classical Scrapie:

- A quantitative comparison of infectivity load in both scenarios is not possible, because there are no data available on the amount of infectious tissues that would be still present on the carcasses of 3 months and 6 months of age lambs and kids, prepared according to the terms of reference (*i.e.* 3 months with head and viscera from the thoracic and abdominal cavity remaining for human consumption, but excluding the spleen and the ileum which is currently removed as SRM; 6 months of age without the head and all the viscera from the thoracic and abdominal cavities).

- There is an increase, between 3 and 6 months of age, of the number of PrP^{res} accumulating lymphoid formations. A part of these newly involved lymphoid formations would remain on dressed carcasses.
- In the worst case scenario, there would be an increase of infectivity level in lymphoid tissue between ages of 3 and 6 months (approximately 10 fold) on a per unit weight basis.
- The level of infectivity in secondary lymphoid tissues that may remain on the dressed carcasses, can reach by 6 months of age a level of infectivity per gram equivalent to 1/50 of what is found in the same amount of brain tissue from a terminally affected sheep.
- Removal of the head and the thoracic and abdominal viscera will result in incomplete removal of the infectivity load at both 3 and 6 months of age.
- In the absence of new quantitative data on the tissue infectivity load in kids and lambs, the risk assessment and procedures for safe sourcing of small ruminant materials proposed in 2002 by the SSC, including the use of the combination of genotype and age as sourcing criteria, remain valid.

RECOMMENDATIONS

- To improve quantitative risk assessments in this field, further experimental work is needed to define the variability and uncertainty of the estimates of relative infectivity titre at different ages in young lambs and kids, and to determine the variability and uncertainty of the weights of lymphoid tissue entering the food chain.

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APPENDIX I. SUMMARY OF STUDIES ON PrP^{RES} DETECTION IN TSE INFECTED FLOCKS

Reference	Infection (natural-experimental)	Genotype of affected animals	PrP ^{RES} positive tissue	Prevalence
Caplazi <i>et al.</i> , 2004	natural	12 x ARQ/ARQ 1 x VRQ/ARQ	CNS, lymphoid tissue (GALT and non-GALT), placenta	16 % (including 9 % preclinical)
Elsen <i>et al.</i> , 1999	'natural' Langlade flock	VRQ, ARQ, AHQ, ARR and combinations	CNS	33 % clinical disease. 76 % positive of the VRQ/VRQ sheep
Diaz <i>et al.</i> , 2005	'natural' Langlade flock	All genotypes, decreasing prevalence with decreasing risk	n.a.	11,03 % clinical
Ersdal <i>et al.</i> , 2003	natural	VRQ/VRQ ARQ/ARQ	CNS, lymphoid tissue	35 % preclinical
Jeffrey <i>et al.</i> , 2002	natural	VRQ/VRQ	CNS, intestine, lymphatic system, cranial mesenteric ganglia	3.1 % preclinical
Langeveld <i>et al.</i> , 2006	a) natural b) natural (active surveillance) c) experimental BSE	a) VRQ/VRQ b) all genotypes c) ARQ/ARQ	a) RLN b) RLN c) RLN	a) n.a. b) n.a. c) 100 %
Ligos <i>et al.</i> , 2006	natural	ARQ/ARQ ; ARQ/AHQ	CNS, PNS, LRS	6.6 % preclinical
Reckzeh <i>et al.</i> , 2007	natural	ARQ/ARQ ; ARQ/AHQ ; ARQ/ARR ; VRQ/ARQ ; VRQ/ARR	CNS, PNS, LRS	22.8 % preclinical
Thorgeirsdottir <i>et al.</i> , 2002	natural	VRQ homo- or hemizygous	CNS	38.5 % preclinical
Roels <i>et al.</i> , 1999	natural (active surveillance)	n.a.	CNS, tonsils	12.12 % (including 6 % preclinical)
Vascellari <i>et al.</i> , 2005	natural	ARQ/ARQ ; ARQ/AHQ ; ARQ/VRQ	CNS, RLN	5.2 % preclinical
Georgsson <i>et al.</i> , 2008	two natural scrapie flocks	ARQ/ARQ; VRQ/ARQ	CNS , LRS	58.3 % and 42.5 % preclinical

APPENDIX II. FURTHER DATA RELEVANT TO THE TIMING AND SPREAD OF INFECTION IN VRQ/VRQ LAMBS

a. Colostrum/Milk as a potential source of infectivity.

Qualitative TSE risk assessments of the safety of milk from sheep and goats have been made in the past, but little published data have been available. Konold and colleagues have recently published a study of the transmission of scrapie from scrapie-affected dams to lambs by feeding from birth milk (and colostrum) taken during the later stages of scrapie-infection in the dams (Konold *et al.*, 2008). Evidence of infection was detected as early as 44-46 days in the distal ileum of two lambs by immunohistochemistry for abnormal PrP (PrP^{res}) and widespread infection inferred by RAMALT⁶ testing by 190-210 days of age. Both donor ewes and lambs were of the susceptible VRQ/VRQ genotype and the donor ewes were sourced from a flock with a ~ 10% prevalence of natural infection. This is directly relevant to the consideration of “a worst case scenario” and confirms previous work indicating lambs can become infected very soon after birth and that, within 6-7 months, the infection can be widely disseminated, at least in lymphoid tissue, in the susceptible animal.

b. The role of blood in spreading the agent within the body.

One of the critical scientific uncertainties (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. In the specific context of this mandate, Andreoletti and co-workers have reported infectivity in blood of pre-clinical VRQ/VRQ lambs at 3 months of age (Andreoletti *et al.*, Neuroprion Edinburgh 2007).

c. Intestine: the anatomical location where infectivity is first detected.

PrP^{res} 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 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^{res} 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^{res} 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^{res} is a poor indicator for the absence of TSE infection in the digestive tract of the lamb.

⁶ RAMALT stands for recto-anal mucosal associated lymphoid tissue.

APPENDIX III. INFECTIVITY OF TISSUES IN SHEEP AND GOATS WITH NATURAL SCRAPIE

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 goats⁷

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

Group	Infectivity Titre	PRE-CLINICAL				CLINICAL	
		SHEEP				SHEEP	GOATS
	(approx.range)	≤8 months.(0/16)	10-14 months (8/15) ⁸	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

⁷ After Hadlow *et al.* (1979, 1980, 1982), Pattison *et al.* (1964, 1972), Groschup *et al.* (1996). Regarding DRG: see text.

⁸ 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.

	Infectivity	PRE-CLINICAL				CLINICAL	
Group	Titre	SHEEP				SHEEP	GOATS
	(approx.range)	≤8 months.(0/16)	10-14 months (8/15) ⁸	25 months (1/13)	> 25 months (1/6)	34-57 months (9/9)	38-49 months (3/3)
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 midbrain), Colon-distal, , LN (s. mammary), Nasal mucosa, Salivary glands, Spinal cord, Thymus	Colostrum	Blood clot, Fetus, Heart, Kidney, Lung, Mammary gland, Muscleskeletal, Ovary, Saliva, Salivary gland, Sem. Vesicle, Testis, Thyroid, Uterus	Blood clot, Bone marrow, Faeces, Kidney, Mammary gland, Milk, Muscle-skeletal, Ovary, Salivary gland, Serum (see report), 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-spinalfluid

LN = Lymph node

BM = Bronchomediastinal

APPENDIX IV. DEVELOPMENT OF A SMALL RUMINANT TSE SQRA.

A. Immuno-histochemical labelling of prion protein (PrP^{res})

Pathogenesis studies conducted in lambs from naturally affected scrapie flocks have provided a comprehensive picture of PrP^{res} dissemination dynamics in VRQ/VRQ lambs (Andreoletti *et al.*, 2000; Van Keulen *et al.*, 2002). It should be noted that in these studies exact age of animals are available, which allows discussing small age variation impact on PrP^{res} dissemination status.

Most of the available scrapie pathogenesis studies were carried out using immunohistochemistry (IHC). While IHC is a valuable technique in term of sensitivity and description of structures involved in accumulation of PrP^{res}, it remains qualitative and subject to variations (according to methodology).

In the study from Andreoletti and colleagues (Table 1), the surrogate marker for infectivity (PrP^{res}) was first detected in ileal Peyer's patches (PP) and ileal mesenteric lymph nodes (MLNs) in one of four animals tested at 2 months of age but in no other of many head, thoracic and abdominal lymphoid tissues. One month later (at 3 months), the infection has spread (or become detectable) in three of four animals into all tested lymphoid tissues apart from the third eyelid or thymus. Thereafter, biological variability is apparent in labelling intensity and numbers of animals affected but generally all animals become infected by 5 months and the level of PrP^{res} in a particular lymphoid tissue reaches a plateau. Although qualitatively different, the study from Van Keulen and colleagues (Table 2) concurs with the work from Andreoletti *et al.*: no PrP^{res} is detected in any of the lymphoid organs of two lambs sacrificed at one month of age but first signs of infection are picked up in head and gut lymph nodes of both animals tested at two months and widespread detection of PrP^{res} is found at three months and thereafter.

In Tables 1 and 2, the tissues containing detectable PrP^{res} which might currently enter the food chain are highlighted in green (scenario 1); the tissues containing detectable PrP^{res} which might enter the food chain if the restrictions were changed to exclude the head, and abdominal and thoracic viscera, and the age limit raised to six months, are highlighted in blue (scenario 2). The orange coloured cells identify those tissues carrying PrP^{res} that would enter in the food chain in both scenarios.

Table 1. PrP^{res} IHC detection in lymphoid tissues from susceptible VRQ/VRQ lambs with natural scrapie (Modified from Andreoletti *et al.*, 2000). Groups of four lambs were scored on the basis of PrP^{res}-labelling intensity and the range of intensity is scored as negative (-), minimal to slight (+), moderate (++) or strong (+++). Tissues with PrP^{res} potentially entering the food chain in scenario 1 (green, spleen and ileum removed as SRM), in scenario 2 (blue, spleen and ileum removed as SRM plus the advised tissues) or in both scenarios (orange) are shown by colour-coding.

Body part / Organ	Age (months)									
	2		3 ¹		4		5		6	
	Positive sheep	PrP ^{res} -labelling	Positive sheep	PrP ^{res} -labelling	Positive sheep	PrP ^{res} -labelling	Positive sheep	PrP ^{res} -labelling	Positive sheep	PrP ^{res} -labelling
Head										
Third eyelid	0/4	-	0/4	-	nd	nd	4/4	+++	3/4	+++
Palatin tonsils	0/4	-	3/4	+++	4/4	+++	4/4	+++	4/4	+++
Retropharyngeal LN	0/4	-	3/4	+++	4/4	++ / +++	4/4	+++	4/4	+++
Mandibular LN	0/4	-	3/4	+++	3/4	++ / +++	4/4	+++	4/4	+++
Parotid LN	0/4	-	3/4	+	2/4	+	4/4	+ / ++	4/4	++
Abdominal and Thoracic Viscera										
Thymus	0/4	-	0/4	-	0/4	-	0/4	-	0/4	-
Mediastinal LN	0/4	-	3/4	+	2/4	+	4/4	+ / ++	3/4	++
Hepatic LN	0/4	-	3/4	+	2/4	+	4/4	+ / ++	3/4	++
Spleen	0/4	-	3/4	+	2/4	+	4/4	+ / ++	3/4	++
PP-duodenum	0/4	-	3/4	+++	3/4	+++	4/4	+++	4/4	+++
MLN-duodenum	0/4	-	3/4	+	2/4	+	4/4	+ / ++	4/4	++
PP-jejunum 25%	0/4	-	3/4	+++	3/4	+++	4/4	+++	4/4	+++
MLN-jejunum 25%	0/4	-	3/4	+++	3/4	+++	4/4	+++	4/4	+++
PP-jejunum 50 %	0/4	-	3/4	+++	4/4	+++	4/4	+++	4/4	+++
MLN-jejunum 50 %	0/4	-	3/4	+++	4/4	+++	4/4	+++	4/4	+++
PP-jejunum 75 %	0/4	-	3/4	+++	4/4	+++	4/4	+++	4/4	+++
MLN-jejunum 75 %	0/4	-	3/4	+++	4/4	+++	4/4	+++	4/4	+++
PP-caecum	0/4	-	3/4	+++	4/4	+++	4/4	+++	4/4	+++
Ileo-coecal LN	0/4	-	3/4	+++	4/4	+++	4/4	+++	4/4	+++
PP-ileum	1/4	++	3/4	+++	4/4	+++	4/4	+++	4/4	+++
MLN-ileum	1/4	+	3/4	+++	4/4	+++	4/4	+++	4/4	+++
Carcass										
Precrural LN	0/4	-	3/4	+	2/4	+	4/4	+++	3/4	+++
Prescapular LN	0/4	-	3/4	+++	2/4	+++	4/4	+++	4/4	+++

* LN, lymph node; MLN, mesenteric lymph node; PP, Peyer's patch; ¹ One of the four 3 month old lambs was negative in all examined tissues; nd, Not determined

Colour Key

	Organ with PrP ^{res} detected potentially entering the food chain in scenario 1 (spleen and ileum removed as SRM)
	Organ with PrP ^{res} detected potentially entering the food chain in scenario 2 (spleen and ileum removed as SRM, plus the advised tissues)
	Organ with PrP ^{res} detected potentially entering the food chain in both scenarios (1 and 2)


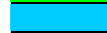

Table 2. PrP^{res} IHC detection in lymphoid tissues from susceptible VRQ/VRQ lambs with natural scrapie (Modified from Van Keulen *et al.*, 2002).

Groups of two lambs were scored on the basis of the percentage of lymphoid follicles positive to PrP^{res}-labelling, and were graded as none detected (-), detected in 0-25% of the lymphoid follicles (+), detected in 25-50% of the lymphoid follicles (++) , detected in 50-75% of the lymphoid follicles (+++) and detected in 75-100% (++++) of the lymphoid follicles. Tissues with PrP^{res} potentially entering the food chain in scenario 1 (green, spleen and ileum removed as SRM), in scenario 2 (blue, spleen and ileum removed as SRM plus the advised tissues) and in both scenarios (orange) are shown by colour-coding.

Body part / Organ	Age (months)									
	1		2		3		4		5	
	Positive sheep	PrP ^{res} -labelling	Positive sheep	PrP ^{res} -labelling	Positive sheep	PrP ^{res} -labelling	Positive sheep	PrP ^{res} -labelling	Positive sheep	PrP ^{res} -labelling
Head										
Third eye lid	0/2	-	0/2	-	1/2	+	2/2	+	0/2	-
Palatine tonsil	0/2	-	2/2	+	1/2	++	2/2	+++	2/2	+++ / ++++
Medial retropharyngeal LN	0/2	-	2/2	+	1/2	++	2/2	+++ / ++++	2/2	+++ / ++++
Mandibular LN	0/2	-	0/2	-	1/2	+	2/2	+ / ++	2/2	+ / +++
Parotideal LN	0/2	-	0/2	-	0/2	-	2/2	++	2/2	++
Abdominal and Thoracic Viscera										
Spleen	0/2	-	0/2	-	1/2	+	2/2	++ / +++	2/2	+
Tracheobronchial LN	0/2	-	0/2	-	0/2	-	2/2	+	2/2	+
Caudal mediastinal LN	0/2	-	0/2	-	1/2	+	2/2	+ / ++	2/2	+ / ++
PPc jejunum 0–25%	0/2	-	0/2	-	1/2	+	2/2	+ / +++	2/2	+++
MLN Cranial jejunal	0/2	-	0/2	-	1/2	+	2/2	+++ / ++++	2/2	++++
PP jejunum 25–50%	0/2	-	0/2	-	2/2	+	2/2	+++ / ++++	2/2	+++
MLN Middle jejunal	0/2	-	0/2	-	2/2	+	2/2	+++ / ++++	2/2	+++ / ++++
PP jejunum 50–75%	0/2	-	0/2	-	2/2	+	2/2	+++ / ++++	2/2	+++
MLN Caudal jejunal	0/2	-	2/2	+	2/2	+	2/2	++++	2/2	++++
PP jejunum 75–100%	0/2	-	2/2	+	2/2	+ / ++	2/2	+++ / ++++	2/2	+++ / ++++
PP ileum	0/2	-	2/2	+	2/2	+ / ++	2/2	++++	2/2	++++
MLN Ileocaecal	0/2	-	2/2	+	2/2	+ / ++	2/2	++++	2/2	++++
PP caecum	0/2	-	0/2	-	1/2	++	2/2	++++	2/2	+++ / ++++
Carcass										
Prescapular LN	0/2	-	0/2	-	1/2	+	2/2	+ / +++	2/2	++ / +++
Prefemoral LN	0/2	-	0/2	-	0/2	-	2/2	+	2/2	+
Popliteal LN	0/2	-	0/2	-	0/2	-	2/2	+	2/2	+
Cranial sternal LN	0/2	-	0/2	-	0/2	-	1/2	+	2/2	++
Medial iliac LN	0/2	-	0/2	-	0/2	-	1/2	++	2/2	+
Superficial inguinal LN	0/2	-	0/2	-	1/2	+	2/2	+	2/2	+

* LN, lymph node; MLN, mesenteric lymph node; PP, Peyer's patch.

Colour Key:

-  Organ with PrP^{res} detected potentially entering the food chain in scenario 1 (spleen and ileum removed as SRM)
-  Organ with PrP^{res} detected potentially entering the food chain in scenario 2 (spleen and ileum removed as SRM, plus the advised tissues)
-  Organ with PrP^{res} detected potentially entering the food chain in both scenarios (1 and 2)

B. ELISA detection and quantitation of PrP^{res} and infectivity titration in laboratory rodents.

According to the prion hypothesis, PrP^{res} is an infectious protein and the causative agent of TSEs (Prusiner, 1982). In TSEs the accumulation of PrP^{res} in tissues of infected individuals is correlated with the presence of infectivity (McKinley *et al.*, 1983; and *inter alia*, Race *et al.*, 2001). In its Opinion on the quantitative risk assessment on the residual BSE risk in sheep meat and meat products (EFSA, 2007b), the BIOHAZ Panel concluded that “*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*”.

Biochemical assays can similarly be used to monitor the timing and relative amounts of infectivity in tissues of TSE affected animals. In naturally and experimentally (orally) infected VRQ/VRQ sheep (the French Langlade Flock) the timing of detection, and the quantity, of PrP^{res} in various tissue including the ileal mesenteric lymph node (Figure 1) and the prescapular lymph node (Figure 2) was established employing a commercial PrP^{res} ELISA test (TeSe sheep and goat – Biorad) using recombinant VRQ ovine prion protein as external standard (research by Andreoletti and colleagues⁹).

Qualitatively, these data confirm the IHC studies showing biological variability, the earlier detection of PrP^{res} (at two months in 4 out of 4 animals) in ileal mesenteric lymph node (iMLN) compared to its detection in pre-scapular lymph node (at 3 months in 3 out of 4 animals) and the levelling off of the accumulation of prions (plateau effect). Based on tissues obtained from the experimentally (orally) infected VRQ/VRQ sheep, the relative change in PrP^{res} in iMLN between three and six months was estimated as ~ 10-fold, while in pre-scapular lymph node similar changes could be seen but with greater variability.

⁹ EU funded research project reference QLK-CT-01309 - ‘BSE in sheep’ – Program Coordinator: Dr. Olivier Andreoletti.

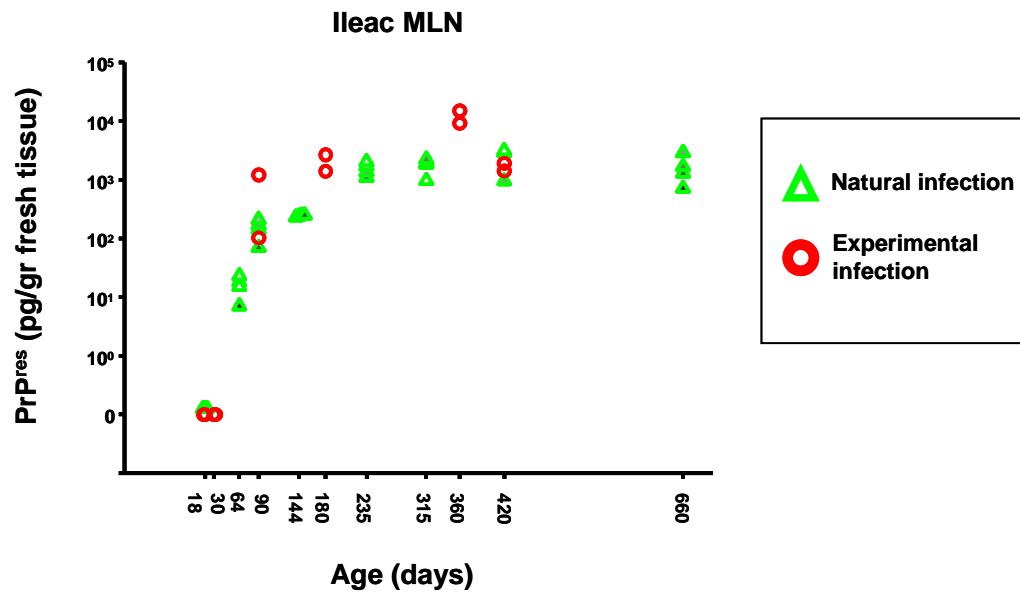


Figure 1. Quantification of PrP^{res} per mg of fresh tissue in the Ileal mesenteric lymph node. Results are expressed with reference to a recombinant PrP external control (in picograms).

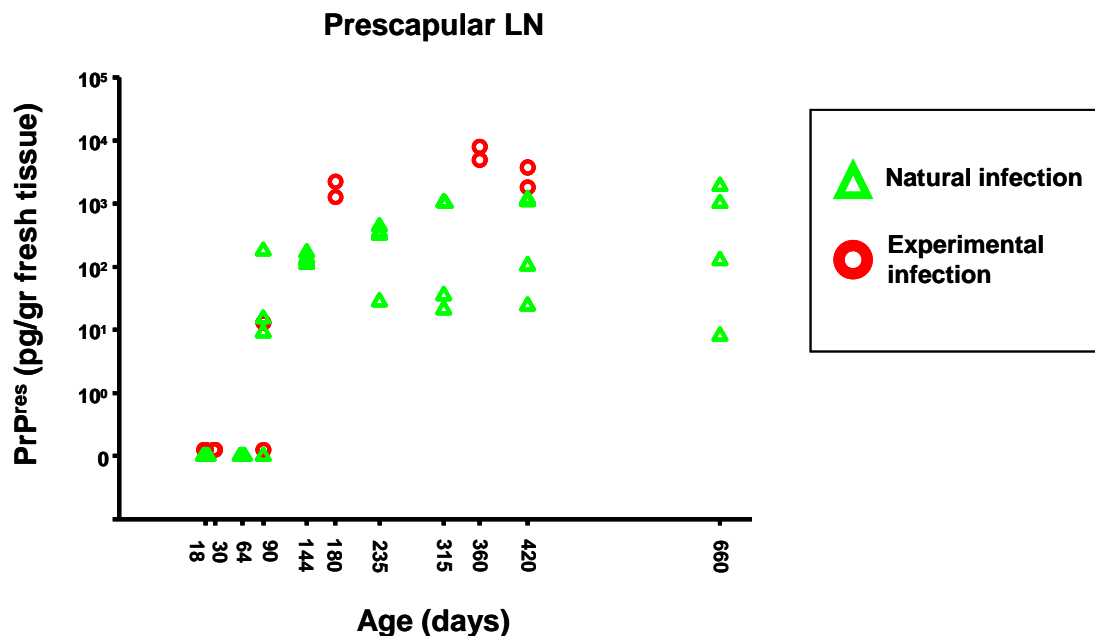


Figure 2. Quantification of PrP^{res} per mg of fresh tissue in the prescapular lymph node. Results are expressed with reference to a recombinant PrP external control scale (in picograms).

This work serves as a guide for more quantitative approaches such as bioassay and quantal titration in laboratory rodents. An example of this is given below in Figure 3, for the ileal mesenteric lymph node from naturally infected VRQ/VRQ sheep in the same study (research by Andreoletti and colleagues¹⁰).

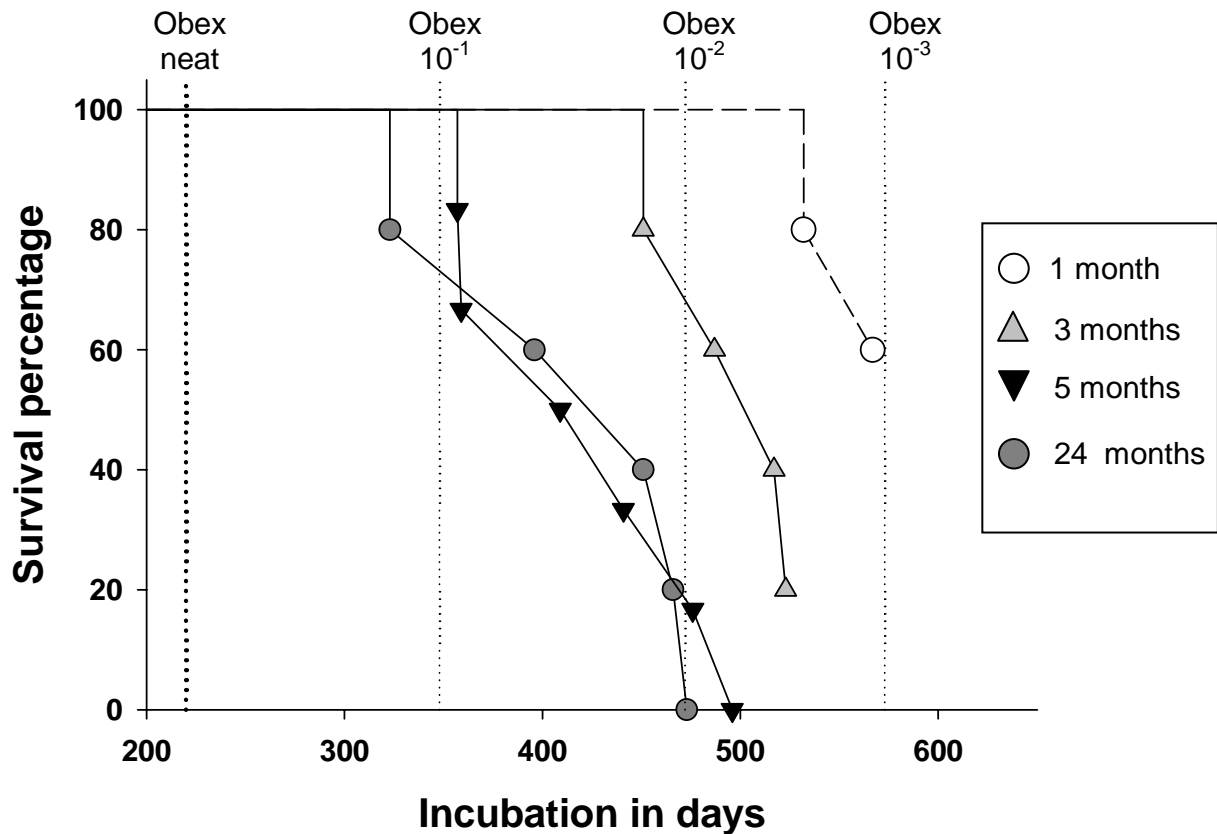


Figure 3. Infectivity titration of an ileal mesenteric lymph node of naturally Classical Scrapie infected sheep culled at different ages.

This Figure shows Kaplan-Meier survival curves of transgenic mice¹¹ (n = 5 or 6) inoculated intra-cerebrally with 10% w/v- homogenate of ileal MLNs taken from naturally-infected VRQ/VRQ sheep at different times during the course of disease: one month (open circle); 3 months (shaded triangle); 5 months (black inverted triangle); end point of disease – terminally affected sheep – 24 months (shaded circle). The survival times (incubation in days) are calibrated against the mean survival times of mice inoculated with different 10-fold serial dilutions of a 10% w/v- homogenate of obex (CNS) from a terminally-affected sheep from the same flock (*i.e.*, affected by the same Scrapie Agent). The superimposition of the survival curves for the 5 and 24 months ileal MLNs indicates similar titres in this tissues, inferring a plateau of infectivity between these time points. This plateau of infectivity is consistent with the IHC and ELISA PrP^{tes} data, which show a similar plateau of PrP^{tes} accumulation between 6 and 24 months. Thus, it can be logically assumed that the infectivity of the ileal MLN of a 6

¹⁰ EU funded research project reference QLK-CT-01309 - ‘BSE in sheep’ – Program Coordinator: Dr. Olivier Andreoletti.

¹¹ Tg338 mice which over-express the ovine VRQ allele in CNS tissue (Le Dur *et al.*, 2005).

months old lamb would be equal or very similar to that of the ileal MLN of a 5 months old lamb. The extended incubation times (and lower attack rates) of the three month and one month samples indicate lower titres at these times in this lymph node at levels (by calibration against the obex) of about 10-folds (at 3 months) and 50-folds (one month) less than the plateau level or about 200-fold and 2000-fold less than the titre of infectivity in the brain (obex) at the terminal stage of disease.¹²

If we assume similar replication kinetics and spread to the other lymph nodes that remain on the carcass and potentially enter the human food chain, then there is an approximate 10 times increase in infectivity (exposure) in moving the age limit from one month to three months, a further 5 to 10 times increase in moving from three months to six months after which levels of infectivity (at ~ 1/50 that found in terminal brain) in lymphoid tissue reach a plateau.

If the weights of infected tissues currently (W1; some of these presented and highlighted in green in Tables 1 and 2) and proposed (W2; some of these presented and highlighted in blue in Tables 1 and 2) to enter the food chain were known then the relative change in human exposure (E) could be expressed, from these assumptions and estimates of relative titre at 3 and 5 months of age, as: $E \sim 10(W2/W1)$.

Further experimental work is needed to define the variability and uncertainty of these estimates of relative titre at different ages in young lambs and kids, and to determine the variability and uncertainty of W1 and W2. These data would allow quantifying the increase of exposure risk linked to the consumption of a certain amount of lymphoid tissue that would remain on the dressed carcass.

¹² Note that infectivity is detected at one month in MLN by tg338 mouse bioassay but not by ELISA. This is consistent with infection at or around birth. These results are also consistent with the reported presence of infectivity without detectable PrP^{res}.