OPINION

of the French Food Safety Agency
regarding a request for a summary of the available data concerning infectivity
in ruminant tissues

1. REVIEW OF THE REQUEST

The French Food Safety Agency (AFSSA) received a request from the Directorate General for Food (DGAL), on 29 April 2009, for a summary of the scientific data concerning infectivity in ruminant tissues.

2. BACKGROUND

A large number of AFSSA opinions, particularly those related to specified risk materials and exposure risks associated with the consumption of certain animal products, are based on the available data regarding the distribution of infectious agents responsible for TSEs [transmissible spongiform encephalopathies] (infectivity) or its abnormal PrP [prion] protein marker (PrPSc [scrapie-associated form of the prion protein]) in tissues of animals exposed naturally or experimentally.

The distribution of infectivity and abnormal PrP protein in the tissues of animals incubating or clinically affected with a TSE may vary according to the host species (and its possible genetic susceptibility factors related to the PrP gene) and the agent involved.

Given the complexity of the available data, the DGAL requested that AFSSA prepare a document summarising the nature of the tissues, organs or products in the different species of domestic ruminants in which the presence of prions has been reported. Ideally, this document should take into account:

i) the nature of the TSE agent involved,

ii) the PRNP genotype (PrP gene) of the host (in species for which this information is relevant).

Where these data are available, the age at which tissues or different products were identified as positive, as well as the levels of infectivity concerned, will be specified.
3. EXPERT ASSESSMENT METHOD

Collective expert assessment was undertaken by the Scientific panel (CES) on TSSEs [Transmissible Subacute Spongiform Encephalopathies], which met on 30 June 2009, 1 October 2009, 14 January 2010, 16 February 2010, and 19 March 2010.

This Opinion is based on a review of the scientific literature as well as data that has not yet been published. The Committee has also taken into account the summary on infectivity of tissues conducted by the WHO in 2006 and updated in 2010.

4. DISCUSSION

AFSSA’s discussion is based on the Opinion of the Scientific panel on TSSEs whose information is presented below:

4.1. Introduction

Studies related to the pathogenesis of prion diseases and the spread of the infectious agent in the body of hosts are primarily based on two approaches:

- the first uses immunochemical detection of the abnormal prion protein (PrPSc) in the tissues of affected individuals (Western Blot, ELISA or Immunohistochemical analyses), a method whose sensitivity has improved significantly over the last decade.

- the second uses the detection of infectivity by bioassay (on ground material from the tissue examined and animal inoculation). For over 40 years, these bioassays were performed on congenic mouse strains. However, the capacity for prion propagation is limited by the species barrier. Thus, some isolates which are highly infectious in the species of origin nonetheless cannot be transmitted to these murine models (Hadlow et al., 1982). For other isolates, it is the ability to detect low levels of infectivity that will be affected by the use of this type of conventional mouse. The recent development of transgenic mouse models for the PRNP gene of different species (sheep, cattle, pig, etc.) has made it possible to go beyond this limit. However, the ability of these models to detect very low levels of infectivity raises questions about the biological relevance of the data obtained. The levels of infectivity observed (and risks of exposure) are then to be considered in reference to those generated by the most infectious tissue (usually brain tissue).

It should also be noted that discrepancies are sometimes observed between the accumulation of PrPSc and the presence of infectivity. In some cases these discrepancies (particularly with respect to atypical scrapie) result from a simple lack of sensitivity of the immunochemical methods used. In other cases, they seem to be related to dissociation between PrP forms resistant to proteinase K (which are targeted by immunochemical methods) and infectivity (Lasmézas et al., 1997).

In addition, a significant portion of the data to which the Committee is referring was obtained as part of experimental inoculations sometimes carried out at high doses. Such exposure conditions must be considered as extreme and therefore unlikely in the context of a natural exposure. Consequently, these data must be used with caution.

Lastly, the distribution of infectivity in affected animals is likely to extend to tissues that are negative as a rule (e.g. mammary tissue) when the disease develops under specific inflammatory conditions, such as mastitis (Heikenwalder et al., 2005, Ligios et al., 2005).

4.2. TSE in cattle (See Annex 1).

Three forms of bovine TSE have been described to date. These include classical BSE (bovine spongiform encephalopathy) and two atypical forms characterised in the last five years, BSE-L and BSE-H (Casalone C et al., 2004; Biacabe A.G. et al., 2004). Because they have only been identified recently, there are very little data on atypical forms of BSE in terms of tissue infectivity in affected animals. Note, however, that experimental transmission studies (in cattle, primates, and conventional or transgenic mice) suggest significant potential infectivity for BSE-L (Lombardi et al., 2008, Beringue et al., 2007 and 2008, Comoy et al., 2008, Kong 2008, Capobianco et al., 2007). Furthermore, unpublished data indicate infectivity in skeletal muscles of cattle infected with BSE-L (Suardi et al., 2009).

Concerning classical BSE, the available data come both from experimental studies on oral inoculation of cattle and measurement of tissue infectivity in naturally affected cattle. The primary results concerning the kinetics of the onset of infectivity or of its associated marker (PrPSc) were initially established as a result of the study of cattle experimentally infected by ingestion of 100 g (per animal) of brainstem tissue from cattle in the final stages of infection (Wells et al., 1998; Grassi et al., 2001; Terry et al., 2003; Wells et al., 2005). Whereas clinical signs of the disease appeared at 36 months in this experiment, infectivity was detected by inoculating RIII wild-type mice in the central nervous system at 32 months, in the distal ileum from 6 to 18 months and after 36 months, as well as in the palatine tonsils at 10 months. These first basic results had been the specific subject of an Opinion of the Scientific Steering Committee on 8 November 2002. More recently, some studies have clarified these results, either by other studies of experimental transmission in cattle, or by applying new infectivity detection models, with the use of transgenic mice expressing bovine prion protein in particular.

4.2.1. Longitudinal studies

Experimental oral infection of cattle with 100 g or by one gram of brainstem tissue from BSE-infected cattle has enabled a comparison to be made of the time elapsed from the onset of the pathological prion protein according to the incubation period (Arnold et al., 2007). Note that experimental infection with one gram of inoculum more closely imitates the circumstances of the natural disease since it is associated with an average incubation period of five years. In these experiments, the animals were sacrificed at various stages of the incubation period and the presence of PrPSc was investigated by different methods (Western blot, ELISA test or Immunohistochemical). This study was supplemented by bioassay experiments on conventional mice (Arnold et al., 2009).

This work makes it possible to estimate that for half of the experimentally infected animals,

- with 1 g: PrPSc would be detectable at the level of the obex 1.7 months before the onset of clinical signs (i.e., 97% of the incubation period).
- with 100 g: PrPSc would be detectable at the level of the obex 9.6 months before the onset of clinical signs (i.e., 79% of the incubation period).

These data suggest that, under natural conditions of contamination, the probability of detecting PrPSc at the level of the obex is restricted to the six months that precede the onset of clinical signs.

For the group of cattle infected by one gram of tissue, there is no difference in the interval of detection of PrPSc between the brain and the spinal cord. In contrast, for cattle infected by 100 g of tissue, detection of PrPSc would most likely occur first in the obex. One month later, on average, it would be detected in the thoracic and spinal cord or 1.3 months later in the midbrain and lumbar spinal cord.

While PrPSc is occasionally detected in these cattle by pre-clinical testing in the stellate and dorsal root ganglia, it is detected only sporadically in the peripheral nerves and the adrenal gland and only
in animals with confirmed clinical signs. The detection of PrP^{Sc} in the trigeminal and dorsal root ganglia appears very late in the incubation period, and later in the cervical than in the thoracic area (Arnold et al., 2007). It is contemporary or subsequent to detection in the corresponding spinal cord area. These data are consistent with a study conducted in cattle naturally affected with BSE, which reports on the detection of PrP^{Sc} by Western blot, with variable intensity depending on the individual, in the thoracic and cervical dorsal root ganglia, trigeminal ganglia, adrenal gland, vagus, and splanchnic and sciatic nerves (Masujin et al., 2007). Compared to the situation with the natural disease, these results suggest that if the animal is infected in the first six months of life, PrP^{Sc} would be detectable in the central nervous system and the dorsal root ganglia in the majority of the animals only after approximately 42 to 48 months.

Concurrent with this work, a study was conducted on two animals experimentally infected with 100 g of brainstem tissue from cattle infected with BSE and sacrificed at 24 and 28 months, with no clinical signs (Hoffmann et al., 2007). These animals correspond to the shortest time of onset of PrP^{Sc} detectable in the obex area after experimental infection with a high oral dose, a period of 32 months having been observed in similar experiments conducted in the United Kingdom. In the nervous system, PrP^{Sc} was detected in the dorsal vagal nucleus and, in the single animal sacrificed at 24 months, in the celiac-mesenteric ganglion complex and the caudal mesenteric ganglion, as well as in the central and lateral intermediate substances of the spinal cord. It was not detected in any of the peripheral nerves examined, nor in the spleen, lymph nodes or bone marrow.

Finally, the potential infectivity of the bone marrow has been reassessed recently by inoculation of sternal marrow in cattle (100 mg / animal via intra-cerebral route), derived from cattle experimentally infected by oral exposure and sacrificed at 22, 26, 32 and 36 months post-infection (Sohn HJ et al., 2009). These experiments failed to detect infectivity.

4.2.2. Quantitation of infectivity levels

Bioassay experiments in RIII wild-type mice inoculated with tissue from cattle infected with 100 g of brainstem from cattle with BSE (Arnold et al., 2009) were used to estimate:

- the rate of increase in infectivity for the central nervous system: the doubling time of infectivity was shown to average 1.2 months (CI 95% 1.1-1.4 months), shorter than previously supposed.
- the titres of infectivity in the dorsal root ganglia, lower than in the central nervous system: between 32 and 40 months, for cervical and thoracic ganglia the titres were respectively 1 and 1.5 log10 mouse i.c./i.p. ID_{50} lower per gram than the titres of the central nervous system (Arnold et al., 2009).
- the pattern of increase in infectivity in the distal ileum: an initial increase in titres up to 14-18 months post exposure (1.59 and 1.58 log10 mouse i.c./i.p. ID_{50}/g), estimated at levels comparable to those of the central nervous system six months prior to the clinical onset of disease. These titres of infectivity decreased beyond 36 months, possibly in connection with involution of lymphoid tissue.

Another study based on a bioassay in a line of transgenic mice expressing bovine prion protein (Tgbov XV mice) was conducted to assess infectivity in tissues from a cow naturally affected with BSE (Buschmann et al., 2005); the bovine transgenic line used in this study was estimated to be 10,000 times more sensitive than the RIII wild-type mouse and 10 times more sensitive than cattle for detecting the agent of BSE. Infectivity is detected in the brainstem, thoracic or lumbar spinal cord, retina and optic nerve, facial and sciatic nerves, as well as in the distal ileum (by comparison with the bioassay in conventional RIII mice, which revealed no infectivity in some of these tissues, such as optic, facial and sciatic nerves, as well as in the distal ileum). In contrast, infectivity remains undetectable in bovine prion protein transgenic mice in the spleen, palatine tonsils, mesenteric lymph node, or in colostrum. It should be noted that one mouse (1/10) developed the disease following inoculation of semitendinosus muscle (but not following inoculation of the long dorsal [longissimus dorsi] muscle).

---

\textsuperscript{2} Infectious dose inducing the death of 50% of mice inoculated by intracerebral or intraperitoneal routes.
Using bioassay, in another strain of bovine prion protein transgenic mice (Tg110), on tissue taken from cattle sacrificed sequentially after experimental oral infection with an individual dose of 100 g (Espinosa et al., 2007), infectivity is detected in the sciatic nerve at 30 and 33 months, whereas it is found in the brainstem, initially to a limited extent at 27 or 30 months, then increasing sharply at 33 months; from these same tissues, infectivity had only been detected in the brain stem from 32 months by bioassays in RIII wild-type mice. Infectivity is also detected in distal ileal Peyer’s patches and palatine tonsils from 20 months and up to 33 months after experimental infection, representing the entire incubation period examined. In contrast, no infectivity was detected in the spleen, skeletal muscle, blood or urine in this experiment.

Finally, it may be remembered that a study conducted on the milk of BSE-infected cows (experimental oral inoculation with 100 g or one gram of inoculum) failed to reveal abnormal prion protein by biochemical methods (Everest et al., 2006).

Furthermore, research on infectivity by bioassay on cattle proved negative (homogenate of tissues from cattle sacrificed periodically after oral contamination, then inoculated into cattle intracerebrally) for some tissues such as thymus, spleen, liver, lungs, lymph nodes, skin, salivary glands, buffy coat3, and peripheral nerves. Indeed, cattle inoculated with homogenates made from these tissues were still free of clinical signs in December 2004, i.e. 71 to 99 months after experimental infection (Wells et al., 2005). The impact of these results must be put into perspective considering that the more sensitive techniques mentioned above have been able to invalidate these results for the peripheral nerves.

4.3. TSEs in small ruminants (See Annex 2).

In small ruminants, three types of TSE are traditionally distinguished: classical scrapie, atypical scrapie and BSE.

It is important to stress that the term classical scrapie is an operational term designating a group of TSE agents that, in small ruminants, is associated with a biochemical signature typical of abnormal PrP as identified by the Western Blot technique. The term classical scrapie encompasses a set of different biological agents whose properties, including their ability to infect the natural host and spread in its body, may vary.

In sheep, there is a major effect of certain polymorphisms of the PRNP gene on the susceptibility/resistance to infection by TSE agents:

- In terms of classical scrapie and BSE, codons 136 (A or V), 154 (R or H) and 171 (R, Q or H) (Clouscard et al., 1995; Hunter et al., 1996) determine susceptibility to infection. Animals of genotypes VRQ/VRQ, ARQ/VRQ and ARQ/ARQ are considered to be the most susceptible to agents of classical scrapie, whereas homozygous or heterozygous AHQ and heterozygous ARR animals exhibit only marginal susceptibility.
- Animals with the AHQ allele and ARQ/ARQ animals are considered to be the most susceptible to infection (experimental) by BSE, while VRQ/VRQ animals seem less susceptible to this agent.
- ARR/ARR animals are considered as being highly resistant to infection by these agents (Hunter et al., 1997; Hunter et al., 1996). However, several experimental or natural cases of infection have been reported in animals of this genotype (Groschup et al., 2007, Ikeda et al., 1995, Houston et al., 2003).
- In terms of atypical scrapie, genetic determinism of susceptibility in sheep is totally different from that of classical scrapie or BSE. A greatly increased risk of developing the disease is associated with alleles AHQ and AF141RQ, while animals of genotype ALRQ/ALRQ and VRQ/VRQ seem to be less at risk. Animals carrying the ARR allele (homozygous or heterozygous) may develop the disease (Arsac et al., 2007; Moreno et al., 2007; Moum et al., 2005).
In goats, the effects of PRNP gene polymorphism on susceptibility to TSE agents are much less well documented than in sheep. However, this area is the subject of intense research activity and the data, while still incomplete, seem to suggest that some polymorphisms could be associated with greater susceptibility/resistance to infection. Thus, mutations R/H154- R/Q211- D/S146 and Q/K222 seem to reduce susceptibility to infection by agents of classical scrapie (Barillet et al., 2009; Gonzalez et al., 2009; Vaccari et al. 2006). Instead, as in sheep, the allele AHQ appears to be associated with an increased risk of developing atypical scrapie.

For the moment, there are no sufficiently robust data to assess the effect of these mutations on resistance to infection by the agent of BSE.

These complex interactions between the genotype of the host and the agent of TSE examined are likely to influence the distribution and kinetics of the spread of prions in the bodies of infected animals.

4.3.1. Classical scrapie

It is commonly accepted that under natural conditions of exposure, contamination by agents of classical scrapie occurs primarily at birth (maternal and lateral transmission) and that the placenta, which can accumulate large amounts of PrPSc, plays a key role in this process (Andréoletti et al., 2002; Pattison and Millson, 1961; Race et al., 1998; Tuo et al., 2002). More recently, the identification of infectivity in colostrum and milk from affected ewes as well as efficient transmission to the lamb by milk from ewes incubating classical scrapie has reinforced this assumption (Konold et al., 2008; Lacroux et al., 2008).

It may be recalled that the incubation period is highly variable, depending on the isolate and genotype considered. Thus, the incubation periods traditionally reported in the literature range from 20-24 months for VRQ/VRQ animals, to 30-36 months for ARQ/ARQ animals, and from 45 to 72 months for heterozygous ARR animals. The earliest ‘natural’ clinical case reported to date was six months old (Adjou, oral communication, NeuroPrion, 2007). After oral inoculation at the age of 14 days (five grams of brain tissue from affected sheep), homozygous VRQ animals develop TSE with an average incubation period of six months, and the first clinical signs being detectable from four months post-infection (Ryder SJ et al., 2009). These data illustrate the potential impact of an experimental inoculation (including oral) on the time of onset of the disease.

Given these factors, AFSSA considers that the most relevant studies in the context of this request are those conducted in naturally affected animals.

Two herds naturally affected with scrapie were used by the French and Dutch teams. The animals studied were of genotype VRQ/VRQ for which the incubation periods are the shortest. A summary of the distribution of infectivity is presented in Annex 2.

These studies, whose results are consistent, have demonstrated that:

- Abnormal PrP is detectable in secondary lymphoid formations adjacent to the ileum at the age of 21 days (Andréoletti et al., 2002).
- PrPSc spreads and accumulates in lymphoid formations of the intestine and mesenteric lymph nodes during the first two months of life (Andréoletti et al., 2000; Andréoletti et al., 2002; Van Keulen et al., 2002, Heggebo et al., 2000).
- In animals over two months old, PrPSc was observed to have spread to all secondary lymphoid structures of the body (Andréoletti et al., 2000; Andréoletti et al., 2002, Van Keulen et al., 2002).

---

4 Opinion of the French Food Safety Agency on the possible animal and public health consequences of new available scientific findings on the intra-species transmission of the classical scrapie agent by milk, dated 8 October 2008.
• Accumulation of PrP\textsuperscript{Sc} in secondary lymphoid organs continues with age, reaching a plateau after six months (Andreoletti et al., 2000). At this stage, infectivity present in lymphoid organs can reach titres 50 times lower than those observed in the brainstem of an animal in the final stage of the disease (with equal tissue mass).

• The central nervous system (brain and spinal cord) becomes positive (PrP\textsuperscript{Sc} and infectivity) between seven and ten months (Andréoletti et al., 2000; Jeffrey et al., 2001; van Keulen et al., 2002).

• The presence of PrP\textsuperscript{Sc} in the skeletal muscle is detectable by the age of 13 months (Andréoletti et al., 2004). In some samples, the levels of infectivity observed in the muscle can be equivalent to those observed in the lymphoid organs of animals older than six months. However, given the heterogeneity of the distribution of the infectious agent in striated muscle tissue, these data should be approached with caution.

• Infectivity is detectable in milk from the first lactation. Infectious titres measured in fractions of milk or colostrum are appreciably lower than those observed in the brain (see Annex 2).

• Infectivity in blood has been reported in sheep at three months of age. This infectivity persists throughout the life of the animals. Data related to infectious blood titres are not available at this stage of experimentation.

Note as well that PrP-res [protease-resistant prion protein] has been reported in the kidneys of sheep affected with classical scrapie (Siso et al., 2006) and in the tongue (Casalone et al., 2005).

4.3.2. Cases in goats

During infection by an agent of classical scrapie, the pattern of dissemination of PrP\textsuperscript{Sc} in the body of goats is generally similar to that described in sheep.

Although some elements related to the kinetics of distribution in the body are available in naturally affected animals, the most complete data come from oral inoculations performed in kids less than two weeks old (Andréoletti – Chauvin Perrin et al., unpublished work).

It may be recalled that, as in sheep, some polymorphisms of the PRNP genes in goats (codons 142, 154, 211 and 222 in the French goat population) seem likely to strongly influence susceptibility to infection and/or the dissemination kinetics of prions in the body. However, the data available at this stage are still too limited to be used here.

Three experiments conducted independently indicate that, within the limits of these studies (animals of the genotype most susceptible to infection I\textsubscript{142}R, I\textsubscript{154}R, I\textsubscript{211}Q, I\textsubscript{222}Q, nature of the agents of classical scrapie, and dose used):

• No accumulation of PrP\textsuperscript{Sc} is detectable in the tissues of animals under three months old;
• PrP\textsuperscript{Sc} is detected in lymphoid formations adjacent to the intestine in animals over three months old;
• PrP\textsuperscript{Sc} is detected in systemic lymphoid formations in animals over six months old;
• PrP\textsuperscript{Sc} is detected in the central nervous system of animals over 12 months old;
• PrP\textsuperscript{Sc} is detected in the skeletal muscles of animals over 18 months old.

At the present time there is no available data related to infectious titres found in these different tissues.

Lastly, preliminary data indicate the presence of infectivity in the milk of goats incubating natural classical scrapie (Andréoletti – Chauvin Perrin et al., unpublished work).
4.3.3. Atypical scrapie

Atypical scrapie was first described in 1998 in Norway (Benestad et al., 2008). The discovery of the prevalence of this TSE agent in small ruminants (approximately half of TSE cases detected in small ruminants in recent years) is directly related to the active TSE epidemiological surveillance programme. Because of the biochemical characteristics associated with PrP\textsuperscript{Sc} in this type of TSE, for atypical scrapie there is a very clear discrepancy between the levels of infectivity detectable by bioassays on transgenic mice and the presence of PrP\textsuperscript{Sc} (high infectious titre lacking detectable PrP\textsuperscript{Sc}).

The few available data indicate an apparent absence of any accumulation of PrP\textsuperscript{Sc} in the peripheral tissues of sheep either naturally or experimentally infected or incubating (natural pre-clinical cases detected through the epidemiological surveillance programme) atypical scrapie.

Preliminary results obtained by bioassay in transgenic mice for the VRQ variant of the ovine PrP gene indicate the presence of prior low levels of infectivity in some lymphoid organs of animals incubating classical scrapie (natural cases) or in striated muscle tissue and the peripheral nervous system of animals clinically infected with the disease (intracerebral inoculation).

At present there is no available data on the impact of polymorphisms of the PrP gene on the distribution of peripheral infectivity. Similarly, we have no evidence for specifying the kinetics and dynamics of distribution of the infectious agent in the body of individuals developing this disease.

4.3.4. BSE

Data on the distribution of the agent responsible for BSE in the tissues of small ruminants concern only those animals inoculated orally. Although some data are available regarding goats or sheep of other genotypes, the critical results available concern sheep of genotype ARQ/ARQ, considered as being highly susceptible to BSE.

In ARQ/ARQ animals experimentally orally infected (5 g of BSE-infected sheep’s brain) the presence of PrP\textsuperscript{Sc}, and/or infectivity has been observed:

- in all lymphoid formations adjacent to the intestine, spleen and mesenteric lymph nodes from four months of age;
- in other secondary lymphoid formations in animals ten months of age;
- In the central nervous system of animals ten months of age;

As in classical scrapie, infectivity could be identified by bioassay in skeletal muscle, milk and blood samples, without infectivity levels or the kinetics of the onset of infectivity in these tissues lending themselves to clear specification at this stage.

Infectivity was also detected in the thymus of sheep infected by this strain (Bellworthy et al., 2005).

5. CONCLUSION

The dynamics of prion distribution in the tissues of small ruminants infected with certain agents of classical scrapie and cattle infected with the classical BSE agent are well documented at this time.

AFSSA places particular emphasis on the complexity of the host-pathogen relationship in terms of TSE, primarily in small ruminants. This complexity is due to the biodiversity of the agents of classical scrapie and the potential impact of polymorphisms of the PRNP gene on the pathogenesis of prion infections. Given the influence of these parameters, but also of the route and pressure of infection on the pathogenesis of TSEs, the available knowledge on which this opinion is based cannot be considered definitive.

With regard to atypical forms of TSE (BSE types H and L, and atypical scrapie) the knowledge we have is extremely fragmented and limited. Current or future experiments should help develop a better understanding of these atypical forms in the next few years with a likely impact on risk assessment of specific dietary exposure to these agents.
The implementation of pathogenesis research on these atypical strains, as part of an epidemiological framework in which the prevalence of these forms of TSE reaches and even surpasses that of classical forms, would provide clarifying data essential to understanding these diseases.

These are AFSSAs conclusions at this time.

The Director General

Marc MORTUREUX

KEY WORDS

KEY words: TSE, infectivity, tissues, SRM [specified risk material], small ruminants.

BIBLIOGRAPHY

Adjou, K; Humeau, G; Messiens, S; El Hachimi, KH; Ouidja, M; Couquet, C; Deslys, JP; Eloit, M; Brugere-Picoux, J. Characterization of a Highly Pathogenic Isolate of Sheep Scrapie in Transgenic Mice Overexpressing Murine PrP; NeuroPrion 2007, Edinburgh, Scotland, UK.


Update of the opinion on TSE infectivity distribution in ruminant tissues initially adopted by the Scientific Steering Committee at its meeting of 10-11 January 2002 and amended at its meeting of 7-8 November 2002.


## ANNEX 1: BOVINE TISSUES

<table>
<thead>
<tr>
<th>Tissue considered</th>
<th>Experimental infection 1g</th>
<th>Experimental infection 100g</th>
<th>Natural conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PrP-res</td>
<td>Infectivity</td>
<td>PrP-res</td>
</tr>
<tr>
<td><strong>Central nervous system</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>42 months&lt;sup&gt;1&lt;/sup&gt;</td>
<td>30 months&lt;sup&gt;1&lt;/sup&gt;</td>
<td>24 months&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>42 months&lt;sup&gt;1&lt;/sup&gt;</td>
<td>30 months&lt;sup&gt;1&lt;/sup&gt;</td>
<td>24 months&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Retina</td>
<td>++</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td><strong>Peripheral nervous system</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorsal root ganglia</td>
<td>42 months&lt;sup&gt;1&lt;/sup&gt; (low)</td>
<td>32 months&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Trigeminal ganglia</td>
<td>48 months&lt;sup&gt;1&lt;/sup&gt;</td>
<td>32 months&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Peripheral nerves</td>
<td>44 months&lt;sup&gt;1&lt;/sup&gt;</td>
<td>35 months&lt;sup&gt;1&lt;/sup&gt;</td>
<td>30 months&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Autonomic nervous system ganglia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lymphoreticular system</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palatine tonsils</td>
<td></td>
<td></td>
<td>10 months&lt;sup&gt;3&lt;/sup&gt;-20 months&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Endocrine system</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>35 months&lt;sup&gt;4&lt;/sup&gt;</td>
<td>+&lt;sup&gt;5&lt;/sup&gt;</td>
<td>+&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Digestive system</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>Negative to 6 months (IHC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>Positive&lt;sup&gt;6&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Skeletal muscles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semitendinosus muscle&lt;sup&gt;7&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tissues with no infectivity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colostrum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesenteric lymph node</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longissimus dorsi muscle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1: Arnold, J Gen Virol. 2007  
2: Espinosa, J Gen Virol. 2007  
3: Hoffmann, J Gen Virol. 2007  
4: Buschmann, J Infect Dis. 2005  
5: Terry, Vet Rec. 2003  
6: Wells, Vet Rec. 1998  
7: Wells et al Vet Rec. 2005  
8: Masujin K, J Gen Virol, 2007  
9: WHO Tables on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies; 2010
# ANNEX 2: OVINE AND CAPRINE TISSUES

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Classical scrapie</th>
<th>Ovine BSE</th>
<th>Atypical scrapie</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Caprines</strong> (R142R154Q222IRRO)</td>
<td>Infectivity</td>
<td>PrP&lt;sup&gt;Sc&lt;/sup&gt;</td>
<td>Infectivity</td>
</tr>
<tr>
<td>Ovines (VRQ/VRQ)</td>
<td>Infectivity</td>
<td>PrP&lt;sup&gt;Sc&lt;/sup&gt;</td>
<td>Infectivity</td>
</tr>
<tr>
<td><strong>Central nervous system</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain (obex)</td>
<td>+</td>
<td>(&gt;12 m- &lt;20m)</td>
<td>+</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>+</td>
<td>(&gt;12 m- &lt;20m)</td>
<td>+</td>
</tr>
<tr>
<td><strong>Peripheral nervous system</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vagus nerve</td>
<td>Not transmitted</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td>Hind limbs</td>
<td>NT</td>
<td>(&gt;12 m-&lt;20m)</td>
<td>+</td>
</tr>
<tr>
<td>Forelimbs</td>
<td>NT</td>
<td>(&gt;12 m-&lt;20m)</td>
<td>+</td>
</tr>
<tr>
<td><strong>Lymphoid tissues</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palatine tonsils</td>
<td>NT</td>
<td>(&gt;6 m-&lt;12m)</td>
<td>+</td>
</tr>
<tr>
<td>Lymph nodes of the head</td>
<td>NT</td>
<td>(&gt;6 m-&lt;12m)</td>
<td>+</td>
</tr>
<tr>
<td>Lymph nodes of the thoracic cavity</td>
<td>NT</td>
<td>(&gt;6 m-&lt;12m)</td>
<td>+</td>
</tr>
<tr>
<td>Mesenteric lymph nodes</td>
<td>NT</td>
<td>(&gt;4 m-&lt;6m)</td>
<td>(&gt;18d - &lt;30d)</td>
</tr>
<tr>
<td>Prescapular lymph nodes&lt;sup&gt;5&lt;/sup&gt;</td>
<td>NT</td>
<td>(&gt;6 m-&lt;12m)</td>
<td>+</td>
</tr>
<tr>
<td>Precrural lymph nodes&lt;sup&gt;6&lt;/sup&gt;</td>
<td>NT</td>
<td>(&gt;6 m-&lt;12m)</td>
<td>+</td>
</tr>
<tr>
<td>Spleen</td>
<td>NT</td>
<td>(&gt;6 m-&lt;12m)</td>
<td>+</td>
</tr>
<tr>
<td>Thymus&lt;sup&gt;7&lt;/sup&gt;</td>
<td></td>
<td>(+)&lt;sup&gt;7&lt;/sup&gt;</td>
<td>+</td>
</tr>
</tbody>
</table>

---

<sup>5</sup> Forelimbs

<sup>6</sup> Hind limbs

<sup>7</sup> Data to be used with caution
<table>
<thead>
<tr>
<th>Tissues</th>
<th>Classical scrapie</th>
<th>Ovine BSE</th>
<th>Atypical scrapie</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Caprines (I142R150R151</td>
<td>Ovines (VRQ/VRQ)</td>
<td>Experimental oral route (ARQ/ARQ)</td>
</tr>
<tr>
<td></td>
<td>IRRQ)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Infectivity</td>
<td>PrP(^{Sc})</td>
<td>Infectivity</td>
</tr>
<tr>
<td>Ingest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>NT</td>
<td>+</td>
<td>(+2m - &lt;3m)</td>
</tr>
<tr>
<td>Jejunum</td>
<td>NT</td>
<td>+</td>
<td>(+2m - &lt;3m)</td>
</tr>
<tr>
<td>Ileum</td>
<td>NT</td>
<td>+</td>
<td>(+2m - &lt;3m)</td>
</tr>
<tr>
<td>Caecum</td>
<td>NT</td>
<td>+</td>
<td>(+2m - &lt;3m)</td>
</tr>
<tr>
<td>Various</td>
<td>Milk</td>
<td>+</td>
<td>(&lt;18m - &lt;21m)</td>
</tr>
<tr>
<td></td>
<td>Colostroma</td>
<td>NT</td>
<td>+ (1st lactation)</td>
</tr>
<tr>
<td></td>
<td>Skeletal muscle</td>
<td>+ (titration in progress)</td>
<td>(+18m - &lt;21m)</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>NT</td>
<td>+ (&lt;3m)</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>NT</td>
<td>+ (&lt;3m)</td>
</tr>
</tbody>
</table>

Sources:

**Caprine classical scrapie:**
- AFSSA –INRA INOC 2005 project / AFSSA INRA INOC 2003 project (personal communication)
- Andreoletti et al J Gen Virol 2000
- Andreoletti et al J Gen Virol 2002
- Andreoletti et al Nature Medicine 2004
- Andreoletti et al personal communication
- Lacroux et al Plos Pathogen 2008
- Van Keulen et al APMIS 2002
- Siso et al. 2006

**Ovine classical scrapie:**
- Andreoletti et al J Gen Virol 2002
- Hunter et al J Gen Virol 2002
- Bellworthy et al Vet Rec 2005

**Experimental ovine BSE:**
- Thuring et al J Comp Pathol 2005
- Van Keulen et al Arch Virol 2005
- Lantier et al communication NeuroPrion 2008 Madrid

**Atypical scrapie:**
- Andreoletti – Benestad – Orge et al personal communication
- Comment: the time intervals listed in these tables can be inferred from several studies.

---

8 The infectious titres measured by intracerebral inoculation in Tg338 transgenic mice in milk and colostrum range between $10^{0.1}$ and $10^{1.6}$ ID50 per mL, compared to a level of $10^{6.8}$ ID50 per g for the brain.