SCIENTIFIC OPINION

Scientific Opinion on BSE/TSE infectivity in small ruminant tissues[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 3]

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ABSTRACT

The objectives addressed were i) to provide an update on TSE (Transmissible Spongiform Encephalopathy) infectivity distribution in small ruminant tissues; and ii) to indicate based on the current epidemiological situation as regards to BSE (Bovine Spongiform Encephalopathy) in the small ruminant population in the EU (European Union), whether a review of the existing SRM (Specified Risk Materials) list for small ruminants should be envisaged with regard to the potential exposure to the BSE agent. The appraisal was addressed by reviewing for Classical scrapie, BSE and Atypical scrapie in small ruminants aspects related to: i) tissue infectivity distribution according to the age and the genotype of sheep and goats; and ii) the infectious load in the different tissues. In order to perform the assessment all the currently available scientific results were reviewed, and data on TSE monitoring in small ruminants in the EU and on small ruminants slaughtered by species and age category in each EU Member State were considered. The reduction of the infectivity associated to the carcass of an infected individual achieved by the current SRM policy in small ruminants for Classical scrapie and BSE was estimated. The total number of Classical scrapie infected sheep and goats that could enter yearly into the food chain was provided. Moreover, considerations about Atypical scrapie were given. A set of simulations allowing estimating the impact of different policy options on the BSE infectious load potentially present in an infected sheep was provided.

KEY WORDS

Bovine Spongiform Encephalopathy (BSE), Classical scrapie, Atypical scrapie, Transmissible Spongiform Encephalopathies (TSEs), Specified Risk Material (SRM), Small Ruminants

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SUMMARY

Following a request from the European Commission (EC), the Panel on Biological Hazards (BIOHAZ Panel) was asked to deliver a scientific opinion on BSE/TSE infectivity in small ruminant tissues.

The most recent scientific opinion on TSE infectivity distribution in small ruminant tissues was published in January 2002 by the Scientific Steering Committees (SSC) and last amended in November 2002[4]. In recent years new scientific data relating to the infectivity of some tissues in small ruminants became available. Some of those findings related to the tissues from sheep and goats might have an impact to the current measures in relation to the Specified Risk Material (SRM) list of the Regulation (EC) 999/2001[5].

Therefore, the EC asked EFSA: i) to update, as regards small ruminants and on the basis of the most recent scientific data, the SSC scientific opinion from 2002 on TSE infectivity distribution in ruminant tissues; ii) to indicate based on the current epidemiological situation as regards BSE in the small ruminant population in EU, whether a review of the existing SRM list for small ruminants should be envisaged with regard to the potential exposure to the BSE agent.

The BIOHAZ Panel addressed the mandate by reviewing individually for Classical scrapie, BSE and Atypical scrapie in small ruminants aspects related to: i) tissue infectivity distribution according to the age and the genotype of sheep and goats; and ii) the infectious load in the different tissues.

In order to perform the assessment all the currently available scientific results were reviewed. Data about the TSE monitoring in small ruminants in the EU were provided by the European Commission and information on small ruminants slaughtered by species and age category in each EU Member State were provided by the EFSA Focal Points Network.

It was emphasized that this assessment required several assumptions. Moreover, the estimates of the infectious load are based on a simple approach using computations based on a low and a high estimate of each of the parameters. This provides order of magnitude estimates of the infectious load of TSE agents entering into the food chain at EU 27 level. This approach could be replaced by a probabilistic model to provide more insight into the uncertainties. However, due to time and resources constraints it was not possible for the BIOHAZ Panel to develop and validate such a probabilistic model within the framework of this mandate.

Considering Classical scrapie in small ruminants it was concluded that the current SRM policy allows a reduction of the relative infectivity associated to the carcass of an infected animal of about 1 $\log_{10}$ (infectious load as expressed in IC ID_{50}[6] in C57Bl6 mice). The infectivity load as expressed in the opinion (IC ID_{50} in C57Bl6 mice) cannot be related to any quantifiable dietary transmission risk in farmed animals or humans.

As regards to Classical scrapie in goats, it was further concluded that, according to the currently available knowledge, goat kids below 3 months of age, even coming from infected herds, represent a negligible source of infectivity for the food chain.

On the basis of data collected between 2007 and 2009, the total number of Classical scrapie infected animals that could enter yearly into the food chain in the EU27 as a whole was estimated to

4 SSC (Scientific Steering Committee), 2002. Update of the Opinion on TSE Infectivity distribution in ruminant tissues. Available at: http://ec.europa.eu/food/fs/sc/ssc/out296_en.pdf

5 Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. OJ L 147, 31.5.2001, p. 1-40.

6 IC ID_{50} : Intra cerebral Infectious Dose 50%

approximately range between 16,000 and 67,000 (most probable estimate 29, 000) for sheep and between 10,000 and 34,000 (most probable estimate 13,000) for goats.

The Panel pointed out that Classical scrapie is present in a majority of EU member states. However because differences in the prevalence of the disease, population size and production system (age at slaughter), there are significant differences between certain member states with regards to Classical scrapie infectivity load that may enter the food chain. This heterogeneity and the differences in consumption pattern between countries and regions mean that the dietary exposure to Classical scrapie cannot be considered to be homogeneous in the EU27.

It was furthermore concluded that at the EU27 level, the current SRM policy in force allows a global reduction of the potential exposure to Classical scrapie which can be estimated to be around 1 $\log_{10}$ (infectious load as expressed in IC ID_{50} in C57Bl6 mice).

When considering BSE in small ruminants, the Panel concluded that with 95% confidence the the number of BSE cases that could enter yearly into the food chain in the EU is ranging between 0 and 240 for sheep and between 0 and 381 for goats. This estimate argues against any current widespread BSE epidemic within the EU small ruminant population.

The BIOHAZ Panel indicated that the current SRM policy allows a reduction of the relative infectivity associated to the carcass of a BSE infected animal of about 1 $\log_{10}$ (infectious load as expressed in IC ID_{50} in C57Bl6 mice). The infectivity load as expressed in the opinion (IC ID_{50} in C57Bl6 mice) cannot be related to any quantifiable dietary transmission risk in farmed animals or humans.

It was further emphasized that preliminary biochemical and immunohistochemical data in goats suggest that there might be no major involvement of the lymphoid tissues in preclinical and clinical phase of the disease after oral experimental challenge. Before more complete information becomes available it is not possible to provide reliable specific estimates of the impact of SRM removal measures on the BSE exposure that would be associated with an infected goat entering into the food chain. The Panel highlighted that in this context the estimates of the impact of SRM removal measures on the BSE exposure provided for BSE in sheep could be considered as a worst case scenario for BSE in goats.

As regards to Atypical scrapie both in sheep and goats it was concluded that low levels of infectivity can be present in peripheral tissues (lymphoid tissues, nerves, skeletal muscle) in preclinical and clinical cases of Atypical scrapie harbouring various genotypes. Consequently SRM measures cannot be assumed to prevent the entry of the Atypical scrapie agent into the food chain.

It was highlighted that there is currently no data on the kinetics of distribution of the Atypical scrapie agent into peripheral tissues of incubating small ruminants and that there are uncertainties on the Atypical scrapie pathogenesis and its true prevalence in the EU small ruminant population. Therefore, the Panel was not in position to provide an assessment of the current Atypical scrapie infectious load entering into the food chain.

In answering to the first Term of Reference, the BIOHAZ Panel revised the TSE tissue infectivity distribution in small ruminants and provided updated information within the body of the opinion (section 2, tables 1 to 12).

Considering the second Term of Reference, the BIOHAZ Panel provided a set of simulations illustrating the impact of different policy options on the BSE infectious load potentially present in an infected sheep. According to these simulations, the use of the dressed carcass[7] only would allow a

7 The carcass of an animal after slaughter excluding head and the spinal cord.

greater reduction of the BSE exposure risk than the current SRM policy measures. The elimination of the ileum has a major impact on the relative reduction of the BSE infectivity load that might enter in the food chain from an animal aged below 12 months. The CNS (Central Nervous System) removal is the most efficient measure to reduce the relative infectivity load associated with a BSE infected small ruminant older than 12 months entering into the food chain.

It was finally indicated that a modification of the SRM list driven only by consideration about BSE will also impact on the dietary exposure to Classical scrapie and Atypical scrapie agents.

The BIOHAZ Panel recommended: i) to update the assessment once data from ongoing experiments will become available; ii) to develop a specific probabilistic model in order to provide more precise estimates of the impact of SRM removal on the infectious load of TSE agents entering into the food chain at EU 27 level; iii) to improve the quality of the data collected on the small ruminant population (e.g. age category and destination of the animal); and iv) to expand the current data collected in the context of the TSE surveillance activities by recording the tested animal age category and the type of rapid test used.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The most recent scientific opinion on TSE infectivity distribution in small ruminant tissues was published in January 2002 and last amended in November 2002. Following the confirmation of BSE in a goat in France on 28 January 2005, the European Commission invited the European Food Safety Authority (EFSA) and its Scientific Panel on Biological Hazards (BIOHAZ) to carry out "a quantitative assessment of risk posed to humans by tissues of small ruminants in case BSE is present in these animal populations".

In addition to legislative measures already in place the EC stepped up its surveillance programme in sheep and goat. The new measures became mandatory in early 2005 and include a three step testing strategy in order to differentiate between scrapie and BSE for all confirmed positive scrapie cases in both sheep and goats and an increase in surveillance focusing on increased testing of goats for both healthy and fallen stock. The other risk management measures in place (i.e. SRM list, rendering conditions) contribute to the further reduction of the risk to the consumer. The results of increased testing and discriminatory testing have not indicated any additional suspect BSE cases in goats or sheep.

In addition, new scientific data relating to the infectivity of some tissues in small ruminants became-available in recent years. Some of those findings related to the tissues from sheep and goats might have an impact to the current measures in relation to the SRM list of the Regulation (EC) 999/2001. Therefore, DG SANCO considers being appropriate to ask EFSA for update of the scientific data and reconsidering the situation in TSE infectivity distribution in the tissues of the small ruminants.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Food Safety Authority is requested:

- To update, as regards small ruminants and on the basis of the most recent scientific data, the scientific opinion from 2002 on TSE infectivity distribution in ruminant tissues;

- To indicate based on the current epidemiological situation as regards BSE in the small ruminant population in EU, whether a review of the existing SRM list for small ruminants should be envisaged.

Clarification on the Terms of Reference

After discussion with the requestor it was agreed to modify the second terms of reference as reported here below:

- To indicate based on the current epidemiological situation as regards BSE in the small ruminant population in EU, whether a review of the existing SRM list for small ruminants should be envisaged ***with regard to the potential exposure to the BSE agent***.

ASSESSMENT

1. Approach to the mandate

In the current opinion, the BIOHAZ Panel has addressed the mandate by reviewing individually for Classical scrapie, BSE and Atypical scrapie aspects of:

- tissue infectivity distribution according to the age and the genotype of sheep and goats;
- infectious load in the different tissues.

The Panel has furthermore attempted to evaluate the impact of the current SRM measures on:

- infectious load that would result from the entry into the food chain of a small ruminant that would be infected by either Classical scrapie, BSE or Atypical scrapie according to its age;
- the global infectious load (for each Classical scrapie, BSE and Atypical scrapie) that enters the food chain yearly in EU.

In the absence of some parameters this assessment required several assumptions notably with regards to the pathogenesis of the disease, the infectious load and tissues mass in animals, prevalence estimates, number and age distribution of small ruminants slaughtered for human consumption in the EU Member States. Wherever possible these assumptions have realistic limits which are based on experimental data where natural disease and/or precise quantitative data are lacking.

Due to the lack of specific data in goats this risk assessment was performed employing certain data from TSE in sheep only.

The estimates of the infectious load are based on a simple approach using computations based on a low and a high estimate of each of the parameters. This provides order of magnitude estimates of the infectious load of TSE agents entering into the food chain at EU 27 level. Even if valuable to estimate the infectious load of TSE agents entering into the food chain at EU 27 level, this approach remains simple. It could be replaced by a probabilistic model to provide more insight into the uncertainties. However, due to time and resources constraints it was not possible for the BIOHAZ Panel to develop and validate such a probabilistic model within the framework of this mandate.

2. TSE infectivity distribution in small ruminant tissues

The WHO expert group on "TSE Tissue infectivity distribution in Transmissible Spongiform encephalopathies", provides and actualizes on a regular basis tables which collates for each relevant species the available information related to infectivity and/or PrP^{Sc} distribution in tissues. These tables also provide a qualitative classification of the tissues according to their infectious load.

The tissue infectivity distribution for scrapie in small ruminants (ovine and caprine animals considered together) according to the World Health Organisation is summarised by the tables provided below (WHO, 2010).

The data entries are shown as follows:

- + Presence of infectivity or PrP^{Sc}
- - Absence of infectivity or PrP^{Sc}
- NT Not tested
- NA Not applicable
- ? Uncertain interpretation
- ( ) Limited or preliminary data
- [ ] Infectivity or PrP^{Sc} data based exclusively on bioassays in transgenic (Tg) mice over-expressing the PrP-encoding gene or PrP^{Sc} amplification methods

Table 1: High infectivity tissues for scrapie in small ruminants (ovine and caprine animals are considered together). Modified from WHO (2010).

Tissues	Infectivity (a)	PrP^{Sc}
Brain	+	+
Spinal cord	+	+
Retina	NT	+
Optic nerve (b)	NT	+
Spinal ganglia	+	+
Trigeminal ganglia	NT	+
Pituitary gland	+	+

(a): most bioassays of sheep and/or goat tissues have been conducted only in mice. Moreover, not all results are consistent for both species.

(b): In experimental models of TSE, the optic nerve has been shown to be a route of neuroinvasion, and contains high titers of infectivity.

Table 2: Lower infectivity tissues for scrapie in small ruminants (ovine and caprine animals are considered together). Modified from WHO (2010).

Tissues	Infectivity (a)	PrP^{Sc}
Peripheral Nervous System		
Peripheral nerves	+	+
Autonomic ganglia	NT	+
Lymphoreticular tissues		
Spleen	+	+
Lymph nodes	+	+
Tonsil	+	+
Nictitating membrane	[+]	+
Thymus	+	+
Alimentary tract		
Esophagus	[+]	+
Fore-stomach	[+]	+
Stomach/ abomasum	[+]	+
Duodenum	[+]	+
Jejunum	[+]	+
Ileum	+	+
Colon/caecum	+	+
Rectum	NT	+
Reproductive tissues		
Placenta	+	+
Ovary	-	-
Uterus	-	-
Other tissues		
Mammary gland/udder(b)	-	+
Skin(c)	-	+
Heart/pericardium	-	NT
Lung	-	-
Liver	+	-
Kidney(d)	[+]	+
Adrenal	+	-
Pancreas	+	NT
Bone marrow	+	NT
Skeletal muscle	[+]	+
Tongue	[+]	+
Blood vessels	NT	+
Nasal mucosa(e)	+	+
Salivary gland	+	NT
Body fluids, secretions and excretions		
CSF	+	-
Blood	+	?
Saliva	-	NT
Milk	+	[+]
Urine	-	-
Feces	-	NT

(a): most bioassays of sheep and/or goat tissues have been conducted only in mice. Moreover, not all results are consistent for both species.

(b): PrP^{Sc} has been detected in scrapie-infected sheep with chronic mastitis, but not from infected sheep without mastitis (Ligios et al., 2005).

(c): studies in hamsters orally infected with scrapie revealed that PrP^{Sc} deposition in skin was primarily located within small nerve fibers.

(d): PrP^{Sc} detected by immunocytochemistry in the renal pelvis of scrapie infected sheep (Siso et al., 2006)

(e): limited chiefly to regions involved in olfactory sensory reception.

Table 3: Tissues with no detected infectivity or PrP^{Sc} for scrapie in small ruminants (ovine and caprine animals are considered together). Modified from WHO (2010).

Tissues	Infectivity (a)	PrP^{Sc}
Reproductive tissues		
Testis	-	-
Prostate/Epididymis/Seminal vesicle	-	-
Semen	-	-
Fetus	-	-
Embryos	?	NT
Other tissues		
Thyroid gland	-	NT
Body fluids, secretions and excretions		
Colostrum	(?)	NT

(a): most bioassays of sheep and/or goat tissues have been conducted only in mice. Moreover, not all results are consistent for both species.

However, these tables do not provide the following elements:

- the distribution of the TSE agent in the tissues according to the host age and genotype;
- the type of TSE agent (Classical scrapie / BSE/ Atypical scrapie); and
- quantitative data related to infectivity load in tissues.

These are crucial parameters for the successful addressing of this assessment.

2.1. Natural TSE and experimental challenges

The investigation of animals naturally affected by TSE remains the most relevant source of information on the distribution of prion in the organs of infected individuals. Dissemination of the Classical scrapie agent in the organs of PrP susceptible sheep naturally exposed to infection has been extensively described by several authors (Andreoletti et al., 2000; van Keulen et al., 2000). However, the possibilities for studying TSE using naturally affected animals are limited. It requires flocks with sufficient disease incidence and implies numerous constraints. Consequently, in order to study TSE agents like BSE (Bellworthy et al., 2005a; Foster et al., 1996; Foster et al., 2001) or Atypical Scrapie for which natural cases cannot prospectively be investigated in flocks, experimental challenge and in particular oral route challenge has been used as a proxy for natural infection.

Oral inoculation is considered to be the most relevant model to mimic natural prion infection in ruminants. All the data related to BSE pathogenesis in sheep and cattle (distribution in tissues and incubation period) were produced using this experimental approach (Bellworthy et al., 2005a; Bellworthy et al., 2005b; Foster et al., 1996; Foster et al., 2001). In cattle the oral challenge experiments performed with low doses (1g or below) reproduced the main phenotypic features observed in field cattle BSE (Wells et al., 2007), which support the relevance of the oral inoculation model in this context. However, it is accepted that oral challenge can reduce the incubation period and accelerates the dissemination of the agent into the organs (Ryder et al., 2009; Tabouret et al., 2010).

Other inoculation routes (intraperitoneal, scarification, etc…) and in particular the intracerebral route are certainly of more limited interest for mimicking the natural infection except in the case of Atypical scrapie where the origin of the disease has been proposed to be a spontaneous disorder of prion protein folding and catabolism starting in the Central Nervous System (CNS).

Consequently in this assessment the data considered were, in order of priority:

- data arising from natural cases;
- the data obtained from oral experimental challenge when data from natural exposed animals were absent (BSE in small ruminants) or insufficient (TSEs in goats);
- the data obtained from other experimental challenge routes in the context of Atypical scrapie.

2.2. Classical scrapie

Classical scrapie in small ruminants is a disease which has been described for several centuries. It was reported for the first time in UK in 1732. This disease affects both sheep and goats and is widespread throughout most of the world (Detwiler, 1992), Australia and New Zealand are the only two countries which are currently considered by the OIE to be free of Classical scrapie.

Classical scrapie is an infectious disease of small ruminants for which susceptibility is influenced by polymorphisms on the gene (PRNP) encoding for PrP protein (EFSA Panel on Biological Hazards, 2006). In sheep, the major polymorphisms associated with susceptibility or resistance are codons 136 (A or V), 154 (R or H) and 171 (R, Q or H) (Clouscard et al., 1995; Hunter et al., 1996). VRQ/VRQ, ARQ/VRQ and ARQ/ARQ genotype animals are considered as the most susceptible to Classical scrapie, whereas homozygous or heterozygous AHQ and heterozygous ARR animals only show a marginal susceptibility. AHQ allele carriers as well as ARQ/ARQ sheep were described to be the most susceptible genotype to experimental BSE, while VRQ/VRQ were reported to be of lower susceptibility. ARR/ARR sheep are considered to be strongly (but not absolutely) resistant to Classical scrapie (Groschup et al., 2007; Hunter et al., 1996; Hunter et al., 1997; Onodera et al., 1994). In goats other PrP polymorphisms (e.g. I/M142- H/R154- R/Q211- D/S 146 and Q/K222) could also impact on individual susceptibility to these TSE agents (Barillet et al., 2009; EFSA, 2009; EFSA Panel on Biological Hazards (BIOHAZ), 2009; Gonzalez et al., 2009; Vaccari et al., 2006).

It is widely accepted that natural exposure to Classical scrapie in affected flocks mainly occurs around birth (Andreoletti et al., 2002; Pattison and Millson, 1961; Race et al., 1998; Tuo et al., 2002). Contamination with Classical scrapie was reported in sheep that were introduced in an infected flock after they reached adulthood (Hourrigan et al., 1979; Hourrigan, 1988; Ryder et al., 2004). The efficacy of such transmission appears to be lower in older animals than in younger. The origin of such contamination remains unclear and both inter-individual horizontal transmission and environmental sources could be involved. The role of the environment as a source of contamination is now unambiguously demonstrated. Dexter et al. (2009) shows infection of naive animals introduced to an infected environment without contact with animals. The policy for eradication that was applied in Iceland since 1947, with the recording of new cases of disease occurring after the culling of infected flocks and restocking with scrapie free animals, strongly support the implication of environment into Classical scrapie natural transmission (Georgsson et al., 2006).

Despite the relative uniformity of the signs in the natural host, Classical Scrapie can be caused by TSE agents that harbour different biological features. Depending on the nature of the infectious agent and on the PrP genotype of the host the dissemination dynamics and kinetics of the infectious agent can be affected.

2.2.1. Classical scrapie in sheep

2.2.1.1. PrP^{Sc} dissemination dynamics in sheep

Most of the published data related to PrP^{Sc} dissemination dynamics in sheep naturally affected with Classical scrapie were obtained in VRQ/VRQ sheep born and raised in three individual flocks (one in the Netherlands, one in France: Langlade flock and one in the UK: Rectory/Ripley flock).

The data indicated that:

- Abnormal PrP can be detected in ileal Gut Associated Lymphoid Tissues (GALT) as early as 21 days old (Andreoletti et al., 2002).

- PrP^{Sc} spreads and accumulates in other GALT formation and in mesenteric lymph node during the two first months of life (Andreoletti et al., 2000; Andreoletti et al., 2002; Heggebo et al., 2000; van Keulen et al., 2002).
- In lambs older than 2 months PrP^{Sc} spreads to all lymph nodes, including those that remain on prepared carcasses (Andreoletti et al., 2000; Andreoletti et al., 2002; van Keulen et al., 2002).
- The amount of PrP^{Sc} in lymphoid formations increases with age before reaching a plateau level around 6 months old (Andreoletti et al., 2000). At the plateau level, the infectivity that is found in lymphoid organs can be equivalent to 1/50 of the infectivity found in the same mass of obex from an animal at the terminal stage of the disease.
- PrP^{Sc} becomes detectable in the CNS (brain and spinal cord) between the age of 7 months and 10 months (Andreoletti et al., 2000; Jeffrey et al., 2001a; van Keulen et al., 2002). PrP^{Sc} accumulates in the CNS following exponential kinetics.
- PrP^{Sc} was identified in skeletal muscle in 13 month old animals but not in 10 month old sheep (Andreoletti et al., 2004).
- PrP^{Sc} was reported in the liver (Kuppfer cells) from sheep at both preclinical and clinical stage of natural scrapie (Everest et al., 2009).
- Infectivity is shed in milk and colostrum from the first lactation. The infectious titre that can be found in a litre of milk is equivalent to the infectivity contained in 0.2 to 6 mg of brain material from a terminally affected sheep. The estimated quantity of milk which is produced yearly by a sheep ranges between 100 and 300 Kg.
- Infectivity was also reported in blood. The infectious agent can be detected in blood as early as 3 months of age and persists throughout the incubation period.

VRQ/VRQ sheep are considered to be the most sensitive to most of TSE agents responsible for Classical scrapie. Moreover it is also considered that the dissemination kinetics of the TSE agent in these animals is more rapid than in other genotypes.

There is a paucity of relevant data related to Classical scrapie dissemination in sheep of other genotypes.

For the genotype ARQ/VRQ there is only one published study (Lacroux et al., 2008) reporting the dynamics of PrP^{Sc} dissemination in sheep naturally affected with Classical scrapie. In this study a cohort of VRQ/VRQ and ARQ/VRQ sheep born and raised in a French flock infected by Classical scrapie (the same flock which provided results in VRQ/VRQ lambs) was investigated. PrP^{Sc} dissemination in both genotypes of animals was similar. However, the dissemination kinetics were slower in ARQ/VRQ animals than in VRQ/VRQ sheep which illustrates the impact of the host genotype on the pathogenesis of the disease.

In ARQ/ARQ sheep (Jeffrey et al., 2001a), at the late preclinical stage and in clinically affected individuals, PrP^{Sc} can be detected (like in VRQ/VRQ) in central nervous system, lymphoreticular system, gastro intestinal tract and peripheral nervous system. In a group of 10 animals harbouring the AxQ/AxQ genotype no PrP^{Sc} was detected by tonsil biopsy at 3 months of age while 2 out of the 10 were positive by 8 months old and 10 out of the 10 were positive by 20 months old. The experimental design and the size of animal groups that were submitted to sequential necropsies do not allow deduction of reliable kinetics of the dissemination of the agent in the organs of ARQ/ARQ sheep. However, these data support the statement that the PrP^{Sc} dissemination scheme in ARQ/ARQ sheep naturally affected with Classical scrapie could be similar to the one reported in VRQ/VRQ and ARQ/VRQ sheep but displays a slightly delayed dynamic.

Under natural exposure conditions heterozygous ARR sheep have a limited susceptibility to Classical scrapie (Elsen et al., 1999; Hunter et al., 1997). A few Classical scrapie clinical cases have been reported on occasion in animals harbouring such genotype. In these animals PrP^{Sc} distribution seemed to be mostly confined to the CNS even if some minimal PrP^{Sc} deposits could be observed in the lymphoreticular system. No information is currently available with regards to the involvement of skeletal muscle in animals of such genotype.

A synthesis of the data related to the publications mentioned in this section is available in Table 4.

In summary, the data that are currently available with regards to Classical scrapie pathogenesis in sheep display a coherent model. However, the knowledge that has been accumulated relies on the study of a limited number of flocks. Considering the potential diversity of TSE agents that are responsible for Classical scrapie and the importance of the interaction between the host genotype and the TSE agent strain properties this knowledge cannot be considered to be definitive. For instance several clinical Classical scrapie cases were reported in ARQ/VRQ and ARQ/ARQ sheep (Jeffrey et al., 2002; Ligios et al., 2006) in the absence of detectable PrP^{Sc} in the lymphoid tissues.

Table 4: PrP^{Sc} detection in naturally affected sheep (synthesis of all the available data mentioned in the publications reported in this section).

Tissue	Genotype VRQ/VRQ		Genotype ARQ/VRQ		Genotype ARQ/ARQ	
	PrP^{Sc}	**Age first detection**	**PrP^{Sc}**	**Age first detection**	**PrP^{Sc}**	**Age first detection**
CNS						
Obex	+	>7 m - <10m	+	>10 - <13 m	+	<21 m
Spinal cord	+	>7 m - <10m	+	NT	NT	
PNS						
Sciatic nerve	+	>10m - <13m			NT	
Brachial nerve	+	>10m - <13m			NT	
Lymphoid Tissues						
Tonsil	+	>21d - <64d		>4m - <7m	+	>3m - <8m
Mandibular and parotideal LN	+	>21d - <64d	NT		+	<21 m
Mediastinal LN	+	>64d - <104d	+	>4m - <7m	NT	
Mesenteric LN	+	>10d - <21d	+	<4m	+	<14 m
Prescapular LN	++	>64d - <90d	+	>4m - <7m	+	<21 m
Precrural LN	+	>64d - <90d	NT		NT	
Spleen	+	>64d - <104d		>4m - <7m	+	<21 m
Intestine						
Duodenum		>2m - <3m	+	<4m	+	<21 m
Jejunum		>2m - <3m	+	<4m	+	<21 m
Ileum	+	>10d - <21d	+	<4m	+	< 21 m
Caecum		>2m - <3m	+	>4m - <7m	+	< 21 m
Other tissues						
Milk	-	1st lactation	-	1st lactation	NT	
Colostrum	-	1st lactation	-	1st lactation	NT	
Skeletal Muscle		>10m - <13m	+	NT	+	NT
Liver	-		-		+	
Blood	+ §		-	NT	-	

+ Presence of PrP^{Sc}
- Absence of PrP^{Sc}
m Months
d Days
NT Not tested
§ PrP^{Sc} detected utilising PMCA

2.2.1.2. Classical scrapie infectivity load in sheep tissues

Historically the data related to infectivity load in tissues of sheep affected with Classical scrapie relied on the studies carried out by Hadlow (1982).

In these experiments:

- a range of tissues from up to 9 Suffolk sheep (aged between 34 and 57 months) and clinically affected with scrapie were end point titrated by intra-cerebral inoculation into C57Bl6 mice (see Tables 5 and 6);
- a more limited range of tissues collected in 9 preclinical Suffolk sheep aged between 10 and 25 months old were end point titrated in C57Bl6 mice.

Table 5: Quantitative distribution of scrapie infectivity in nervous tissue of Suffolk sheep affected with natural scrapie. Modified from Hadlow et al. (1982).

Age (months)	Infectivity titre*									
	Cerebral cortex	**Corpus striatum**	**Dience phalon**	**Mid-brain**	**Medulla oblongata**	**Cerebellar cortex**	**Cervical spinal cord**	**Sciatic nerve**	**Pituitary gland**	**Cerebro -spinal fluid**
36	1.0	1.1	3.8	3.1	4.3	3.5	2.9	0.6	ND	ND
37	4.5	3.1	5.3	5.8	4.0	6.1	4.0	1.5	1.6	0.4
34	4.3	3.5	5.1	5.7	4.4	6.5	4.5	2.5	ND	0.8
38	1.8	2.5	3.7	4.4	4.3	3.8	4.1	1.9	ND	2.2
34	0.8	1.6	3.8	3.9	3.7	3.7	2.7	0.6	1.0	ND
57	5.1	4.6	5.7	5.7	5.6	6.5	5.5	2.0	0.8	0.8
36	1.1	3.1	4.0	3.1	3.7	1.5	2.4	ND	1.5	ND
46	2.1	3.8	4.8	5.3	4.7	5.0	4.6	2.2	ND	ND
37	5.0	NT	NT	5.9†	NT	5.8	4.5	2.5	1.8	ND

ND: no infectivity detected
NT: not tested
* expressed as the log 50% mouse intracerebral lethal dose/30 mg of tissue
†Pool of corpus striatum, diecephalon, midbrain and medulla oblongata.

In these animals if medulla oblongata (obex area) is considered as the baseline, relative infectivity of cerebral and cerebellum ranged between 200 folds and 1/2000 of the baseline.

Table 6: Infectivity titres (bio-assayed in mice) in tissues from up to 9 Suffolk sheep (34- 57 months old) at the clinical stage of natural scrapie. Modified from SSC opinion (2002).

Tissue	Infectivity titre (a) ± Standard Error of the Mean	N° of samples
CNS		
Brain	5.6 ± 0.2	51
Spinal cord	5.4 ± 0.3	9
PNS		
Sciatic nerve	3.1 ± 0.3	9
Lymphoid Tissues		
Tonsil	4.2 ± 0.4	9
Lymph nodes	4.2 ± 0.1	45
Spleen	4.5 ± 0.3	9
Thymus	2.2 ± 0.2	9
Bone marrow	<2.0 ± 0.1	3
Intestine		
Proximal colon	4.5 ± 0.2	9
Distal colon	<2.7 ± 0.2	9
Ileum	4.7 ± 0.1	9
Other tissues		
Liver	<2.0 ± 0.1	9
Lung	<2.0	9
Pancreas	<2.1 ± 0.1	9
Mammary gland	<2.0	7
Milk	--	
Heart muscle	<2.0	9
Skeletal muscle	<2.0	9
Blood clot	<1.0	9
Kidney	<2.0	9
Serum	--	
Testis	<2.0	1

(a): Titres are expressed as arithmetic means of log 10 mouse i/c. LD 50/g or ml of tissue (+ve > 2.0).

According to these experiments, using brain stem as the baseline, the relative infectious titre at clinical stage of the disease can be estimated:

- Ileum, proximal colon, lymph nodes, spleen, tonsil 1/50;
- sciatic nerve 1/500;
- thymus 1/1000 ;
- kidney, liver, skeletal muscle <1/3000.

In preclinically affected sheep (at about half of the incubation period: 8 animals 10-14 months old and one 25 months old) the infectious titres that were observed in the different lymphoreticular structures varied between zero and 1/50 of the level of infectivity found in the brain of a clinically affected sheep.

This experiment remains a reference in the field and its results are still sound and valid. However, when these studies were carried out the PrP gene and the impact of its polymorphisms on scrapie pathogenesis in sheep were unknown.

It is not possible to establish the genotype of these sheep retrospectively, however it has been shown that the Suffolk breed (in Europe at least) has very low (lower than 0.0015, or in some countries zero)

frequency of the V136 allele (EU research project FAIR CT97-3305), and one report on scrapie in US Suffolks failed to find any V136 in 31 positive cases. So it is a reasonable assumption that affected animals in the Hadlow study are likely to be AA at 136, and QQ at 171 (Westaway et al., 1994). Moreover, the power of the titration tool that was used by Hadlow and colleagues is 2 $\log_{10}$. Additionally, the transmission barrier has probably hampered the efficacy of the propagation. This limitation has certainly precluded the detection of infectivity in tissues like skeletal muscle, blood and in milk, in which more recent experiments in the natural host and transgenic rodent models have revealed the presence of infectivity.

End point titration is an expensive and time consuming approach. Consequently investigations performed using these approaches are extremely limited. Recently alternative approaches were proposed to the use of end point titration in conventional mice**.**

According to the prion hypothesis, PrP^{Sc} is an infectious protein and the causative agent of TSEs (Prusiner, 1982). In natural TSEs the accumulation of PrP^{Sc} in tissues of infected individuals is correlated with the presence of infectivity (McKinley et al., 1983; 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 considered 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^{Sc} in various tissues has been determined (See Annex IV, B of EFSA, 2008).

This biochemical approach indicated that:

- in lymphoid tissue and obex PrP^{Sc} accumulation is exponential;
- in lymphoid tissues PrP^{Sc} accumulation reaches a plateau level in animals older than six months; and
- this plateau level is equivalent to 1/10 to 1/100 of the amount that accumulates in the obex of terminally affected animals;
- in skeletal muscle from preclinical and clinically affected sheep rare samples were found to contain between 1/2500 -1/5000 of the amount that accumulates in the obex of terminally affected animals.

Another approach which was proposed to estimate the TSE infectivity level in a tissue homogenate is to compare the incubation period observed in bioassay with the sample of interest with a reference curve obtained by end point titrating of a brain homogenate (same TSE agent) (Dickinson and Fraser, 1969; Dickinson et al., 1969; Heikenwalder et al., 2007; Prusiner et al., 1982; Tixador et al., 2010). This approach was for instance used for estimating the infectious titre of milk collected in TSE affected sheep (Lacroux et al., 2008).

Using this methodology, infectivity levels were evaluated in various tissues from naturally exposed VRQ/VRQ sheep (Langlade flock) (See Annex IV, B of EFSA, 2008).

The results obtained indicated that in Mesenteric lymph node infectivity is:

- at 1 month old equivalent to 1/1000;
- at 3 months old equivalent to 1/200;
- at 6 months old and terminal phase of the disease equivalent to 1/20

of the infectivity observed in the brainstem of a terminally affected individual.

These figures are similar to those obtained by comparing the relative PrP^{Sc} measured in both type of tissues.

Infectivity was also estimated using the same approach in different tissues (including skeletal muscle) from one VRQ/VRQ animal at 13 months old, and one VRQ/VRQ animal at 22 months old (clinical) (see Table 7).

Table 7: Estimation of the infectivity level in different tissues of two VRQ/VRQ sheep using the method of the incubation period in bioassay

Status	Age in months	Tissue	N° mice	Incubation period in days +/- SD	Infectious titre	Ratio tissue investigated/ obex of terminally affected sheep
Clinical	22	Obex	6/6	221+/-20	$10^{6.8}$	1
		Spleen	6/6	431+/-32	$10^{5.5}$	1/20
		Semi membranous muscle	6/6	436+/-66	$10^{5.4}$	1/20
Preclinical	13	Psoas muscle	6/6	453+/-32	$10^{5.5}$	1/20
		Ileal lymph node	6/6	429+/-65	$10^{5.6}$	1/16
		Obex	6/6	552+/-72	$10^{3.8}$	1/1000

Skeletal muscle samples collected in preclinical (13 months) and clinically affected sheep with natural scrapie (Langlade flock) were chosen from the study carried out by Andreoletti et al. (Andreoletti et al., 2004). They correspond to samples that were found positive using biochemical PrP^{Sc} assays.

The observed incubation periods indicate that the infectivity level in skeletal muscle can be equivalent to about 1/20 of the infectivity found in the obex of a terminally affected animal (Lacroux et al., 2010).

However, considering the great heterogeneity of the prion distribution in skeletal muscle (positive structure: muscle spindles) this value cannot be directly inferred to the whole muscle mass of an individual. In their study Andreoletti et al. (2004) screened about 100 aliquots for each investigated skeletal muscle in naturally infected animals to identify one positive. Together with the findings reported by Hadlow et al. (1982), these data lead the BIOHAZ Panel to assume that in skeletal muscle mean infectivity level, if any is present, remains below 1/2500 of the infectivity found in the obex from a terminally affected animal.

2.2.2. Classical scrapie in goats

2.2.2.1. PrP^{Sc} dissemination dynamics in goats

In France, investigations were carried out in two herds highly affected with Classical scrapie (Project: UMR INRA1225 - AFSSA Niort). In the first herd 19 animals belonging to different age cohorts were selected on the basis of a positive PrP^{Sc} tonsil biopsy. These animals and appropriate age/genotype matched controls were culled and a large panel of tissues sampled for PrP^{Sc} detection by immunohistochemistry. The results indicated that following natural exposure the infection occurred through the Gut Associated Lymphoid Tissues (GALT) before PrP^{Sc} dissemination to the other

secondary lymphoid organs. PrP^{Sc} entry into the nervous system occurs at gut level through the enteric plexi of the autonomic system. Central nervous system invasion occurs at both the obex and thoracic spinal cord level through the autonomic nerve roots (EFSA Panel on Biological Hazards (BIOHAZ), 2009). Similar investigations were carried out in the UK (Gonzalez et al., 2009): two scrapie – affected goat herds were culled in 2008, and brain and lymphoid tissues examined for the prion protein by immunohistochemistry (PrP^{d} IHC), and PrP^{Sc} ELISA and Western blot. The results obtained so far are consistent.

Additionally, goat kids (n=54) were orally challenged with Classical scrapie around birth (1.5g brain homogenate from naturally affected goats administered through natural suckling) (Project UMR INRA1225 - AFSSA Niort).

In a first experiment, groups of wild type genotype challenged (n=3 or 4) and control animals (n=1) were sequentially killed at 21 days, 1 month, 4 months, 12 months and 21 months post challenge. A last group (n=4) was observed until the occurrence of clinical signs (41 months post challenge).

In a second experiment groups of wild type genotype challenged (n=3 or 4) and control animals (n=1) were sequentially killed at 30 days, 3 months, 6 months, 12 months, 18 months and 30 months post challenge. A last group (n=6) was observed until the occurrence of clinical signs (36-40 months post challenge).

In these experiments:

- there was lack of detectable PrP^{Sc} in animals of 3 months old or younger;
- PrP^{Sc} was detected in GALT in 4 month old animals;
- PrP^{Sc} can be detected in lymphoid tissues that are not associated with the digestive tract in animals that are older than 6 months;
- The Central Nervous System (CNS) remained PrP^{Sc} negative at 12 months old but was found positive (obex) in animals 18 months and older; and
- PrP^{Sc} is detectable in skeletal muscle of goats older than 21 months (18 month old animals negative).

A synthesis of the data related to the publications mentioned in this section is available in Table 8.

An experiment aiming at determining the impact of I142M, Q211R and K222Q heterozygosis on the pathogenesis of Classical scrapie is currently ongoing (See EFSA Panel on Biological Hazards (BIOHAZ), 2009). The first available results (12 months post challenge) confirm that:

- I142M polymorphism is not associated with strong resistance to oral infection but delays the dissemination of the agent in the organs of exposed goats; and
- R211Q and Q222K heterozygosis seems to be associated with a certain level of resistance against oral infection to the scrapie agent used in this study.

However, at the moment the available information remains too incomplete to include them in this assessment.

Table 8: PrP^{Sc} detection in orally inoculated goats (synthesis of all the available data mentioned in the publications reported in this section).

Tissue	Genotype $I_{142}R_{154}R_{211}Q_{222}$/IRRQ	
	PrP^{Sc}	Age at first detection
CNS		
Obex	+	>12 m - <20m
Spinal cord	+	>12 m - <20m
PNS		
Vagal nerve	+	
Sciatic nerve	+	>12 m - <20m
Brachial nerve	+	>12 m - <20m
Lymphoid Tissues		
Tonsil	+	>6 m - <12m
Mandibular and parotideal LN	+	>6 m - <12m
Mediastinal LN	+	>6 m - <12m
Mesenteric LN	+	>4 m - <6m
Prescapular LN	++	>6 m - <12m
Precrural LN	+	>6 m - <12m
Spleen	+	>6 m - <12m
Intestine		
Duodenum		>4m - <6m
Jejunum		>4m - <6m
Ileum	+	>3m - <4m
Caecum		>4m - <6m
Other tissues		
Milk	NT	
Colostrum	NT	
Skeletal Muscle	+	>18m - <21m
Blood	NT	

+ Presence of PrP^{Sc}
- Absence of PrP^{Sc}
m Months
NT Not tested

Available data seems to indicate that Classical scrapie dissemination in the organs of goats is very similar to that observed in sheep.

However, as described in sheep, there is a still uncharacterized diversity in the agents that can cause Classical scrapie in goats. This diversity in interaction with the goat PrP genotype may impact on the kinetics of the dissemination and on the distribution of the TSE agent in the organs. For instance development of clinical Classical scrapie was recently reported, in the absence of PrP^{Sc} accumulation in lymphoid tissues in some naturally affected goats (Gonzalez et al., 2009; Konold et al., 2007a).

2.2.2.2. Classical scrapie infectivity load in goat tissues

The only available data on infectivity load in tissues of goats affected with Classical scrapie are those published by Hadlow (1980). In this study some tissues from up to 3 goats (aged between 38 and 49 months) and clinically affected with scrapie were end point titrated by intra-cerebral inoculation into C57Bl6 mice (see Tables 9 and 10).

Table 9: Quantitative distribution of scrapie infectivity in nervous tissue of goats affected with natural scrapie. Modified from Hadlow et al. (Hadlow et al., 1980).

Age (months)	Infectivity titre*										
	Cerebral cortex (parietal)	Corpus striatum	Diencephalon	Mid-brain	Medulla oblongata	Cerebellar cortex	Cervical spinal cord	Lumbar spinal cord	Sciatic nerve	Pituitary gland	Cerebro-spinal fluid
38	4.8	4.5	5.4	6.2	5.8	5.7	4.8	5.4	2.3	3.5	1.0
38	4.2	3.5	4.4	5.0	4.6	4.9	3.8	4.6	2.5	3.5	0.6
49	4.8	4.4	5.7	5.9	5.0	5.7	4.2	4.5	1.5	3.1	UN

* expressed as $\log_{10}$ mouse intracerebral LD_{50}/30 mg of tissue
UN: infectivity was detected only in undiluted fluid expressed as the log 50% mouse intracerebral lethal dose/30 mg of tissue

According to these results which remain limited the infectious titre in the different parts of the brain seems to be relatively homogenous.

Table 10: Infectivity titres (bio-assayed in mice) in tissues from up to 3 goats (38-49 months old) at the clinical stage of natural scrapie. Modified from SSC opinion 2002.

Tissue	Infectivity titre (a) ± Standard Error of the Mean	N° of samples
CNS		
Brain	6.5 ± 0.2	18
Spinal cord	6.1 ± 0.1	6
PNS		
Sciatic nerve	3.6 ± 0.3	3
Lymphoid Tissues		
Tonsil	5.1 ± 0.1	3
Lymph nodes	4.8 ± 0.1	3
Spleen	4.5 ± 0.1	3
Thymus	<2.3 ±0.2	3
Bone marrow	<2.0	3
Intestine		
Proximal colon	4.7 ± 0.2	3
Distal colon	3.3 ± 0.5	
Ileum	4.6 ± 0.3	3
Other tissues		
Liver	--	
Lung	<2.1 ± 0.1	
Pancreas	--	
Mammary gland	<2.0	3
Milk	<1.0	3
Heart muscle	--	
Skeletal muscle	<2.0	1
Blood clot	<1.0	3
Kidney	<2.0	3
Serum	<1.0	3
Testis	<2.0	1

(a): Titres are expressed as arithmetic means of log 10 mouse i/c. LD 50/g or ml of tissue (+ve > 2.0).

Using this data, considering the brainstem as baseline, the relative infectious titres in different tissues at clinical stage of the disease can be estimated:

- cerebellar cortex and spinal cord 1;
- cerebral cortex about 1/10;
- ranging in ileum, proximal colon, lymph nodes, spleen, tonsil between 1/800 and 1/60;
- thymus 1/10000.

2.2.3. Classical scrapie conclusions

- PrP^{Sc} dissemination and distribution in the organs of VRQ/VRQ sheep naturally infected with some scrapie agents is well documented.
- Data related to other sheep PrP genotypes are limited.
- PrP^{Sc} dissemination and distribution in the organs of wild type genotype goats is well documented. However, most of the knowledge relies on experimental challenge.
- There are some data available with regards to the level of infectivity that accumulates in the tissue of sheep naturally infected with some Classical scrapie agents.
- There are few data available with regards to the level of infectivity that can be found in certain tissues of goats affected with natural Classical scrapie.
- Considering the diversity of the TSE agent causing Classical scrapie the current knowledge cannot be considered to encompass all the possible variability in terms of distribution/kinetics of accumulation of the agent into small ruminant organs.

2.3. BSE

BSE agent possible spread in small ruminants has been considered as a major threat over the last 15 years.

To date, there has been:

- no report of naturally occurring BSE in sheep in the commercial situation,
- one confirmed case of natural BSE in a goat was reported in France 2002 (Eloit et al., 2005).

Both sheep and goats have been shown to be susceptible to the BSE agent and in the absence of natural cases to be studied, all the knowledge related to BSE pathogenesis in small ruminants relies on experimental challenges in sheep (Bellworthy et al., 2008; Bellworthy et al., 2005b; Gonzalez et al., 2005; Jeffrey et al., 2001b; van Keulen et al., 2008a) and goats EU "goatBSE project (FOOD-CT-2006-36353)[8].

Like the situation in natural scrapie, PRNP polymorphisms have a major impact on BSE susceptibility and dissemination of the agent in the organs. However, PRNP genotypes that are associated with the highest susceptibility in the context of BSE in sheep are different from those observed for natural scrapie. Moreover it is now well documented that BSE agent can propagate in sheep bearing the ARR/ARR genotype after oral exposure (Andreoletti et al., 2006; Lantier et al., 2008).

The low natural prevalence of BSE in a number of species (including human) other than cattle that were exposed to cattle BSE suggests the existence of real barrier to transmission of this disease under natural conditions. However, recently the BSE agent in sheep was described to harbour a higher virulence and capacities to cross the transmission barrier than the original BSE cattle agent (Espinosa et al., 2007; Espinosa et al., 2009). These observations suggest that exposure to small ruminant passaged BSE agent might result in a higher transmission rate in a third species compared to that observed with cattle BSE.

8 Details available at http://www.goatbse.eu

Recently presented data suggest that BSE adapted in small ruminants might have a higher efficacy to cross the human species barrier (as modelled in transgenic mice expressing the human PrP Met 129 gene) than cattle BSE (Plinstone et al., 2010). Currently the minimum BSE infectious dose that would allow to infect a human being remains unknown.

2.3.1. BSE in sheep

The distribution of PrP^{Sc} in sheep experimentally infected with BSE is very similar to that observed in sheep with Classical scrapie. It involves the lymphoreticular system, the peripheral nervous system, enteric nervous system, muscle, blood and Central nervous system (Foster et al., 1993; Jeffrey et al., 2001b; van Keulen et al., 2008b). More recently scant PrP^{Sc} deposits have been detected in the liver of clinical and preclinical and ARQ/ARQ BSE (Everest et al., 2009), which is consistent with earliest report of infectivity presence in this tissue (Bellworthy et al., 2005b).

The presence of PrP^{Sc} was described in lymphoreticular tissues from sheep clinically affected with ARR/ARR and VRQ/VRQ genotype (Andreoletti et al., 2006; Bellworthy et al., 2008) although with greatly prolonged incubation periods compared to ARQ/ARQ or AHQ/AHQ sheep.

Together studies published by (Bellworthy et al., 2005b; van Keulen et al., 2008a) and the data presented by Lantier et al. (Lantier et al., 2008) provides an overall picture of the dissemination kinetics of the BSE agent in the organs of orally challenged ARQ/ARQ sheep (see Table 11).

Table 11: PrP^{Sc} detection in ARQ/ARQ BSE orally inoculated sheep (synthesis of all the available data mentioned in the publications reported in this section).

Tissue	Genotype ARQ/ARQ	
	PrP^{Sc}	Age at first detection
CNS		
Obex	+	>6 m - <9m
Spinal cord	+	>6 m - <9m
PNS		
Vagal nerve	+	
Sciatic nerve	+	
Brachial nerve	+	
Lymphoid Tissues		
Tonsil	+	>4m - <6m
Mandibular and parotid LN	+	>4m - <10m
Mediastinal LN	+	>4m - <10m
Mesenteric LN	+	<4m
Prescapular LN	+	>4m - <10m
Precrural LN	+	>4m - <10m
Spleen	+	<4m
Intestine		
Duodenum		<4m
Jejunum		<4m
Ileum	+	<4m
Caecum		<4m
Other tissues		
Milk	-	
Colostrum	-	
Skeletal Muscle	+	>10m - <19m
Blood	NT	

+ Presence of PrP^{Sc}
- Absence of PrP^{Sc}
m Months
NT Not tested

Quantitative data on the amount of PrP^{Sc} or infectivity that can be found in tissues from BSE incubating or affected ewes remain extremely limited. The only data set providing comprehensive elements relate to the kinetics of PrP^{Sc} in different tissues from BSE incubating and affected animals were obtained in the framework of an EU project (BSE in sheep) and already used in a former EFSA opinion (EFSA, 2007b). This data set is shown in Table 12.

Table 12: PrP^{Sc} quantification in experimental BSE sheep tissues. Modified form the EFSA Scientific Opinion on "Quantitative risk assessment on the residual BSE risk in sheep meat and meat products" (EFSA, 2007b).

Tissue	Animal	Age		
		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	-	-	1124 pg/mg
	Animal 2	-	17 pg/mg	1011 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:

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

2.3.2. BSE in goats

Until recently, there was no specific data describing the pathogenesis of the BSE agent following oral exposure of goats. In that context, BSE risk assessments undertaken so far in that species have relied on the assumption that BSE in goats would behave similarly to natural scrapie in goats and experimental BSE in sheep (EFSA, 2005a).

Within the framework of the European GoatBSE project (FOOD-CT-2006-36353; www.goatBSE.eu), oral challenge experiments of BSE to goats were performed using either cattle BSE isolate or experimental goat BSE isolate (INRA; the University of Edinburgh; Friedrich-Loeffler Institute).

Animals harbouring various PRNP genotypes were inoculated; the (expected) susceptible wild-type $I_{142}R_{211}Q_{222}$/IRQ, the animals with lower susceptibility genotypes $I_{142}Q_{211}Q_{222}$/IRQ, $I_{142}R_{211}K_{222}$/IRQ and $M_{142}R_{211}Q_{222}$/IRQ were used (EFSA Panel on Biological Hazards (BIOHAZ), 2009).

Serial kills of a defined number of animals were performed at 6 and 12 and 24 months post infection. To date, PrP^{Sc} was detected in the brainstem (PTA-immunoblot and imunohistochemistry) of four wild-type goats 24 months post infection, which indicates a BSE-infection.

Additionally in these animals PrP^{Sc} deposition (Immunohistochemistry) was found in the gut (GALT) and the peripheral nervous system of the time point killed animals, but not in other lymphoid tissues.

These PrP^{Sc} detection results (Immunohistochemistry) are consistent with a lack of major involvement of the lymphoreticular tissues. In order to confirm these results, a panel of relevant tissues are currently being tested by bioassay ovine PrP overexpressing mice (TgshpXV).

In the meanwhile available results remain too preliminary to draw definitive conclusions. However, if confirmed, they would signify that BSE pathogenesis in sheep and goats might be dissimilar. Under such scenario the use of data collected in sheep infected with BSE could not anymore be considered pertinent to assess BSE risk in goat, other than to assume that extrapolating from sheep data would give a worst case scenario.

2.3.3. BSE conclusions

- In sheep:
    - Dissemination and distribution of PrP^{Sc} in the organs of orally challenged sheep bearing the ARQ/ARQ genotype is well documented.
    - The kinetics of distribution of the BSE agent in sheep harbouring other genotypes is less or not documented.
    - There is little information available on the infectivity titer in the tissues of BSE affected sheep at the different stages of the disease.
- In goats:
    - Preliminary data, after oral experimental challenge, suggest that there is apparently no major involvement of the lymphoid tissues in the preclinical and clinical phase. However, these data need to be completed and confirmed.
    - Pathogenesis data collected in sheep can be considered as a worst case scenario for BSE in goats.
    - There is no information available on the infectivity titer in the tissues of BSE affected goats at the different stages of the disease.

2.4. Atypical scrapie

In 1998, the molecular and histopathological spectrum of TSEs in sheep was extended by the discovery in Norway of an experimentally-transmissible, PrP-related, neurological disease of sheep that was clearly distinguishable from Classical scrapie and was therefore considered to be an "atypical" form of scrapie (Benestad et al., 1999 and 2003). The PrP genetic sensitivity to Atypical scrapie is different from that observed in Classical scrapie and BSE. While a clearly increased risk for developing Atypical scrapie is associated with AF141Q and AHQ alleles, the VRQ allele seems to be at lower risk. Strikingly ARR allele carriers (both homozygous and heterozygous) can develop the disease (Arsac et al., 2007; EFSA Panel on Biological Hazards, 2006; Moreno et al., 2007; Moum et al., 2005).

Atypical scrapie has now been identified in most EU member states (Benestad et al., 2008; Benestad et al., 2003; Buschmann et al., 2004a; Buschmann et al., 2004b; De Bosschere et al., 2004; Gavier-Widen et al., 2004; Onnasch et al., 2004; Orge et al., 2004) and this TSE form has also been reported in countries like Canada (Mitchell et al.), USA (Loiacono et al., 2009), New Zealand (Kittelberger et al., In press) and Australia[9].

The transmissibility of the Atypical scrapie agent by the intracerebral route is clearly established in both rodent models (transgenic animals expressing the ovine PrP gene) and sheep (Le Dur et al., 2005; Simmons et al., 2007; Simmons et al., 2010a). However, the contagiousness of Atypical scrapie in natural conditions is still debated.

Data from an oral challenge study indicates that very early oral exposure (within 24 hours of birth) can lead to successful transmission in AHQ homozygous homologous challenges as least, with clinical disease developing on one animal by 24 months of age (Simmons et al., 2010b).

The analysis of the data collected through the active surveillance program (Fediaevsky et al., 2010; Fediaevsky et al., 2009) seems to indicate that the capacity of Atypical scrapie cases to transmit disease to other sheep under field conditions is low and possibly nil. In most of the Atypical scrapie flocks, only a single case is found. However, there are reports of several cases in sheep that originated from the same flock (Konold et al., 2007b; Onnasch et al., 2004) and of Atypical scrapie cases detected in flocks affected by Classical scrapie (Orge et al., 2010). This, coupled with our lack of knowledge of the pathogenesis of Atypical scrapie, the distribution of prions within an affected animal and the sensitivity of tests to detect pre- or sub-clinical stages of this disease means that we cannot be certain that this disease is non-contagious in all natural circumstances.

2.4.1. Atypical scrapie in sheep

Data about Atypical/Nor98 scrapie agent distribution in the organs are limited (Benestad et al., 2003; Buschmann et al., 2004a; Nentwig et al., 2007; Simmons et al., 2007; Vidal et al., 2008) but seem to indicate, as far as no detectable abnormal PrP has been found in peripheral tissues, that this infectious agent would be restricted to the central nervous system.

Recently a study aiming at comparing PrP^{Sc} biochemical detection sensitivity and bioassay has shown that biochemical PrP^{Sc} detection remains negative with samples that contained massive amount of Atypical scrapie agents (up to $10^{6.2}$ ID50). Similar experiments carried out with two different Classical scrapie agents indicated in that the lowest level of infectivity that the same PrP^{Sc} tool were able to detect was equivalent to 10^2 ID50 (see Appendix I).

9 www.animalhealthaustralia.com.au/fms/Animal%20Health%20Australia/ADSP/AHSQ/AHSQ%20Q1%202010.pdf

In that context the absence of detectable abnormal PrP in peripheral tissues of atypical cases cannot be considered as a reliable basis for excluding the possible presence of this agent in tissues of affected individuals.

Table 13: Infectivity and PrP^{Sc} distribution in naturally and experimentally (IC challenge) sheep positive for Atypical scrapie (Lacroux et al., 2010).

Tissue	Infectivity	PrPSc
CNS		
Obex	+ (Nat and Exp)	+ (Nat and Exp)
Spinal cord	+(Nat and Exp)	+/-(Nat and Exp)
PNS		
Vagal nerve		
Sciatic nerve	+ (Exp)	+/- (Exp)
Brachial nerve	+ (Exp)	+/- (Exp)
Lymphoid Tissues		
Tonsil	+ (Nat)	- (Exp)
Mandibular and parotid LN	+ (Nat)	- (Exp)
Mediastinal LN	NT	- (Exp)
Mesenteric LN	NT	- (Exp)
Prescapular LN	+ (Nat)	- (Exp)
Precrural LN	NT	- (Exp)
Spleen	NT	- (Exp)
Intestine		
Duodenum	NT	- (Exp)
Jejunum	NT	- (Exp)
Ileum	NT	- (Exp)
Caecum	NT	- (Exp)
Other tissues		
Milk	NT	NT
Colostrum	NT	NT
Skeletal Muscle	+ (Exp)	- (Exp)
Blood	NT	NT

+ Presence of infectivity/PrP^{Sc}
- Absence of infectivity/PrP^{Sc}
Exp Experimental infection
Nat Natural infection
NT Not tested

2.4.1.1. Atypical scrapie TSE agent load in sheep tissues

Recently, tissues (CNS, lymphoid, peripheral nerves and skeletal muscle) collected from field (either clinical or preclinical n=7) or experimental cases were bioassayed in transgenic ovine mice. The assayed peripheral tissues were found negative for PrP^{Sc} (IHC and WB) while the CNS of all the cases were positive (Lacroux et al., 2010).

In a significant proportion of natural cases (5/7 natural cases- including an ARR/ARR animal) infectivity was detected in peripheral tissues. Similarly in experimental cases (IC route) at clinical stage of the disease the presence of infectivity was demonstrated in peripheral nerves and muscle .

In all the cases the detected levels of infectivity were low (close to the limit of the detection of the bioassay (LD_{50}). The infectious titre in investigated brain samples (equivalent weight) were about 10^4 to 10^6 fold higher than those observed in the positive peripheral tissues (see Appendix I).

Bioassay in transgenic ovinised mice has also revealed the presence of infectivity in the ileum of sheep in the pre-clinical phase of disease, with a negative brain, following oral challenge with Atypical scrapie. No PrP^{Sc} could be detected in these tissues by IHC or ELISA (Simmons et al., 2010b).

2.4.2. Atypical scrapie in goats

To date Atypical scrapie cases in goats has been reported in several EU countries. There is no available information related to the pathogenesis of this TSE form in goats. While in sheep several projects related to Atypical scrapie are ongoing, in goats no specific on-going project has been identified. In the absence of specific data related to goat species, the ability of Atypical scrapie to accumulate in peripheral tissues of goat should be considered similar to that reported for sheep.

2.4.3. Atypical scrapie conclusions

- Atypical scrapie pathogenesis (origin of the disease, contagiousness) remains mostly unknown.
- In sheep:
    - low levels of infectivity can be present in peripheral tissues (lymphoid tissues, nerves, skeletal muscle) in preclinical and clinical cases of Atypical scrapie in sheep of various genotypes;
    - there is currently no sufficient data on the kinetics of distribution of the Atypical scrapie agent into peripheral tissues of preclinical infected animals.
- In goats there is currently no data on the presence of infectivity in peripheral tissues and on the kinetics of distribution of the Atypical scrapie agent within infected animals.

3. TSE epidemiological situation in the small ruminant European population

Since 2002, data on the epidemiology of Classical scrapie have been collected at European level through the mandatory active surveillance program on healthy animals slaughtered for human consumption and dead animals collected as fallen stock. Animals over 18 months are screened for TSE cases by analysis of brainstem samples with rapid tests detecting PrP^{Sc}.

Non negative samples are confirmed mostly using Western Blot or immunohistochemistry. The first series of analysis allows the confirmatory diagnostic of TSE and, since 2007, most often the diagnostic of Atypical scrapie at the same time. If a TSE different from Atypical scrapie is confirmed, discriminatory tests are conducted to distinguish Classical scrapie from BSE. Only if BSE cannot be ruled out, further tests are carried out (biochemistry and bioassay). In Cyprus, the cases were not systematically typed before 2008.

For each type of TSE, the crude prevalence is estimated in this section as the number of cases divided by the number of tests recommended for the considered type of TSE. The 95% confidence interval is estimated according to binomial distribution. The accuracy of the prevalence estimate (size of the confidence intervals) depends strongly on the number of animals tested which was mostly determined according to the size of the sheep or goat population.

The confidence intervals take into account the uncertainty on the prevalence estimate due to random sampling but does not account for biases.

The active surveillance in slaughterhouse should be based on random selection of animals, however many biases could occur. At slaughterhouse the main biases include over or under representation of some geographical regions, non random timing of sampling such as systematic same week days for sampling, sampling rate related to flock size or commercial pattern, biases on animal age or general appearance (Del Rio Vilas et al., 2007; Fediaevsky et al., 2009; Tongue et al., 2008).

The overall sensitivity of the active surveillance should be considered separately for each type of TSE.

3.1. Classical scrapie epidemiological situation in the small ruminant European population

3.1.1. Apparent prevalence at slaughterhouse

In sheep, the overall apparent prevalence of TSE cases compatible with Classical scrapie, excluding Cyprus and Slovenia which presented very specific situations, decreased from 2002 to 2009. Although this trend may not be similar in all the member states (Del Rio Vilas et al., 2007; Fediaevsky et al., 2008), it indicates that current exposure to infected animals should be assessed with the most recent data. The most recent data could have indicated lower levels of infection in countries where the decline of Classical scrapie is particularly marked. However increased window of observation period also contribute to gain confidence on prevalence estimates. Therefore, in this analysis, data from 2007 to 2009 were included; data from 2010 were not included because it was not available for the same period of time for all the countries

From 2007 to 2009, 22 countries detected TSE cases in sheep compatible with Classical scrapie[10] for a total number of 145/721,892 cases (0.02%, 95%CI: 0.017 – 0.024) detected in sheep slaughtered for human consumption, excluding Cyprus because of untyped TSE cases. Only 14 cases were detected at slaughterhouse in 2009. Because of the genetic breeding program for resistance to scrapie for several years, the proportion of sheep carrying ARR alleles should increase in the coming years and therefore the prevalence rates of Classical scrapie is expected to further decrease in the coming years.

During the same period, 11 countries detected TSE cases compatible with Classical scrapie in goats[11] for a total number of 358/302,643 (0.12% 95%CI: 0.11 – 0.13) cases detected at slaughterhouse. Most of the cases (90%) were detected in Cyprus and no case was detected in 2009 at slaughterhouse.

Details on the apparent prevalence rates in the different Member States are provided in Table 14 and in Table 15.

10 Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

11 Cyprus, Finland, France, Greece, Italy, Norway, Portugal, Romania, Slovenia, Spain, United Kingdom

Table 14: Apparent prevalence rates of Classical scrapie at slaughter house from 2007 to 2009 in sheep (Source: European Commission)

Member State	N° cases reported as Classical scrapie	N° of rapid tests	Prevalence rate (per 10,000 animals tested)	95% confidence interval
Austria	0	557	0.0	0.0 ; 66.0
Belgium	0	6757	0.0	0.0 ; 5.5
Bulgaria	2	34702	0.6	0.1 ; 2.1
Cyprus	108	13726	244.8	219.6 ; 272.0
Czech Republic	0	1632	0.0	0.0 ; 22.6
Denmark	0	67	0.0	0.0 ; 535.6
Estonia	0	2441	0.0	0.0 ; 15.1
Finland	0	1944	0.0	0.0 ; 19.0
France	11	101918	1.1	0.5 ; 1.9
Germany	9	34964	2.6	0.1 ; 0.5
Greece	3	18321	1.6	0.3 ; 4.8
Hungary	4	13706	2.9	0.8 ; 7.5
Ireland	10	46821	2.1	1.0 ; 3.9
Italy	29	107386	2.7	1.8 ; 3.9
Latvia	0	1413	0.0	0.0 ; 26.1
Lithuania	0	7492	0.0	0.0 ; 4.9
Luxembourg	0	703	0.0	0.0 ; 52.3
Malta	0	56	0.0	0.0 ; 637.5
Netherlands	15	36166	4.1	2.3 ; 6.8
Norway	0	26794	0.0	0.0 ; 1.4
Poland	0	16080	0.0	0.0 ; 2.3
Portugal	4	153490	0.3	0.1 ; 0.7
Romania	5	27247	1.8	0.6 ; 4.3
Slovakia	18	5012	35.9	21.3 ; 56.7
Slovenia	12	342	350.9	182.6 ; 604.9
Spain	0	19104	0.0	0.0 ; 1.9
Sweden	0	7437	0.0	0.0 ; 5.0
United Kingdom	23	49407	4.7	3.0 ; 7.0

Table 15: Apparent prevalence rates of Classical scrapie at slaughter house from 2007 to 2009 in goats (Source: European Commission)

Member State	N° cases reported as Classical scrapie	N° of rapid tests	Prevalence rate (per 10,000 animals tested)	95% confidence interval
Austria	0	133	0.0	0.0 ; 273.5
Belgium	0	534	0.0	0.0 ; 68.8
Bulgaria	0	4367	0.0	0.0 ; 8.4
Cyprus	344	8625	398.8	358.5 ; 442.3
Czech Republic	0	57	0.0	0.0 ; 626.7
Denmark	0	30	0.0	0.0 ; 1105.7
Estonia	0	44	0.0	0.0 ; 804.2
Finland	0	77	0.0	0.0 ; 467.8
France	6	153741	0.4	0.1 ; 0.8
Germany	0	4229	0.0	0.0 ; 871.0
Greece	3	8469	3.5	0.7 ; 10.3
Hungary	0	264	0.0	0.0 ; 138.8
Ireland	0	1	0.0	0.0 ; 9750.0
Italy	1	38952	0.3	0.0 ; 1.4
Latvia	0	34	0.0	0.0 ; 1028.2
Lithuania	0	269	0.0	0.0 ; 133.2
Luxembourg	0	892	0.0	0.0 ; 41.3
Malta	0	15	0.0	0.0 ; 2180.2
Netherlands	0	15222	0.0	0.0 ; 2.4
Norway	0	3083	0.0	0.0 ; 12.0
Poland	0	151	0.0	0.0 ; 229.3
Portugal	0	17205	0.0	0.0 ; 2.1
Romania	0	1047	0.0	0.0 ; 35.2
Slovakia	0	1	0.0	0.0 ; 9750.0
Slovenia	0	54	0.0	0.0 ; 660.3
Spain	0	15649	0.0	0.0 ; 2.4
Sweden	0	11	0.0	0.0 ; 2849.1
United Kingdom	6	1467	40.3	14.8 ; 87.6

Because the number of sheep tested in Malta and of goats tested in many countries[12] is very small, the confidence intervals of the prevalence rates are wide and this indicates high uncertainty on the real level of infection.

Despite the apparent rarity of Classical scrapie in most countries, it is important to consider the heterogeneity of the prevalence rates between geographical areas (countries or areas within countries). According to the data from 2007 to 2009, 3 groups of countries could be distinguished: countries with no case detected at slaughterhouse, countries with low prevalence rates and countries with high prevalence rates (see Appendix II for details). However within countries with low prevalence rates, prevalence could be high in some specific areas. The prevalence of clinically and sub-clinically affected animals in Classical scrapie-affected flocks has been investigated by different research

12 Austria,Czech Republic, Estonia, Finland, Ireland, Latvia, Malta, Poland, Slovakia, Slovenia, Sweden

groups in different European countries and it varied between 3% (Jeffrey et al., 2002) and 41% (Ligios et al., 2006). Evidence for similar prevalence differences were obtained from affected goat herds (Gonzalez et al., 2009).

The sensitivity of the diagnostic method is not perfect due to the intrinsic capacity of the rapid test to detect PrP^{Sc} whenever it is present, and due to the variability of the presence of PrP^{Sc} in brainstem samples according to the incubation stage of the disease. Different studies indicated that the diagnostic method sensitivity was poor in pre-clinically affected animals, namely those that should be submitted as healthy slaughter. A study by Andreoletti et al.[13] provided quantitative estimates of the sensitivity of the method in pre-clinically affected goats of different ages (see Appendix II for details). Based on these estimates and in the absence of more complete information in sheep, the overall sensitivity of the diagnostic method was estimated to 50%, meaning that only one case out of two would be detected among animals tested by the active surveillance programme at slaughterhouse.

3.1.2. Conclusions

- There is a decline of the apparent prevalence of Classical scrapie at EU level during the period 2002 – 2009.
- The prevalence of Classical scrapie in sheep and goats can be very different according to the MS, hence it is probable that the same applies to the dietary exposure to Classical scrapie in the different MS.
- Due to the surveillance methodology applied and Classical scrapie pathogenesis (i.e. the overall diagnostic sensitivity depends on the age of the animal tested which is not documented) there is a likely underestimation of the prevalence.

3.2. BSE epidemiological situation in the small ruminant European population

The Opinion adopted on 25 January 2007 by 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 products" already addressed the question of the prevalence of BSE in sheep in the EU (EFSA, 2007b).

The assessment was based on the data collected within the framework of the TSEs in small ruminants carried out in the EU that included, since 2005, that each positive index case detected in an affected flock was required to be further analysed using tests capable of *in vitro* discrimination of BSE and scrapie in sheep. The prevalence was calculated both in the healthy slaughter exit stream and in all exit streams (some data were obtained by retrospective testing of passive surveillance cases identified between 1998-2004 (Stack et al., 2006)).

In the model 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 could be estimated using the assumption of a binomial distribution.

The model assumes that specificity of the screening tests is 100% (EFSA, 2005a) 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%. The sensitivity and specificity of the discriminatory tests were both assumed to be 100%.

13 PrioNet conference 2009, Banf, Canada. Available on-line at: www.neuroprion.org/en/home.html

Within the framework of this new opinion the same model was used using the TSE in small ruminants surveillance data collected over the period 2001-2009.

According to data provided by the European Commission on 25th March 2010, during the period 2002 – 2009 4,321,320 TSE tests were carried out on sheep in EU27 in all exit streams in the context of TSE surveillance. 17,479 animals were found TSE positive, and among them, 5533 were subjected to discriminatory testing. No case of BSE was identified by discriminatory assays among these cases. During the same period 1,297,380 TSE tests were carried out on goats in EU27 in all exit streams in the context of TSE surveillance. 4,492 animals were found TSE positive, and among them, 469 were subjected to discriminatory testing. One case of BSE was identified by discriminatory assays among these cases in France in a goats slaughtered in 2002 (Eloit et al., 2005).

The model used in the EFSA 2007 opinion was rerun taking into consideration these updated data and a rapid test sensitivity of 90% and 50%; the results are provided in Fig. 1, 2, 3 and 4 here below.

According to the model there is a 95% confidence that the number of BSE cases in the EU27 small ruminant population is:

- equal to or below 2.4 BSE cases per million sheep with a most probable value of 0, under the assumption of a 90% sensitivity of the screening test;
- equal to or below 4.2 BSE cases per sheep with a most probable value of 0, under the assumption of a 50% sensitivity of the screening test.
- equal to or below 29.9 BSE cases per million goats with a most probable value of 8.2, under the assumption of a 90% sensitivity of the screening test;
- equal to or below 53.7 BSE cases per million goats with a most probable value of 14.7, under the assumption of a 50% sensitivity of the screening test.

The higher most probable values and larger confidence intervals for goats is mostly due to the smaller sample size of TSE testing for goats and to the discovery of one BSE case in goats.

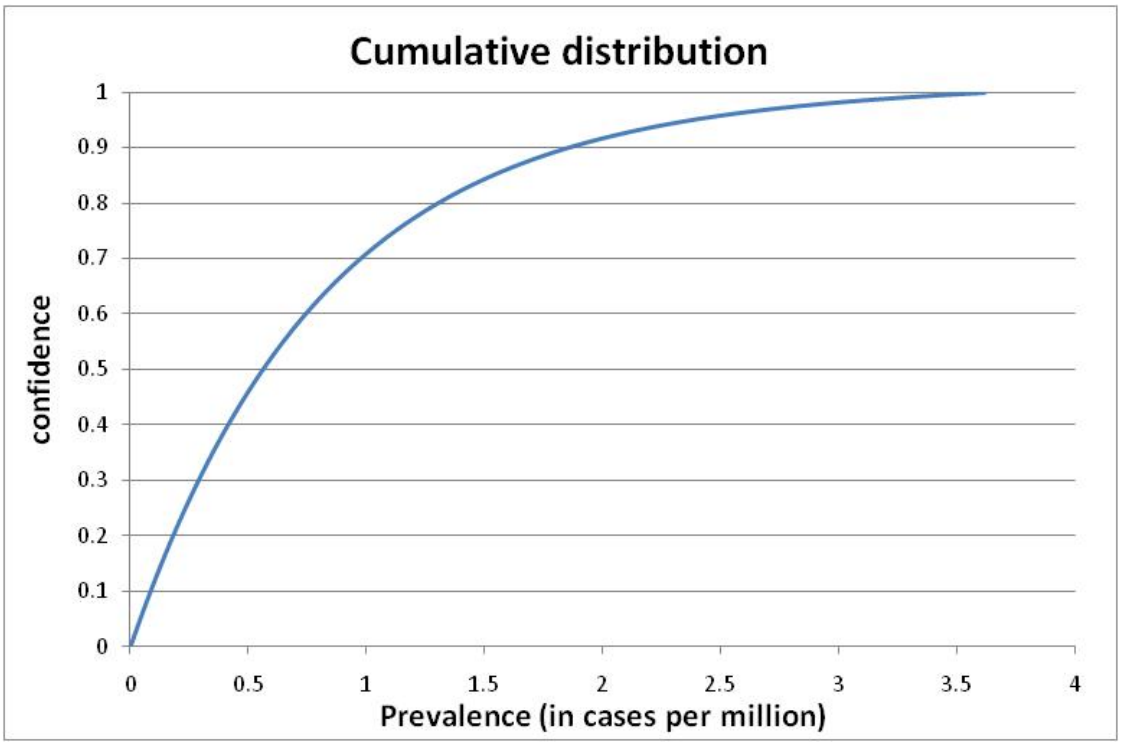

Figure 1: Cumulative uncertainty distribution of the BSE prevalence in the EU27 sheep population in all exit streams, under the assumption of a 90% sensitivity of the screening test.

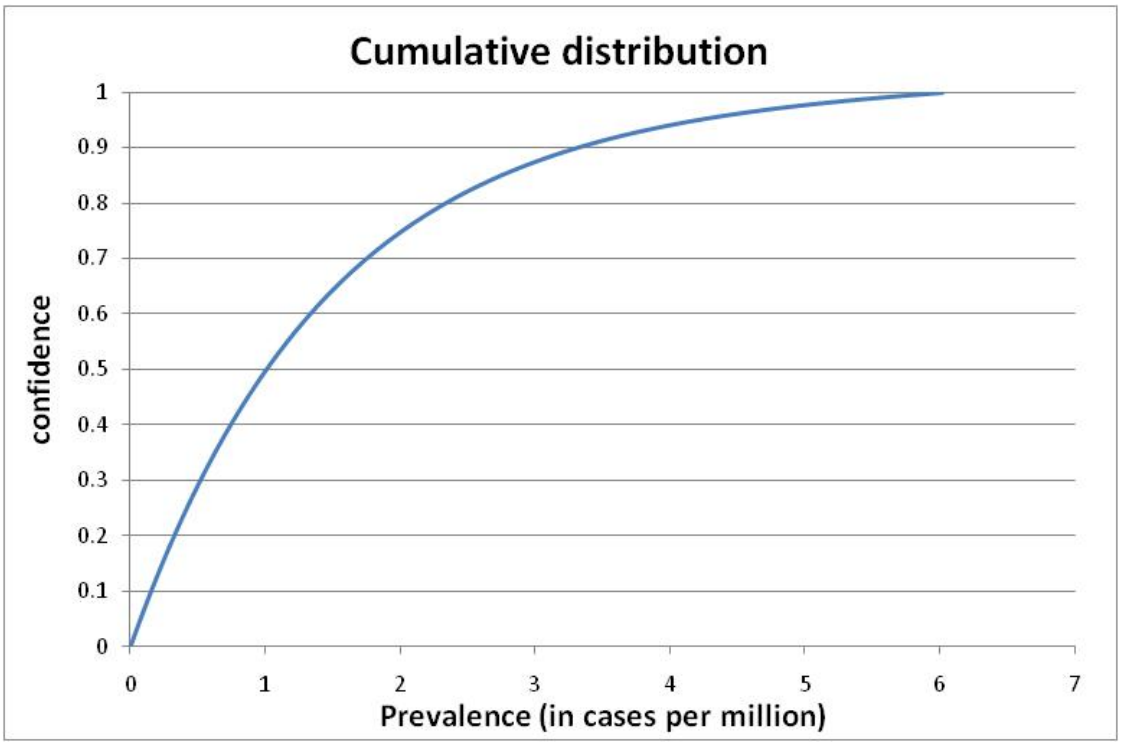

Figure 2: Cumulative uncertainty distribution of the BSE prevalence in the EU27 sheep population in all exit streams, under the assumption of a 50% sensitivity of the screening test.

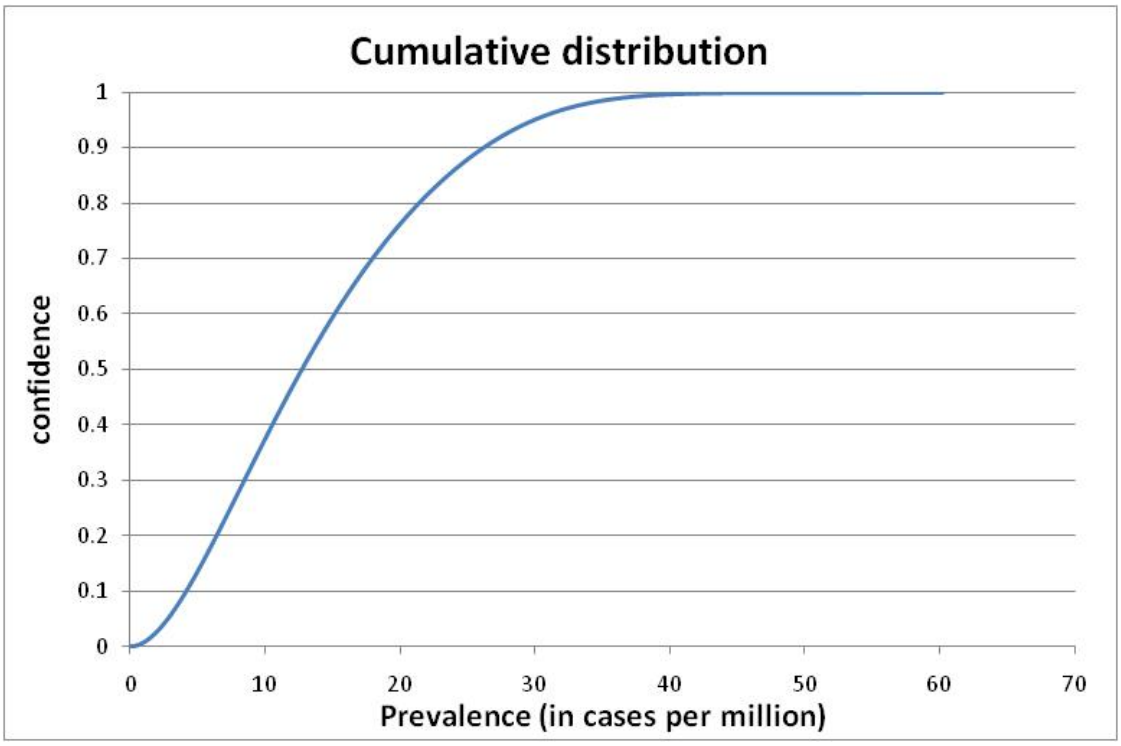

Figure 3: Cumulative uncertainty distribution of the BSE prevalence in the EU27 goat population in all exit streams, under the assumption of a 90% sensitivity of the screening test.

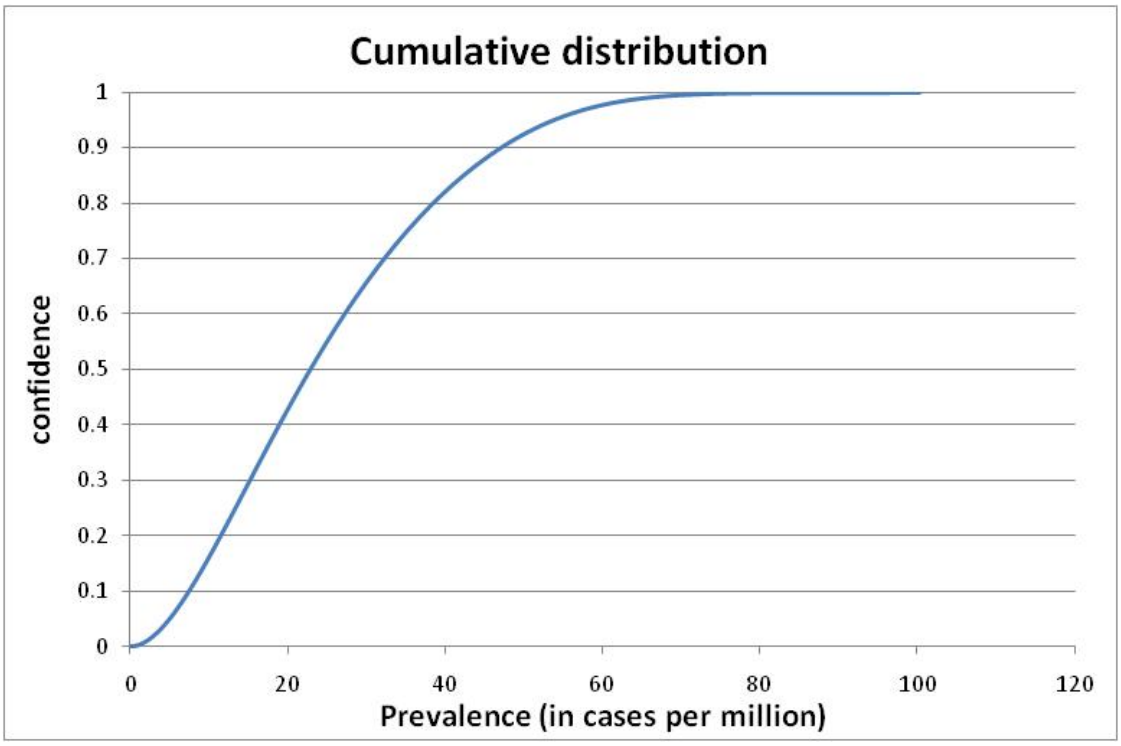

Figure 4: Cumulative uncertainty distribution of the BSE prevalence in the EU27 goat population in all exit streams, under the assumption of a 50% sensitivity of the screening test.

These estimates of the BSE prevalence allow excluding the existence of any widespread BSE epidemics within the EU small ruminant population.

However in its 2007 Opinion on "Certain aspects related to the risk of Transmissible Spongiform Encephalopathies (TSEs) in ovine and caprine animals" and in its January 2008 "Scientific and technical clarification in the interpretation and consideration of some facets of the conclusions of its Opinion of 8 March 2007 on certain aspects related to the risk of Transmissible Spongiform Encephalopathies (TSEs) in ovine and caprine animals" (EFSA, 2007a; EFSA Panel on Biological Hazards, 2008) the EFSA Scientific Panel on Biological Hazards indicated:

- *"Current discriminatory tests as described in the EC legislation to be used for discrimination between Scrapie and BSE appear, up to now, to be reliable for the differentiation of BSE from Classical and Atypical Scrapie. However, at the current stage of scientific knowledge, neither their diagnostic sensitivity nor their specificity can be assumed to be perfect."*

- *"discriminatory tests cannot be considered to be perfect because of the current lack of understanding of both the true biodiversity of TSE agents in ovine and caprine animals and how the agents interact in case of co-infection"*

Since 2007, some results obtained in sheep experimentally co-infected with BSE and different scrapie agents brought a proof of concept of the limits of biochemical discriminatory tests used for identifying BSE agents in scrapie/BSE co-infected animals ruminants (Lantier et al., 2009).

3.2.1. Conclusions

- According to the model developed by EFSA in its opinion of January 2007 the maximum number of BSE cases in the EU27 sheep population is equal to or below 4.2 per million sheep with a most probable value of 0, under the assumption of a 50% sensitivity of the screening test.

- According to the model developed by EFSA in its opinion of January 2007 the maximum number of BSE cases in the EU27 goat population is equal to or below 53.7 per million goats with a most probable value of 14.7, under the assumption of a 50% sensitivity of the screening test.

- There are uncertainties related to the technical limits of the methodology applied to detect BSE in sheep (discriminatory assay).

3.3. Atypical scrapie epidemiological situation in the small ruminant European population

According to the data transmitted to the European Commission, to date, 18 European countries have reported Atypical scrapie in sheep[14] and only 4 in goats[15]. As in previous studies, the detection of Atypical scrapie cases was strongly associated with the use of rapid tests recommended for detection of Atypical scrapie in brainstem samples (Fediaevsky et al., 2008). Since 2007 among the 27 Member States, in sheep, only 12 cases / 393,014 tests were associated with tests not recommended for detection of Atypical scrapie in brainstem samples, while 680 cases / 1,030,545 tests were associated with tests recommended for detection of Atypical scrapie in brainstem samples.

From 2007 to 2009, the apparent prevalence of Atypical scrapie in healthy slaughtered sheep was similar or higher than the prevalence of Classical scrapie in healthy slaughtered sheep in 8 countries[16]

14 Belgium, Bulgaria, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Netherlands, Norway, Poland, Portugal, Slovakia, Spain, Sweden, United Kingdom

15 France, Italy, Portugal, Spain

16 Bulgaria, France, Hungary, Italy, Norway, Portugal, Sweden, United Kingdom

suggesting that Atypical scrapie represents a significant proportion of TSE infected small ruminants that enter into the EU food chain.

Based on rapid tests recommended for detection of Atypical scrapie in brainstem samples, the apparent prevalence of Atypical scrapie at slaughterhouse from 2007 to 2009, ranged from 0.005 (1/18,321) in Greece to 0.23 (32/13,706) in Hungary (see Appendix II for details) in sheep and from 0.004 (2/54955) in France to 0.015 (4/27,093) in Italy in goats. The overall prevalence of Atypical scrapie was estimated to be about 8 cases per 10,000 tested sheep and 1 case per 10,000 tested goats.

There are an important number of caveats to consider in the estimation of the epidemiological situation of Atypical scrapie in Europe, in addition to the general considerations on the limits of active surveillance, which are common to Classical and Atypical scrapie. The apparent prevalence rates of Atypical scrapie vary with the age of the tested animals. However, the data available are insufficient to estimate accurate age-specific prevalences. The brainstem sample is inconstantly associated with the detection of PrP^{Sc} in infected animals (Benestad et al., 2008) and not all the approved rapid tests are recommended for the detection of Atypical scrapie in brainstem sample (EFSA, 2005b). Moreover, some recent results by (Lacroux et al., 2010) indicate that PrP^{Sc} could remain undetected in lymphoreticular system (LRS) tissues with important titers of infectivity suggesting that any diagnostic method based on PrP^{Sc} detection could result in an unknown proportion of undetected cases.

Therefore because of these important limits, the results of the TSE surveillance give indications on the epidemiology of Atypical scrapie but cannot be used to derive an accurate level of prevalence in animals slaughtered for human consumption.

However, assuming that the underestimation of the number of cases due to diagnostic impairment remains similar, the apparent prevalences of Atypical scrapie appeared relatively homogeneous in space and time (Fediaevsky et al., 2010; Fediaevsky et al., 2008; McIntyre et al., 2008), specially compared with Classical scrapie. These results that suggested a non contagious disease in natural conditions need to be consolidated with the more recent data on peripheral infectivity.

Since the aetiology of Atypical scrapie infected animals presents too many unknowns the assumption that the apparent prevalence in animals over 18 months old reflects prevalence in younger animals only relies on analogy with Classical scrapie.

3.3.1. Conclusions

- The presently detected prevalence of Atypical scrapie seems to be stable and comparable between MS (about 8 positive per 10,000 sheep and 1 positive per 10,000 goats tested at slaughterhouse).

- Because of the limits of PrP^{Sc} based detection methodology to identify Atypical scrapie agent and the uncertainties on Atypical scrapie pathogenesis in its natural host it remains impossible to assess the sensitivity of the currently applied surveillance.

4. Impact of SRM measures on exposure to TSE agents associated to an infected individual entering into the food chain.

4.1. The SRM tissue list

According to Annex V, point 1., letter (b) of Regulation (EC) 999/2001[17] (the TSE Regulation), as amended, the current list of Specified Risk Material (SRM) for ovine and caprine animals at EU level consists of:

- the spleen and ileum of animals of all ages;
- the skull including the brain and eyes, the tonsils and the spinal cord of animals aged over 12 months or which have a permanent incisor erupted through the gum.

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, 2002) but are beyond the scope of this risk assessment.

4.2. Estimation of at risk tissue mass and choice of the animal age classes

4.2.1. Estimation of at risk tissue mass

There are major differences in term of tissues weight in sheep and goat according to the age, the breed and the sex of the considered individual. For instance lambs and goats kids that are slaughtered for consumption between 3 weeks old and 5 weeks old weaning in southern Europe harbours a body weight from 7 to 15 Kg while and adult Suffolk sheep can reach 80-100 Kg. Similarly a Latcha dairy adult dairy sheep (Spain) will have a total weight of 30-40 Kg when a Ile de France will be between 60 and 80 Kg.

Additionally, according to the immunological status the lymphoreticular system activity and mass can vary significantly. For instance, while in lambs below 6 months the ileal Peyer's patches represent one of the major lymphocentres, in older animals it will reduce dramatically. Similarly thymus will go through a progressive involution in animals older than 4-5 months old.

Consequently, the current assessment relies on the use of approximate values (low and high) that despite being as realistic as possible, cannot be considered to be accurate. Some of the estimated values (like Ileal peyer's patch mass, skeletal muscle for instance) were elaborated by the experts on the basis of published data. For other tissues like the lymphnodes or nerves the chosen values relies on experts estimates.

4.3. Sheep production/consumption pattern and choice of animal age classes

In the EU the consumption, and consequently the production system, of sheep is very different according to the country.

In most of the Southern EU countries (Spain – Portugal – Italy) very young lambs/goat kids (usually less than a month old) represent an important part of the market. Since the local production is unable

17 Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. OJ L 147, 31.5.2001, p. 1-40.

to fulfil the demand a part of these animals are imported from other countries (like France). These lambs and goat kids usually correspond to dairy flocks in which mother/offspring separation occurs between 3 and 4 weeks of age, at the moment when commercial milking is initiated.

In other countries like France/ Belgium the lambs are mostly culled between the age of 3-6 months. In Northern countries (UK, Ireland, Germany for instance) the lambs are culled older (6-12 months).

Whatever the production system and consumption habits, a part of the adult sheep population is eliminated each year (20-30% in high performance flocks). In dairy sheep flocks and goat herds a significant proportion of the adult population is replaced each year and the age of the majority of the animals is generally below 5 years. In meat producing flocks the ewes are generally kept longer and the renewal rate is generally lower than in dairy flocks and the mean age of the producing animals can exceed 6 -7 years old.

Taking into account:

- the consumption/production parameters,
- the main step of the TSE agent dissemination in the organs of sheep and goat,
- the age for the retrieval of SRM in small ruminants as stated by the EU regulation,

the Panel decided to consider in this assessment four different age groups. Below 3 months, from 3 to less than 6 months old, from 6 to less than 12 months old and from 12 months and above.

Table 16: Estimated tissue weight in small ruminants at different age categories

		Age category			
		<3M	**3M - <6M**	**6M - <12M**	**=>12M**
Total weight *, **, [1, 2]		9-12 kg	25 -40 kg	30-50kg	35-80Kg
Carcass weight [1,2]		5-7 kg	15-20 Kg	15-25g	20-40Kg
Brain[6]		80-100 g	80-150 g	100-200g	100-200g
Spinal cord *, [6]		30-60 g	30-75g	50-100g	75-100 g
Peripheral nervous system*:	Dorsal root ganaglia nerves , autonomic ganglia	25-50g	25-50g	40-80g	40-80g
Head lymphoid formation *, [3]:	third eyelid, Retropharyngeal LN, parotid LN –– tonsil – mandibular LN – tonsil	15-30g	30-100g	30-100g	30-100g
Abdominal and thoracic cavity lymph node *, [3]:	Mediastinal, broncheal, retrohepatic, pancreatic, abomasal, mesenteric, inguino-femoral	15-50g	50-150g	50-150g	50-150g
Thymus*		20-80g	20-80g	20-50g	0-20g
Spleen*		50-100g	100-300g	100-300g	100-300g
Limbs LN*, [3]:	brachial, popliteal, precrural, Prescapular	20-50g	20-60g	20-60g	20-60g
GALT [4]:	Ileal Peyer Patches	60-150g	60-150g	5-60g	0-20g
	Other peyer Patches , colon follicles, rectal tonsil	5-20g	5-20g	5-20g	5-20g
Skeletal muscle (40-50% of carcass weight) [5]		2-3.5 Kg	6-10 Kg	6-12.5 Kg	10-20 Kg

*assumption by the Biohaz panel's experts

** unofficial technical information obtained from sheep and goat industry

[1] Croissance, engraissement et qualité des caracasses. Proceeding 'journées de la recherche ovine et caprine INRA – 1974

[2] Crapelet C. Traité d'élevage moderne Tome IV- Le mouton. Vigot et frères 1964

[3] Baum B, Mohr A, Pfaffl M, Bauer J and Hewicker-Trautwein M, 2005. Morphological findings in lymphatic tissues of sheep following oral application of the immunosuppressive mycotoxin mycophenolic acid. Mycopathologia, 160, 167-175.

[4] Reynolds JD and Morris B, 1983. The evolution and involution of Peyer's patches in fetal and postnatal sheep. Eur J Immunol, 13, 627-635.

[5] Jackson SP, Miller MF and Green RD, 1997. Phenotypic characterization of Rambouillet sheep expressing the Callipyge gene .3. Muscle weights and muscle weight distribution. Journal of Animal Science, 75, 133-138.

[6] Pruwhon M étude des caractéristiques de croissance des principaux tissues entre 25 et 150 jours chez les agneaux appartenant à 5 génotypes: Romanov, BerrichonxRomanov, texelx Romanov, Lacaunex Romanov et Charmois xRomanov. Proceeding CIHEAM - Options Méditerranéennes 1981. I.N.R.A. Station de Physiologie Animale 34060 Montpellier-Cedex

4.4. Estimation of SRM measure impact according to the age and the type of agent

4.4.1. General assumption applied to the quantification model

Considering the data available several general assumptions had to be made in order to carry out this assessment:

- The infectious load values that were used are the one described by Kimberlin (1994) and Hadlow (1982). These values were completed for muscle using an estimate as described in section 2 (infectious titre varying in clinically affected sheep between 0 and 1/2500 of the one observed in the brain from terminally affected sheep).

- There is evidence that kidney and liver from affected (incubating) sheep can in certain conditions accumulate TSE agent in natural scrapie infection context. There is no data about the timescale of this, or on the level of infectivity to consider. Despite these tissues being edible they were not integrated into this assessment.

- Thymus from Classical scrapie and BSE affected sheep is known to contain infectivity. In the absence of specific data on the kinetics of accumulation of TSE agents in this tissue, it was considered to behave similarly to the other thoracic lymphoreticular formations.

- Blood is known to be infectious in sheep incubating scrapie and BSE. However, due to the current lack of infectious titre estimate (probably low), this tissue was not included into the assessment.

- The autonomic nervous system within the digestive tract is known to accumulate PrP^{Sc} in both BSE and scrapie incubating and affected animals. In the absence of quantitative data related to the mass of these structures at the different levels of the digestive tract and of the infectivity specifically associated with this structure, they were not considered into this assessment.

The infectivity in the different tissues at the different considered age and for each type of TSE agent (BSE, Classical scrapie) was estimated by combining:

- the PrP^{Sc} accumulation kinetics in the different tissues as described in section 2.

- the infectivity level per mass unit of the different tissues as described in Tables 6, 10 and 12

- the mass of each tissue as described in the section 4.3

The estimate of the infectivity load per animal (in the different age classes) was calculated as the sum of the infectivity load contained in the different tissues.

For each TSE agent (BSE, Classical scrapie), age class, and small ruminants species the total infectivity amount that is associated to an animal before and after the retrieval of the SRM tissues was calculated in order to estimate the impact of SRM policy on food chain protection.

For each tissue, each agent and each class a low and a high values are provided.

- the low value correspond to the combination of the lowest estimated tissue mass and infectivity level in the considered tissue,

- the high value correspond to the combination of the highest estimated tissue mass and infectivity level in the considered tissue.

The tables reporting the quantification model are included in the body of the text.

In all cases the quantification model and its output should be considered as an approach to evaluate the magnitude of the exposure risk reduction that can be expected from the SRM policy under the different scenarios that were considered. The model cannot be considered to reflect directly the reality and will benefit from recalculation when further data related to infectivity load in certain tissues (digestive autonomic nervous system, blood, thymus…) and dissemination of TSE agent in tissues will become available.

4.4.2. Classical scrapie in sheep

For this assessment the distribution scheme of the TSE agent that was used is the one described in VRQ/VRQ sheep (section 2). This scenario can be considered as the worst case scenario. It however corresponds to the data that were used in previous assessments that were considered to design the SRM policy currently in force (EFSA, 2008; SSC, 2002).

Table 17 reports the quantification model for Classical scrapie in sheep.

Table 17: Quantification model to estimate the SRM measure impact for Classical scrapie in sheep

Tissue	<3 months						3 - <6 months						6 - <12 months						>= 12 months					
	Mass range (g)		Infectivity range (% final titre in the organ)		total infectivity range (ID50 IC in C57Bl6)		Mass range (g)		Infectivity range (% final titre in the organ)		total infectivity range (ID50 IC in C57Bl6)		Mass range (g)		Infectivity range (% final titre in the organ)		total infectivity range (ID50 IC in C57Bl6)		Mass range (g)		Infectivity range (% final titre in the organ)		total infectivity range (ID50 IC in C57Bl6)	
	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high
Brain	80	100	0%	0%	0.0E+00	0.0E+00	80	150	0%	0%	0.0E+00	0.0E+00	100	200	0%	10%	0.0E+00	7.3E+02	100	200	10%	100%	3.6E+02	8.0E+07
Spinal cord	30	60	0%	0%	0.0E+00	0.0E+00	30	75	0%	0%	0.0E+00	0.0E+00	50	100	0%	10%	0.0E+00	3.6E+02	75	100	10%	100%	2.7E+02	4.0E+07
Peripheral nervous system	25	50	0%	0%	0.0E+00	0.0E+00	25	50	0%	0%	0.0E+00	0.0E+00	40	80	0%	0%	0.0E+00	0.0E+00	40	80	0%	100%	0.0E+00	1.0E+05
Head lymphoid formation	15	30	0%	50%	0.0E+00	3.8E+03	30	60	50%	100%	3.8E+03	9.5E+05	30	60	100%	100%	4.8E+05	9.5E+05	30	60	100%	100%	4.8E+05	9.5E+05
Abdominal and thoracic cavity lymph node	15	50	0%	25%	0.0E+00	5.6E+02	50	150	25%	100%	5.6E+02	2.4E+06	50	150	100%	100%	7.9E+05	2.4E+06	50	150	100%	100%	7.9E+05	2.4E+06
Thymus	20	80	0%	100%	0.0E+00	1.3E+04	20	80	100%	100%	3.2E+03	1.3E+04	20	50	100%	100%	3.2E+03	7.9E+03	0	20	100%	100%	0.0E+00	3.2E+03
Spleen	50	100	0%	30%	0.0E+00	2.2E+03	100	300	30%	100%	2.2E+03	9.5E+06	100	300	100%	100%	3.2E+06	9.5E+06	100	300	100%	100%	3.2E+06	9.5E+06
Limbs LN	20	50	0%	10%	0.0E+00	1.3E+02	20	60	10%	100%	5.3E+01	9.5E+05	20	60	100%	100%	3.2E+05	9.5E+05	20	60	100%	100%	3.2E+05	9.5E+05
GALT ileal	60	150	0%	60%	0.0E+00	9.9E+04	60	150	60%	100%	4.0E+04	7.5E+06	5	60	100%	100%	2.5E+05	3.0E+06	5	20	100%	100%	2.5E+05	1.0E+06
GALT other	5	20	0%	30%	0.0E+00	5.1E+02	5	20	30%	100%	1.3E+02	1.0E+06	5	20	100%	100%	2.5E+05	1.0E+06	5	20	100%	100%	2.5E+05	1.0E+06
Skeletal muscle	2000	3500	0%	0%	0.0E+00	0.0E+00	3000	10000	0%	0%	0.0E+00	0.0E+00	6000	12500	0%	0%	0.0E+00	0.0E+00	10000	20000	0%	100%	0.0E+00	2.5E+06
Total infectious load					0.0E+00	1.2E+05					5.0E+04	2.2E+07					5.3E+06	1.8E+07					5.2E+06	1.4E+08
SRM Infectious load					0.0E+00	1.0E+05					4.2E+04	1.7E+07					3.4E+06	1.2E+07					3.4E+06	1.3E+08
Non SRM infectious					0.0E+00	1.8E+04					7.7E+03	5.3E+06					1.8E+06	5.3E+06					1.8E+06	8.9E+06
Reduction % associated with SRM retrieval					Not applicable	85					84	76					65	70					65	94

Within the limits and under the assumptions described in section 2 and section 4

- According to the calculations carried out in the present model the current SRM removal allows a reduction of the infectivity associated to the carcass of an infected individual:
    - by a range between 85% and 100% in animals below 3 months of age;
    - by a range between 76% and 84% in animals from 3 months till less than 6 months of age;
    - by a range between 65% and 70% in animals from 6 months till less than 12 months of age;
    - by a range between 65% and 94% in animals from 12 months of age;
- Considering the limitations of the used calculation approach the effect of SRM removal at individual level for an animal aged below 3 months, between 3 and 6 months, between 6 and 12 months and above 12 months can be considered to range around 1 $\log_{10}$ reduction (infectious load as expressed in IC ID_{50} in C57Bl6 mice).
- It can be estimated that there is, after SRM removal, the same amount of infectivity in one animal aged above 12 months as in:
    - 10^2 to 10^7 infected lambs aged below 3 months (from which SRM would have been removed);
    - up to 1,200 infected lambs aged between 3 and 6 months (from which SRM would have been removed);
    - up to 5 infected animals aged between 6 and 12 months (from which SRM would have been removed).

4.4.3. Classical scrapie in goats

The distribution scheme of the TSE agent that was used is the one described in orally challenged wild type goat (section 2). This scenario can be considered as the worst case scenario.

Table 18 reports the quantification model for Classical scrapie in goats.

Table 18: Quantification model to estimate the SRM measure impact for Classical scrapie in goats

Tissue	<3 months						3 - <6 months						6 - <12 months						>= 12 months					
	Mass range (g)		Infectivity range (% final titre in the organ)		total infectivity range (ID50 IC in C57Bl6)		Mass range (g)		Infectivity range (% final titre in the organ)		total infectivity range (ID50 IC in C57Bl6)		Mass range (g)		Infectivity range (% final titre in the organ)		total infectivity range (ID50 IC in C57Bl6)		Mass range (g)		Infectivity range (% final titre in the organ)		total infectivity range (ID50 IC in C57Bl6)	
	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high
Brain	80	100	0%	0%	0.0E+00	0.0E+00	80	150	0%	0%	0.0E+00	0.0E+00	100	200	0%	0%	0.0E+00	0.0E+00	100	200	0%	100%	0.0E+00	6.3E+08
Spinal cord	30	60	0%	0%	0.0E+00	0.0E+00	30	75	0%	0%	0.0E+00	0.0E+00	50	100	0%	0%	0.0E+00	0.0E+00	75	100	0%	100%	0.0E+00	1.3E+08
Peripheral nervous system	25	50	0%	0%	0.0E+00	0.0E+00	25	50	0%	0%	0.0E+00	0.0E+00	40	80	0%	0%	0.0E+00	0.0E+00	40	80	0%	100%	0.0E+00	3.2E+05
Head lymphoid formation	15	30	0%	0%	0.0E+00	0.0E+00	30	60	0%	0%	0.0E+00	0.0E+00	30	60	0%	100%	0.0E+00	3.8E+06	30	60	50%	100%	7.5E+03	3.8E+06
Abdominal and thoracic cavity lymph node	15	50	0%	0%	0.0E+00	0.0E+00	50	150	0%	20%	0.0E+00	1.4E+03	50	150	20%	100%	4.6E+02	9.5E+06	50	150	75%	100%	2.0E+05	9.5E+06
Thymus	20	80	0%	0%	0.0E+00	0.0E+00	20	80	0%	0%	0.0E+00	0.0E+00	20	50	100%	100%	3.2E+03	7.9E+03	0	20	100%	100%	0.0E+00	3.2E+03
Spleen	50	100	0%	0%	0.0E+00	0.0E+00	100	300	0%	0%	0.0E+00	0.0E+00	100	300	0%	100%	0.0E+00	9.5E+06	100	300	50%	100%	1.8E+04	9.5E+06
Limbs LN	20	50	0%	0%	0.0E+00	0.0E+00	20	60	0%	0%	0.0E+00	0.0E+00	20	60	0%	100%	0.0E+00	3.8E+06	20	60	50%	100%	5.0E+03	3.8E+06
GALT ileal	60	150	0%	0%	0.0E+00	0.0E+00	60	150	0%	75%	0.0E+00	5.0E+05	5	60	75%	100%	1.7E+04	3.0E+06	5	20	100%	100%	2.5E+05	1.0E+06
GALT other	5	20	0%	0%	0.0E+00	0.0E+00	5	20	0%	50%	0.0E+00	4.5E+03	5	20	50%	100%	1.1E+03	1.0E+06	5	20	100%	100%	2.5E+05	1.0E+06
Skeletal muscle	2000	3500	0%	0%	0.0E+00	0.0E+00	6000	10000	0%	0%	0.0E+00	0.0E+00	60000	12500	0%	0%	0.0E+00	0.0E+00	10000	20000	0%	100%	0.0E+00	2.5E+06
Total infectious load					0.0E+00	0.0E+00					0.0E+00	5.1E+05					2.1E+04	3.1E+07					7.3E+05	7.9E+08
SRM Infectious load					0.0E+00	0.0E+00					0.0E+00	5.0E+05					1.7E+04	1.2E+07					2.7E+05	7.7E+08
Non SRM infectious					0.0E+00	0.0E+00					0.0E+00	5.8E+03					4.7E+03	1.8E+07					4.6E+05	2.2E+07
Reduction % associated with SRM retrieval					Not applicable	Not applicable					Not applicable	99					78	41					37	97

Within the limits and under the assumptions described in section 2 and section 4:

- According to the currently available knowledge, goat kids below 3 months of age even coming from Classical scrapie infected herds represent a negligible source of infectivity for the food chain.
- According to the calculations carried out in the present model the current SRM retrieval allows a reduction of the infectivity associated to the carcass of an infected individual:
    - by a range between 99% and 100% in animals from 3 months till less than 6 months of age;
    - by a range between 41% and 78% in animals from 6 months till less than 12 months of age;
    - by a range between 37% and 97% in animals from 12 months of age.
- Considering the limitations of the used calculation approach the effect of SRM removal at individual level for an animal aged between 3 and 6 months, between 6 and 12 months and above 12 months can be considered to range around 1 $\log_{10}$ reduction (infectious load as expressed in IC ID_{50} in C57Bl6 mice).
- It can be estimated that there is, after SRM removal, the same amount of infectivity in one animal aged above 12 months as in:
    - 10^2 to 10^7 infected goat kids aged between 3 and 6 months (from which SRM would have been removed);
    - up to 10^4 infected animals aged between 6 and 12 months (from which SRM would have been removed).

4.4.4. BSE in sheep

The distribution scheme of the TSE agent that was used is the one described in orally challenged ARQ/ARQ sheep (section 2). This scenario can be considered as the worst case scenario

In the absence of specific data, the infectious load values are the one described by Kimberlin and Hadlow with natural scrapie.

Table 19 reports the quantification model for BSE in sheep.

Table 19: Quantification model to estimate the SRM measure impact for BSE in sheep

Tissue	<3 months						3 - <6 months						6 - <12 months						>= 12 months					
	Mass range (g)		Infectivity range (% final titre in the organ)		total infectivity range (ID50 IC in C57Bl6)		Mass range (g)		Infectivity range (% final titre in the organ)		total infectivity range (ID50 IC in C57Bl6)		Mass range (g)		Infectivity range (% final titre in the organ)		total infectivity range (ID50 IC in C57Bl6)		Mass range (g)		Infectivity range (% final titre in the organ)		total infectivity range (ID50 IC in C57Bl6)	
	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high
Brain	80	100	0%	0%	0.0E+00	0.0E+00	80	150	0%	0%	0.0E+00	0.0E+00	100	200	0%	10%	0.0E+00	7.3E+02	100	200	10%	100%	3.6E+02	8.0E+07
Spinal cord	30	60	0%	0%	0.0E+00	0.0E+00	30	75	0%	0%	0.0E+00	0.0E+00	50	100	0%	10%	0.0E+00	3.6E+02	75	100	10%	100%	2.7E+02	4.0E+07
Peripheral nervous system	25	50	0%	0%	0.0E+00	0.0E+00	25	50	0%	0%	0.0E+00	0.0E+00	40	80	0%	0%	0.0E+00	0.0E+00	40	80	0%	100%	0.0E+00	1.0E+05
Head lymphoid formation	15	30	0%	0%	0.0E+00	0.0E+00	30	60	0%	30%	0.0E+00	1.1E+03	30	60	30%	100%	5.5E+02	9.5E+05	30	60	100%	100%	4.8E+05	9.5E+05
Abdominal and thoracic cavity lymph node	15	50	0%	5%	0.0E+00	8.1E+01	50	150	5%	75%	8.1E+01	2.1E+05	50	150	75%	100%	7.1E+04	2.4E+06	50	150	100%	100%	7.9E+05	2.4E+06
Thymus	20	80	0%	0%	0.0E+00	0.0E+00	20	80	0%	100%	0.0E+00	1.3E+04	20	50	100%	100%	3.2E+03	7.9E+03	0	20	100%	100%	0.0E+00	3.2E+03
Spleen	50	100	0%	30%	0.0E+00	2.2E+03	100	300	30%	75%	2.2E+03	7.1E+05	100	300	75%	100%	2.4E+05	9.5E+06	100	300	100%	100%	3.2E+06	9.5E+06
Limbs LN	20	50	0%	0%	0.0E+00	0.0E+00	20	60	0%	75%	0.0E+00	8.5E+04	20	60	75%	100%	2.8E+04	9.5E+05	20	60	100%	100%	3.2E+05	9.5E+05
GALT ileal	60	150	0%	50%	0.0E+00	3.4E+04	60	150	50%	100%	1.3E+04	7.5E+06	5	60	100%	100%	2.5E+05	3.0E+06	5	20	100%	100%	2.5E+05	1.0E+06
GALT other	5	20	0%	30%	0.0E+00	5.1E+02	5	20	30%	100%	1.3E+02	1.0E+06	5	20	100%	100%	2.5E+05	1.0E+06	5	20	100%	100%	2.5E+05	1.0E+06
Skeletal muscle	2000	3500	0%	0%	0.0E+00	0.0E+00	6000	10000	0%	0%	0.0E+00	0.0E+00	6000	12500	0%	0%	0.0E+00	0.0E+00	10000	20000	0%	100%	0.0E+00	2.5E+06
Total infectious load					0.0E+00	3.6E+04					1.6E+04	9.5E+06					8.4E+05	1.8E+07					5.2E+06	1.4E+08
SRM Infectious load					0.0E+00	3.6E+04					1.6E+04	8.2E+06					4.9E+05	1.2E+07					3.4E+06	1.3E+08
Non SRM infectious					0.0E+00	6.0E+02					2.1E+02	1.3E+06					3.5E+05	5.3E+06					1.8E+06	7.9E+06
Reduction % associated with SRM retrieval					Not applicable	98					99	86					58	70					65	94

Within the limits and under the assumptions described in section 2 and section 4

- According to the calculations carried out in the present model the current SRM removal allows a reduction of the infectivity associated to the carcass of an infected individual:
    - by a range between 98% to 100% in animals below 3 months of age;
    - by a range between 86% to 99% in animals from 3 months till less than 6 months of age;
    - by a range between 58% to 70% in animals from 6 months till less than 12 months of age;
    - by a range between 65% to 94% in animals from 12 months of age;
- Considering the limitations of the used calculation approach the effect of SRM removal at individual level for an animal aged below 3 months, between 3 and 6 months, between 6 and 12 months and above 12 months can be considered to range around 1 $\log_{10}$ reduction (infectious load as expressed in IC ID_{50} in C57Bl6 mice).
- It can be estimated that there is, after SRM removal, the same amount of infectivity in one animal aged above 12 months as in:
    - 10^3 to 10^7 infected lambs aged below 3 months (from which SRM would have been removed);
    - 1 to 10^5 infected lambs aged between 3 and 6 months (from which SRM would have been removed);
    - up to 25 infected animals aged between 6 and 12 months (from which SRM would have been removed).

4.4.5. BSE in goat

Considering,

- the results recently obtained in goat following oral challenge which seem to indicate that the BSE pathogenesis in this species might be different from the one described in sheep,
- the lack of comprehensive data on the infectivity/PrP^{Sc} level and distribution of the BSE agent in goats,

it is not possible at this stage to carry out a pertinent assessment of the efficacy that SRM measures would have to limit/prevent the entry into the food chain of the BSE agent associated with a BSE infected goat.

Such assessment should be carried out when complete results from the pathogenesis studies of BSE in goat (TSE goat EU project) become available.

However, under the hypothesis that BSE agent does not accumulate substantially in lymphoid tissues of infected goats, the current SRM policy could be considered more efficient for limiting the BSE agent entry into the food chain in goat species than in sheep.

4.4.6. Atypical scrapie in sheep and goats

The current knowledge related to the pathogenesis of Atypical scrapie in small ruminants remains insufficient to assess the quantitative impact of SRM retrieval measures on the entry into the food chain of this TSE agent.

However, considering the reported detection of Atypical scrapie infectivity in lymphoid tissues, peripheral nervous system and some lymphoid tissues from natural and experimental Atypical scrapie cases, the SRM measures cannot be considered to have absolute efficacy for prevention of the entry of this TSE agent into the food chain.

5. Impact of SRM measures on the global infectivity load entering into the food chain

5.1. Number of animals slaughtered per age category

On 20th May 2010 EFSA requested to its Focal Points Network, which acts as the interface between EFSA and national food safety authorities in the EU Member States, data related to the n° of small ruminants (sheep and goats) slaughtered at MS level by species, age category and year.

Taking into account the difficulty to provide data on small ruminants slaughtered by age category, although the age categorisation to estimate the total infectivity load at individual level considered in this assessment was taking into account four age categories (less than 3 months, from 3 to less than 6 months, from 6 to less than 12 months and from 12 months of age; see section 4.3 "Sheep production/consumption pattern and choice of animal age classes"), it was requested to the Focal Points Network to consider the following age categories: below 3 months, 3 months – below 12 months, 12 months and above. Moreover, when the numbers by species or age category would not have been available, it was requested to the Network to provide an estimation of the proportion of animals slaughtered in these three categories over the total number of slaughtered small ruminants.

The data concerning the number of small ruminants slaughtered in 2009, together with the related assumptions, are shown in Tables 20 and 21.

For some countries, the number of slaughtered small ruminants was probably not directly recorded and this figure was probably estimated on the basis of carcasses weights. Moreover, for some member states (with low small ruminants population), the number of sheep over 18 months tested at slaughterhouse (as reported to the EU commission) apparently exceeded the recorded slaughtered population of sheep aged over 12 months. These discrepancies point out the relative reliability of the estimated size of the small ruminant population in some member states.

Consequently, the data related to the number of small ruminants slaughtered in each Member states used in this assessment must be considered as indicative and should not be considered to reflect the exact reality.

Table 20: N° of sheep slaughtered by Member State and age category in 2009

Member State	N° of slaughtered sheep			
	below 3 months	3 months – below 12 months	12 months and above	Total
Austria	23,011	207,100	59,977	290,088
Belgium	548	28,897	105,453	134,898
Bulgaria	141,293	430,805	2,747	574,845
Cyprus	23,000	100,703	11,722	135,425
Czeck Republic	6,830	33,329	16,092	56,251
Denmark[1]	0	62,991	26,996	89,987
Estonia	0	4,031	1,985	6,016
Finland[2]	84	26,037	10,097	36,218
France[3]	1,159,436	2,705,352	554,455	4,419,243
Germany[4]	0	825,009	20,709	1,045,718
Greece[5]	296,941	5,641,875	909,545	6,848,361
Hungary	5,314	930	7,042	13,286
Ireland[6]	0	2,313,578	440,681	2,754,259
Italy[7]	5,320,492	592,817	647,747	6,561,056
Latvia	93	5,569	3,620	9,282
Lithuania	65	3,970	1,367	5,402
Luxembourg[8]	575	1,342	310	2,227
Malta[9]	0	41	1,612	1,653
Netherlands[10]	0	595,500	75,400	670,900
Poland[11]	0	29,264	15,769	45,033
Portugal[12]	451,805	580,498	72,642	1,104,945
Romania[13]	1,652,300	1,652,300	2,334,400	5,639,000
Slovakia	69,673	4,051	7,291	81,015
Slovenia[14]	9,291	317	0	9,608
Spain	4,096,735	6,643,355	332,168	11,072,258
Sweden	5,000	215,888	33,782	254,670
Uk	0	13,213,700	2,168,000	15,381,700
EU27	13,262,487	35,919,248	8,061,609	57,220,344

1 Considering the fact that Denmark declared that only few animals were slaughtered in the age category below 3 months it was assumed that all the lambs were in the age category 3 months - below 12 months

2 88 animals for which the age category is unknown were not considered.

3 France did not provide the number of sheep in the age categories below 3 months and 3 months - below 12 months but only the total number of slaughtered sheep below 12 months. It was assumed that within these animals 30% were in the age category below 3 months and 70% were in the age category 3 months - below 12 months.

4 Germany did not provide the number of sheep in the age categories below 3 months and 3 months - below 12 months but only the total number of slaughtered lambs. It was assumed that all the lambs were in the age category 3 months - below 12 months

5 Greece did not provide the number of sheep in the age categories below 3 months and 3 months - below 12 months but only the total number of slaughtered sheep below 12 months. By analogy with the age distribution in Cyprus in previous years it was assumed that only few animals between them (5%) were slaughtered in the age category below 3 months.

6 Since in Ireland the percentage of sheep slaughtered below 3 months of age has declared to be less than 1%, this has considered to be negligible and the total number of sheep has been considered composed by animals slaughtered in the two older age categories.

7 Italy provided data till 2007. Data from 2007 have been used as a proxy for 2009. Italy did not provide data per age category as requested by EFSA. The age categories provided by Italy were: 3 months, > 3 months and 12 months. It was assumed these age categories were corresponding respectively to the age categories of below 3 months, 3 month - below 12 months and 12 months and above.

8 All the data for Luxembourg have been obtained through Eurostat (source: Meat production and foreign trade (annual data) [apro_mt_pann] Last update: 02-07-2010). The same age distribution of France for animals below 12 months of age was used.

9 All the data for Malta have been obtained through Eurostat (source: Meat production and foreign trade (annual data) [apro_mt_pann] Last update: 12-07-2010). Given the low number of animals slaughtered in Malta and the fact that Malta did not provided estimates for the age categories of the slaughtered sheep it was assumend that all the lambs were in the age category 3 months - below 12 months

10 The Netherlands did not provide the number of sheep in the age categories below 3 months and 3 months - below 12 months but only the total number of slaughtered sheep below 12 months. It was assumed that all the lambs were in the age category 3 months - below 12 months.

11 All the data for Poland have been obtained through Eurostat (source: Meat production and foreign trade (annual data) [apro_mt_pann] Last update: 12-07-2010). It was assumed that all the lambs were in the age category 3 months - below 12 months.

12 Portugal provided data till 2008. Data from 2008 have been used as a proxy for 2009.

13 All the data for Romania have been obtained through Eurostat (source: Meat production and foreign trade (annual data) [apro_mt_pann] Last update: 14-07-2010). Data for 2009 were not available; data from 2008 have been used as a proxy for 2009. It was assumed that 50% of the lambs were in the age category below 3 months and 50% in the age category 3 months - below 12 months.

14 Slovenia did not provide the number of sheep slaughtered per each of the age category 3 month - below 12 months and 12 months and above but only the total number of sheep above 3 months of age. It was assumed that all these animals were in the age category 3 months - below 12 months.

Table 21: N° of goats slaughtered by Member State and age category in 2009

Member State	N° of slaughtered goats			
	below 3 months	3 months – below 12 months	12 months and above	Total
Austria	25,106	6,276	9,894	41,276
Belgium	3,166	1,919	158	5,243
Bulgaria	961	3,193	188	4,342
Cyprus	21,000	92,618	12,990	126,608
Czeck Republic	2,416	1,961	1,659	6,036
Denmark[1]	0	1,451	622	2,073
Estonia[2]	0	0	0	0
Finland[3]	2,009	0	72	178
France[4]	623,813	32,832	119,082	775,727
Germany[5]	0	22,257	5,564	27,821
Greece[6]	160,438	3,048,325	521,927	3,730,690
Hungary	0	0	95	95
Ireland[7]	0	0	0	0
Italy[8]	264,051	29,339	28,320	321,710
Latvia	0	4	42	47
Lithuania	0	0	0	0
Luxembourg[9]	272	114	68	454
Malta[10]	0	70	629	699
Netherlands[11]	53,394	0	27,506	80,900
Poland[12]	0	4,493	2,419	6,912
Portugal[13]	136,050	5,728	1,432	143,211
Romania[14]	170,500	170,500	341,000	682,000
Slovakia	62	4	17	83
Slovenia[15]	286	164	0	450
Spain[16]	946,697	88,897	118,914	1,154,508
Sweden	0	436	337	773
Uk[17]	0	7,581	1,244	8,825
EU27	2,246,060	3,682,324	1,194,179	7,099,661

1 Considering the fact that Denmark declared that only few animals were slaughtered in the age category below 3 months it was assumed that all the lambs were in the age category 3 months - below 12 months.
2 Estonia declared that less than 1% of all the slaughtered small ruminants were goats and, among them, all were in the age category 12 months and above. Considering that, it was assumed that all the slaughtered small ruminants in Estonia were sheep.
3 1 animal for which the age category is unknown was not considered.
4 France did not provide the number of goats in the age categories below 3 months and 3 months - below 12 months but only the total number of slaughtered goats below 12 months. It was assumed that within these animals 95% were in the age category below 3 months and 5% were in the age category 3 months - below 12 months.
5 Germany did not provide the age distribution for goats. By analogy with sheep the age distribution was assumed to be 80% per goats slaughtered in the age category 3 months - below 12 months and 20% in the age category 12 months and above.
6 Greece did not provide the number of goats in the age categories below 3 months and 3 months - below 12 months but only the total number of slaughtered goats below 12 months. By analogy with the age distribution in Cyprus in previous years it was assumed that only few animals between them (5%) were slaughtered in the age category below 3 months.
7 Given the very low number of goats slaughtered in Ireland and the fact that the age distribution was not available for goats, the total number of goats was added to the total number of sheep and the same age distribution as for sheep was assumed. The total number of slaughtered goats in 2009 in Ireland is 173.
8 Italy provided data till 2007. Data from 2007 have been used as a proxy for 2009. Italy did not provide the age distribution for slaughtered goats below 12 months. It was assumed that in goats slaughtered below 12 months of age the same age distribution of slaughtered sheep below 12 months of age can be applied in Italy.
9 All the data for Luxembourg have been obtained through Eurostat (source: Meat production and foreign trade (annual data) [apro_mt_pann] Last update: 02-07-2010). The same age distribution of France for animals below 12 months of age was used.
10 Given the low number of animals slaughtered in Malta, the fact that Malta did not provide estimates for the age categories of the slaughtered goats and by analogy with sheep it was assumed that the goat kids were 10% of the total number of slaughtered goats and all of them were in the age category 3 months - below 12 months
11 It was assumed that all the animals reported as goats by the Netherlands were in the age category 12 months and above.
12 All the data for Poland have been obtained through Eurostat (source: Meat production and foreign trade (annual data) [apro_mt_pann] Last update: 12-07-2010). By analogy with sheep the age distribution was assumed to be 65% per goats slaughtered in the age category 3 months - below 12 months and 35% in the age category 12 months and above.
13 Portugal provided data till 2008. Data from 2008 have been used as a proxy for 2009.
14 All the data for Romania have been obtained through Eurostat (source: Meat production and foreign trade (annual data) [apro_mt_pann] Last update: 14-07-2010). Data for 2009 were not available; data from 2008 have been used as a proxy for 2009. By analogy with sheep the age distribution was assumed to be 25% in the age category below 3 months of age, 25% in the age category 3 months - below 12 month and 50% in the age category above 12 months.
15 Slovenia did not provide the number of goats slaughtered per each of the age categories 3 month - below 12 months and 12 months and above but only the total number of goats above 3 months of age. It was assumed that all these animals were in the age category 3 months - below 12 months.
16 Spain did not provided the age distribution for 2009. Data from 2008 have been used as a proxy for 2009.
17 UK did not provide the age distribution for goats. The same age distribution as for sheep was assumed.

5.2. Classical scrapie

5.2.1. Method and assumptions

The number of infected animals entering the food chain was estimated in each Member states considering separately sheep and goats from each defined age categories (below 3 months, between 3 to less than 12 months and from 12 months).

The total number of infected animals entering the food chain in EU 27 was considered to be the sum of the infected animals entering the food chain in each member state.

The number of infected animals entering into the food chain was estimated for each TSE agent / age category / country/ species, by multiplying the estimated prevalence of each TSE form with the number of slaughtered animals in each age category. The estimated prevalence value applied in this assessment corresponds to the observed prevalence in slaughtered animals (as provided by the active surveillance) corrected by the relative sensitivity of the system (50% sensitivity- see section 3.1.1).

This approach relies on several assumptions:

- The number of slaughtered animals in each age category is correctly estimated. As previously mentioned, the number of slaughtered sheep estimated in some countries could be discrepant with the number of sheep tested. Since it occurred only in countries with small sheep population, the impact of this error can be considered to remain limited.

- The exchange of live animals between countries has no impact on the estimated TSE prevalence.

- The number of cases detected and removed from the food chain is negligible compared to the number of undetected cases.

- For each species and countries the prevalence rate is similar in all three considered age categories. This assumption relies on the fact that in small ruminants most of the animals are considered to acquire the infection in their first weeks of life.

- In each member state the age structure of slaughtered animals is geographically homogenous. A major limit to this assumption is the possible existence of different type of productions (meat / dairy or light carcass lamb / heavy lambs for instance) in the different regions of a country displaying different prevalence rates. However, considering the data currently available it is not possible at current stage to correct such potential bias.

- The tested population is representative of the slaughtered population. This is the basic assumption to infer the prevalence rates on the general population. As previously mentioned there are a number of limits to this assumption but few means to mitigate. The numbers of cases entering the food chain are estimated in each country based on homogeneous prevalences within countries. Doing so, relatively high prevalences could be applied to important regions of sheep production where the disease could be actually absent. Conversely, facilities slaughtering animals originating from infected areas could issue a relatively important number of infected carcasses.

- The prevalence estimates during the period 2007 - 2009 apply for the current situation.

5.2.2. Assessment of the number of infected animals entering the food chain

The estimated number of Classical scrapie infected sheep and goats entering the food chain is given in Tables 22 and 23. The statistical variability of the estimated prevalence rates are reflected through the lower and upper limits of the confidence intervals, which does not account for systematic biases.

Table 22: Estimated number of Classical scrapie infected sheep entering the food chain per year (in brackets lower and upper estimates)

Member State	below 3 months	3 months – below 12 months	12 months and above	Total	Cases potentially entering the food chain (%)
Austria	0 (0 - 304)	0 (0 - 2734)	0 (0 - 792)	0 (0 - 3830)	0%
Belgium	0 (0 - 1)	0 (0 - 32)	0 (0 - 115)	0 (0 - 147)	0%
Bulgaria	16 (2 - 59)	50 (6 - 179)	0 (0 - 1)	66 (8 - 239)	0%
Cyprus	362 (300 - 437)	1599 (1314 - 1911)	186 (153 - 222)	2131 (1767 - 2570)	7%
Czeck Republic	0 (0 - 31)	0 (0 - 150)	0 (0 - 73)	0 (0 - 254)	0%
Denmark	0 (0 - 0)	0 (0 – 6748)	0 (0 - 2892)	0 (0 - 9639)	0%
Estonia	0 (0 - 0)	0 (0 - 12)	0 (0 - 6)	0 (0 - 18)	0%
Finland	0 (0 - 0)	0 (0 - 99)	0 (0 - 38)	0 (0 - 137)	0%
France	250 (125 - 448)	584 (292 - 1045)	120 (60 - 214)	954 (476 - 1707)	3%
Germany	0 (0 - 0)	425 (20 - 81)	114 (5 - 22)	538 (25 - 102)	2%
Greece	97 (20 - 284)	1848 (381 - 5399)	298 (61 - 870)	2243 (463 - 6553)	8%
Hungary	3 (1 - 8)	1 (0 - 1)	4 (1 - 11)	8 (2 - 20)	0%
Ireland	0 (0 - 0)	988 (474 - 1817)	188 (90 - 346)	1177 (564 - 2163)	4%
Italy	2874 (1925 - 4127)	320 (214 - 460)	350 (234 - 502)	3544 (2373 - 5089)	12%
Latvia	0 (0 - 0)	0 (0 - 29)	0 (0 - 19)	0 (0 - 48)	0%
Lithuania	0 (0 - 0)	0 (0 - 4)	0 (0 - 1)	0 (0 - 5)	0%
Luxembourg	0 (0 - 6)	0 (0 - 14)	0 (0 - 3)	0 (0 - 23)	0%
Malta	0 (0 - 0)	0 (0 - 5)	0 (0 - 206)	0 (0 - 211)	0%
Netherlands	0 (0 - 0)	494 (276 - 815)	63 (35 - 103)	557 (312 - 918)	2%
Poland	0 (0 - 0)	0 (0 - 13)	0 (0 - 7)	0 (0 - 21)	0%
Portugal	24 (6 - 60)	30 (8 - 77)	4 (1 - 10)	58 (16 - 147)	0%
Romania	606 (197 - 1415)	606 (197 - 1415)	857 (278 - 1999)	2070 (672 - 4829)	7%
Slovakia	500 (297 - 790)	29 (17 - 46)	52 (31 - 83)	582 (345 - 919)	2%
Slovenia	652 (339 - 1124)	22 (12 - 38)	0 (0 - 0)	674 (351 - 1162)	2%
Spain	0 (0 - 1582)	0 (0 - 2565)	0 (0 - 128)	0 (0 - 4276)	0%
Sweden	0 (0 - 5)	0 (0 - 214)	0 (0 - 34)	0 (0 - 253)	0%
UK	0 (0 - 0)	12303 (7799 - 18458)	2018 (1280 - 3028)	14321 (9079 - 21486)	49%
EU27	**5385 (3212 - 10680)**	**19299 (11011 - 44363)**	**4254 (2230 - 11726)**	**28937 (16453 - 66769)**	100%

Table 23: Estimated number of Classical scrapie infected goats entering the food chain per year (in brackets lower and upper estimates)

Member State	below 3 months	3 months – below 12 months	12 months and above	Total	Cases potentially entering the food chain (%)
Austria	0 (0 - 1374)	0 (0 - 343)	0 (0 - 541)	0 (0 - 2258)	0%
Belgium	0 (0 - 44)	0 (0 - 26)	0 (0 - 2)	0 (0 - 72)	0%
Bulgaria	0 (0 - 2)	0 (0 - 5)	0 (0 - 0)	0 (0 - 7)	0%
Cyprus	1675 (1506 - 1858)	7388 (6641 - 8193)	1036 (931 - 1149)	10099 (9078 - 11200)	78%
Czeck Republic	0 (0 - 303)	0 (0 - 246)	0 (0 - 208)	0 (0 - 757)	0%
Denmark	0 (0 - 0)	0 (0 - 321)	0 (0 - 138)	0 (0 - 458)	0%
Estonia	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0%
Finland	0 (0 - 188)	0 (0 - 0)	0 (0 - 7)	0 (0 - 195)	0%
France	49 (18 - 106)	3 (1 - 6)	9 (3 - 20)	61 (22 - 132)	0%
Germany	0 (0 - 0)	0 (0 - 3877)	0 (0 - 969)	0 (0 - 4846)	0%
Greece	114 (23 - 332)	2160 (445 - 6309)	370 (76 - 1080)	2643 (545 - 7722)	21%
Hungary	0 (0 - 0)	0 (0 - 0)	0 (0 - 3)	0 (0 - 3)	0%
Ireland	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0%
Italy	14 (0 - 76)	2 (0 - 8)	1 (0 - 8)	17 (0 - 92)	0%
Latvia	0 (0 - 0)	0 (0 - 1)	0 (0 - 9)	0 (0 - 10)	0%
Lithuania	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0%
Luxembourg	0 (0 - 2)	0 (0 - 1)	0 (0 - 1)	0 (0 - 4)	0%
Malta	0 (0 - 0)	0 (0 - 30)	0 (0 - 274)	0 (0 - 305)	0%
Netherlands	0 (0 - 26)	0 (0 - 0)	0 (0 - 13)	0 (0 - 39)	0%
Poland	0 (0 - 0)	0 (0 - 206)	0 (0 - 111)	0 (0 - 317)	0%
Portugal	0 (0 - 58)	0 (0 - 2)	0 (0 - 1)	0 (0 - 61)	0%
Romania	0 (0 - 1199)	0 (0 - 1199)	0 (0 - 2399)	0 (0 - 4797)	0%
Slovakia	0 (0 - 121)	0 (0 - 8)	0 (0 - 32)	0 (0 - 162)	0%
Slovenia	0 (0 - 38)	0 (0 - 22)	0 (0 - 0)	0 (0 - 59)	0%
Spain	0 (0 - 446)	0 (0 - 42)	0 (0 - 56)	0 (0 - 544)	0%
Sweden	0 (0 - 0)	0 (0 - 248)	0 (0 - 192)	0 (0 - 440)	0%
Uk	0 (0 - 0)	61 (22 - 133)	10 (4 - 22)	71 (26 - 155)	1%
EU27	**1851 (1547 - 6172)**	**9613 (7110 - 21228)**	**1427 (1015 - 7235)**	**12891 (9672 - 34635)**	100%

Because the low number of animals tested in some countries and/or species (in particular goats), the upper limits of the confidence interval of the estimated number of infected animals that may enter the food chain are clearly overestimated. Nevertheless, these figures indicate that several thousands of sheep and goats infected with Classical scrapie enter yearly in the food chain in the EU 27.

According to the Classical scrapie surveillance data collected between 2007 and 2009, Classical scrapie is endemic in a majority of EU member states. However, because differences in the prevalence of the disease, population size and production system (age at slaughter), there are significant differences between certain member states with regards to Classical scrapie infected sheep that are susceptible to enter the food chain.

Similarly, because the heterogeneous distribution of the goat population in Europe and significant differences of the Classical scrapie prevalence in this species between countries, Classical scrapie infected goats that are susceptible to enter the food chain originate almost exclusively from some member states.

5.2.3. Assessment of infectivity load entering the food chain

The total amount of infectivity load entering the food chain was estimated, for each EU 27 member states and for EU 27 as a whole, as the number of infected animals in each age category (less than 3 months, between 3 and 12 months and 12 months and above) multiplied by the infectivity load (total, associated with SRM and not associated with SRM) estimated in section 4, in each particular age category.

In section 4, the animal aged between 3 to 6 months and the animals aged between 6 and 12 months were considered separately. However the available data related to the numbers of slaughtered animals reported animals aged between 3 months to 12 months as a single group.

In this assessment the infectivity load values inferred for the category 3 months to less than 12 months was set to the infectivity load of the category 6 to less than 12 months which was the worst case scenario.

For each value the lower bound of the 95% CI confidence interval was associated with the smallest estimate of the infective load and the upper bound of the 95% CI confidence was associated with the highest estimate of the infective load and the mean number of cases (observed prevalence) was associated with the mean infectivity load interval.

Details for countries and EU27 for sheep and goats are provided Tables 24 and 25.

Table 24: Classical scrapie infectivity load in sheep slaughtered for human consumption before SRM removal per year and age category in EU member states (in brackets lower and upper estimates). The infectivity load is expressed as number of IC ID_{50} per gram in C57Bl mice. Please note that the IC ID_{50} in C57Bl6 mice cannot be related to any quantifiable dietary transmission risk in farmed animals or humans.

Member State	below 3 months	3 months – below 12 months	12 months and above	Total	Infectivity load potentially entering the food chain (%)
Austria	0.00E+00 (0.00E+00 - 3.65E+07)	0.00E+00 (0.00E+00 - 4.92E+10)	0.00E+00 (0.00E+00 - 1.11E+11)	0.00E+00 (0.00E+00 - 1.60E+11)	0%
Belgium	0.00E+00 (0.00E+00 - 7.18E+04)	0.00E+00 (0.00E+00 - 5.68E+08)	0.00E+00 0.00E+00 - 1.61E+10)	0.00E+00 (0.00E+00 - 1.67E+10)	0%
Bulgaria	9.77E+05 (0.00E+00 - 7.06E+06)	5.79E+08 (3.19E+07 - 3.23E+09)	2.30E+07 (1.99E+05 - 1.60E+08)	6.02E+08 (3.21E+07 - 3.40E+09)	0%
Cyprus	2.17E+07 (0.00E+00 – 5.24E+07)	1.86E+10 (6.96E+9 – 3.44E+10)	1.35E+10 (7.95E+8 - 3.11E+10)	3.22E+10 (7.76E+9 – 6.76E+10)	6%
Czeck Republic	0.00E+00 (0.00E+00 - 3.70E+06)	0.00E+00 (0.00E+00 - 2.71E+09)	0.00E+00 (0.00E+00 - 1.02E+10)	0.00E+00 (0.00E+00 - 1.29E+10)	0%
Denmark	0.00E+00 (0.00E+00 - 0.00E+00)	0.00E+00 (0.00E+00 - 1.21E+11)	0.00E+00 (0.00E+00 - 4.05E+11)	0.00E+00 (0.00E+00 - 5.26E+11)	0%
Estonia	0.00E+00 (0.00E+00 - 0.00E+00)	0.00E+00 (0.00E+00 - 2.19E+08)	0.00E+00 (0.00E+00 - 8.39E+08)	0.00E+00 (0.00E+00 - 1.06E+09)	0%
Finland	0.00E+00 (0.00E+00 - 3.82E+04)	0.00E+00 (0.00E+00 - 1.78E+09)	0.00E+00 (0.00E+00 - 5.36E+09)	0.00E+00 (0.00E+00 - 7.14E+09)	0%
France	1.50E+07 (0.00E+00 - 5.37E+07)	6.80E+09 (1.55E+09 - 1.88E+10)	8.69E+09 (3.11E+08 - 3.00E+10)	1.55E+10 (1.86E+09 - 4.88E+10)	3%
Germany	0.00E+00 (0.00E+00 - 0.00E+00)	4.95E+09 (1.05E+08 - 1.46E+09)	8.25E+09 (2.75E+07 - 3.03E+09)	1.32E+10 (1.32E+08 - 4.48E+09)	2%
Greece	5.83E+06 (0.00E+00 - 3.41E+07)	2.15E+10 (2.02E+09 - 9.72E+10)	2.16E+10 (3.19E+08 - 1.22E+11)	4.32E+10 (2.34E+09 - 2.19E+11)	8%
Hungary	1.86E+05 (0.00E+00 - 9.53E+05)	6.32E+06 (7.84E+05 - 2.50E+07)	2.98E+08 (5.82E+06 - 1.47E+09)	3.05E+08 (6.61E+06 - 1.50E+09)	0%
Ireland	0.00E+00 (0.00E+00 - 0.00E+00)	1.15E+10 (2.51E+09 - 3.27E+10)	1.37E+10 (4.69E+08 - 4.85E+10)	2.52E+10 (2.98E+09 - 8.12E+10)	5%
Italy	1.72E+08 (0.00E+00 - 4.95E+08)	3.73E+09 (1.14E+09 - 8.28E+09)	2.54E+10 (1.22E+09 - 7.03E+10)	2.93E+10 (2.35E+09 - 7.91E+10)	5%
Latvia	0.00E+00 (0.00E+00 - 5.81E+04)	0.00E+00 (0.00E+00 - 5.23E+08)	0.00E+00 (0.00E+00 - 2.64E+09)	0.00E+00 (0.00E+00 - 3,17E+09)	0%
Lithuania	0.00E+00 (0.00E+00 - 7.66E+03)	0.00E+00 (0.00E+00 - 7.04E+07)	0.00E+00 (0.00E+00 - 1.88E+08)	0.00E+00 (0.00E+00 - 2.59E+08)	0%
Luxembourg	0.00E+00 (0.00E+00 - 7.22E+05)	0.00E+00 (0.00E+00 - 2.53E+08)	0.00E+00 (0.00E+00 - 4.54E+08)	0.00E+00 (0.00E+00 - 7.08E+08)	0%
Malta	0.00E+00 (0.00E+00 - 0.00E+00)	0.00E+00 (0.00E+00 - 9.41E+07)	0.00E+00 (0.00E+00 - 2.88E+10)	0.00E+00 (0.00E+00 - 2.89E+10)	0%
Netherlands	0.00E+00 (0.00E+00 - 0.00E+00)	5.75E+09 (1.47E+09 - 1.47E+10)	4.54E+09 (1.82E+08 - 1.44E+10)	1.03E+10 (1.65E+09 - 2.91E+10)	2%
Poland	0.00E+00 (0.00E+00 - 0.00E+00)	0.00E+00 (0.00E+00 - 2.42E+08)	0.00E+00 (0.00E+00 - 1.01E+09)	0.00E+00 (0.00E+00 - 1.25E+09)	0%
Portugal	1.41E+06 (0.00E+00 - 7.24E+06)	3.52E+08 (4.37E+07 - 1.39E+09)	2.75E+08 (5.36E+06 - 1.36E+09)	6.29E+08 (4.91E+07 - 2.76E+09)	0%
Romania	3.64E+07 (0.00E+00 - 1.70E+08)	7.06E+09 (1.04E+09 - 2.55E+10)	6.22E+10 (1.45E+09 - 2.80E+11)	6.93E+10 (2.49E+09 - 3,06E+11)	13%
Slovakia	3.00E+07 (0.00E+00 - 9.48E+07)	3.39E+08 (9.15E+07 - 8.27E+08)	3.80E+09 (1.62E+08 - 1.16E+10)	4.17E+09 (2.53E+08 - 1.25E+10)	1%
Slovenia	3.91E+07 (0.00E+00 - 1.35E+08)	2.59E+08 (6.14E+07 - 6.90E+08)	0.00E+00 (0.00E+00 - 0.00E+00)	2.98E+08 (6.14E+07 - 8.25E+08)	0%
Spain	0.00E+00 (0.00E+00 - 1.90E+08)	0.00E+00 (0.00E+00 - 4.62E+10)	0.00E+00 (0.00E+00 - 1.80E+10)	0.00E+00 (0.00E+00 - 6.43E+10)	0%
Sweden	0.00E+00 (0.00E+00 - 5.95E+05)	0.00E+00 (0.00E+00 - 3.85E+09)	0.00E+00 (0.00E+00 - 4.69E+09)	0.00E+00 (0.00E+00 - 8.55E+09)	0%
Uk	0.00E+00 (0.00E+00 - 0.00E+00)	1.43E+11 (4.13E+10 - 3.32E+11)	1.47E+11 (6.65E+09 - 4.24E+11)	2.90E+11 (4.80E+10 - 7.56E+11)	54%
EU27	**3.23E+08 (0.00E+00 - 1.28E+09)**	**2.25E+11 (5.84E+10 - 7.98E+11)**	**3.09E+11 (1.16E+10 - 1.64E+12)**	**5.34E+11 (6.99E+10 - 2.44E+12)**	100%

Table 25: Classical scrapie infectivity load in goats slaughtered for human consumption before SRM removal per year and age category in EU member states (in brackets lower and upper estimates). The infectivity load is expressed as number of IC ID_{50} per gram in C57BL mice. Please note that the IC ID_{50} in C57Bl6 mice cannot be related to any quantifiable dietary transmission risk in farmed animals or humans.

Member State	below 3 months	3 months – below 12 months	12 months and above	Total	Infectivity load potentially entering the food chain (%)
Austria	0 (0 - 0)	0.00E+00 (0.00E+00 - 1.06E+10)	0.00E+00 (0.00E+00 - 4.28E+11)	0.00E+00 (0.00E+00 - 4.38E+11)	0%
Belgium	0 (0 - 0)	0.00E+00 (0.00E+00 - 8.19E+08)	0.00E+00 (0.00E+00 - 1.72E+09)	0.00E+00 (0.00E+00 - 2.54E+09)	0%
Bulgaria	0 (0 - 0)	0.00E+00 (0.00E+00 - 1.65E+08)	0.00E+00 (0.00E+00 - 2.48E+08)	0.00E+00 (0.00E+00 - 4.14E+08)	0%
Cyprus	0 (0 - 0)	1.15E+11 (1.39E+08 - 2.54E+11)	4.10E+11 (6.80E+08 - 9.08E+11)	5.24E+11 (8.19E+08 - 1.16E+12)	74%
Czeck Republic	0 (0 - 0)	0.00E+00 (0.00E+00 - 7.62E+09)	0.00E+00 (0.00E+00 - 1.64E+11)	0.00E+00 (0.00E+00 - 1.72E+11)	0%
Denmark	0 (0 - 0)	0.00E+00 (0.00E+00 - 9.95E+09)	0.00E+00 (0.00E+00 - 1.09E+11)	0.00E+00 (0.00E+00 - 1.19E+11)	0%
Estonia	0 (0 - 0)	0.00E+00 (0.00E+00 - 0.00E+00)	0.00E+00 (0.00E+00 - 0.00E+00)	0.00E+00 (0.00E+00 - 0.00E+00)	0%
Finland	0 (0 - 0)	0.00E+00 (0.00E+00 - 0.00E+00)	0.00E+00 (0.00E+00 - 5.32E+09)	0.00E+00 (0.00E+00 - 5.32E+09)	0%
France	0 (0 - 0)	3.97E+07 (1.97E+04 - 1.73E+08)	3.67E+09 (2.49E+06 - 1.60E+10)	3.71E+09 (2.51E+06 - 1.62E+10)	1%
Germany	0 (0 - 0)	0.00E+00 (0.00E+00 - 1.20E+11)	0.00E+00 (0.00E+00 - 7.66E+11)	0.00E+00 (0.00E+00 - 8.86E+11)	0%
Greece	0 (0 - 0)	3.35E+10 (9.35E+06 - 1.96E+11)	1.46E+11 (5.57E+07 - 8.53E+11)	1.80E+11 (6.50E+07 - 1.05E+12)	25%
Hungary	0 (0 - 0)	0.00E+00 (0.00E+00 - 0.00E+00)	0.00E+00 (0.00E+00 - 2.08E+09)	0.00E+00 (0.00E+00 - 2.08E+09)	0%
Ireland	0 (0 - 0)	0.00E+00 (0.00E+00 - 0.00E+00)	0.00E+00 (0.00E+00 - 0.00E+00)	0.00E+00 (0.00E+00 - 0.00E+00)	0%
Italy	0 (0 - 0)	2.34E+07 (8.01E+02 - 2.60E+08)	5.75E+08 (2.69E+04 - 6.40E+09)	5.98E+08 (2.77E+04 - 6.66E+09)	0%
Latvia	0 (0 - 0)	0.00E+00 (0.00E+00 - 2.68E+07)	0.00E+00 (0.00E+00 - 6.82E+09)	0.00E+00 (0.00E+00 - 6.85E+09)	0%
Lithuania	0 (0 - 0)	0.00E+00 (0.00E+00 - 0.00E+00)	0.00E+00 (0.00E+00 - 0.00E+00)	0.00E+00 (0.00E+00 - 0.00E+00)	0%
Luxembourg	0 (0 - 0)	0.00E+00 (0.00E+00 - 2.90E+07)	0.00E+00 (0.00E+00 - 4.44E+08)	0.00E+00 (0.00E+00 - 4.73E+08)	0%
Malta	0 (0 - 0)	0.00E+00 (0.00E+00 - 9.45E+08)	0.00E+00 (0.00E+00 - 2.17E+11)	0.00E+00 (0.00E+00 - 2.18E+11)	0%
Netherlands	0 (0 - 0)	0.00E+00 (0.00E+00 - 0.00E+00)	0.00E+00 (0.00E+00 - 1.05E+10)	0.00E+00 (0.00E+00 - 1.05E+10)	0%
Poland	0 (0 - 0)	0.00E+00 (0.00E+00 - 6.39E+09)	0.00E+00 (0.00E+00 - 8.77E+10)	0.00E+00 (0.00E+00 - 9.40E+10)	0%
Portugal	0 (0 - 0)	0.00E+00 (0.00E+00 - 7.61E+07)	0.00E+00 (0.00E+00 - 4.85E+08)	0.00E+00 (0.00E+00 - 5.61E+08)	0%
Romania	0 (0 - 0)	0.00E+00 (0.00E+00 - 3.72E+10)	0.00E+00 (0.00E+00 - 1.89E+12)	0.00E+00 (0.00E+00 - 1.93E+12)	0%
Slovakia	0 (0 - 0)	0.00E+00 (0.00E+00 - 2.51E+08)	0.00E+00 (0.00E+00 - 2.56E+10)	0.00E+00 (0.00E+00 - 2.58E+10)	0%
Slovenia	0 (0 - 0)	0.00E+00 (0.00E+00 - 6.71E+08)	0.00E+00 (0.00E+00 - 0.00E+00)	0.00E+00 (0.00E+00 - 6.71E+08)	0%
Spain	0 (0 - 0)	0.00E+00 (0.00E+00 - 1.30E+09)	0.00E+00 (0.00E+00 - 4.43E+10)	0.00E+00 (0.00E+00 - 4.56E+10)	0%
Sweden	0 (0 - 0)	0.00E+00 (0.00E+00 - 7.70E+09)	0.00E+00 (0.00E+00 - 1.52E+11)	0.00E+00 (0.00E+00 - 1.59E+11)	0%
Uk	0 (0 - 0)	9.49E+08 (4.72E+05 - 4.12E+09)	3.97E+09 (2.69E+06 - 1.72E+10)	4.92E+09 (3.16E+06 - 2.13E+10)	1%
EU27	**0 (0 - 0)**	**1.49E+11 (1.49E+08 - 6.58E+11)**	**5.64E+11 (7.41E+08 - 5.72E+12)**	**7.13E+11 (8.90E+08 - 6.37E+12)**	100%

Table 26: Classical scrapie infectivity load potentially entering the food chain per year in the EU 27 with and without SRM removal. The infectivity load is expressed as number of IC ID_{50} per gram in C57Bl6 mice. Please note that the IC ID_{50} in C57Bl6 mice cannot be related to any quantifiable dietary transmission risk in farmed animals or humans.

	Amount of infectvity load	% of total	Remaining infectivity load after SRM removal	% of total remaining infectivity
Sheep				
<3 months	3.23E+08 (0.00E+00 - 1.28E+09)	00% (00% - 00%)	4.58E+07 (0.00E+00 - 1.82E+08)	00% (00% - 00%)
3 - <12 months	2.25E+11 (5.84E+10 - 7.99E+11)	42% (83% - 33%)	6.85E+10 (1.98E+10 - 2.35E+11)	75% (83% - 69%)
>= 12 months	3.09E+11 (1.16E+10 - 1.64E+12)	58% (17% - 67%)	2.28E+10 (4.01E+09 - 1.04E+11)	25% (17% - 31%)
Goats				
<3 months	0.00E+00 (0.00E+00 - 0.00E+00)	00% (00% - 00%)	0.00E+00 (0.00E+00 - 0.00E+00)	00% (00% - 00%)
3 - <12 months	1.49E+11 (1.49E+08 - 6.58E+11)	21% (17% - 10%)	8.65E+10 (3.34E+7 - 3.82E+11)	84% (07% - 71%)
>0 12 months	5.64E+11 (7.41E+08 - 5.72E+12)	79% (83% - 90%)	1.6E+10 (4.67E+08 - 1.59E+11)	16% (93% - 29%)

At the EU27 level, the current SRM policy in force allows a global reduction of the potential exposure to Classical scrapie in a proportion ranging from:

- 64 to 84% (most likely value: 81%) in sheep; and
- 44 to 91% (most likely value: 85%) in goats.

The youngest categories do not contribute significantly to the total amount of infectivity entering into the food chain.

Before the SRM removal the main part of the infectivity that could enter into the food chain is due to animals aged 12 months and above. After SRM removal, the animals aged 3 to 12 months represent a higher infectious load than animals older than 12 months.

In infected lambs aged below 3 months the SRM removal measures in force allow a reduction of the infectivity load entering the food chain of about 85%.

Goat kids below 3 months of age even coming from Classical scrapie infected flocks represent a negligible source of infectivity for the food chain.

In infected sheep and goats aged between 3 and 12 months the SRM removal measures in force allow a reduction of the infectivity load entering the food chain ranging between 40 and 70%.

In infected sheep and goats aged over 12 months the SRM removal measures in force allow more than 1 $\log_{10}$ reduction of the infectivity load potentially entering the food chain.

Although Classical scrapie is much more common in sheep than in goats in most countries, the total infectivity load entering the food chain is higher in goats than in sheep. This is explained by the high level of infection in goats in Cyprus and in Greece combined with the higher individual infective load in goats. However reasoning on EU food chain, Classical scrapie is more common in sheep and sheep consumption is more common. This stresses the importance of the geographical heterogeneity of the exposure.

However there are a number of factors that differ between the amount of infectivity entering the food chain and the food exposure such as: the different dietary habits, the possible number of small ruminants entering the food chain without being slaughtered at slaughterhouse therefore with all SRM.

6. Considerations about the revision of the SRM list for small ruminants with regard to the potential exposure to the BSE agent

6.1. Estimation of the total number of small ruminant BSE cases entering the food chain per year

The number of BSE cases entering the food chain in EU 27 is estimated as the number of sheep and goats slaughtered for each age category times the prevalence in sheep and goats (Table 27).

Table 27: Lower and upper estimates of the number of BSE infected small ruminants entering the food chain in EU27 by age category per year

	below 3 months	3 to 12 months	over 12 months	total
Sheep	0 - 56	0 – 151	0 - 34	0 - 240
Goats	0 - 712	0 - 189	0 - 64	0 - 381

According to these figures, the total number of BSE cases in small ruminants that might enter the food chain in the EU27 remains limited. Consequently, if it would occur, the dietary exposure risk to small ruminants BSE agent would probably remain restricted in term of geographical cluster and population. This heterogeneity of the exposure led the BIOHAZ Panel to not consider appropriate to provide a global estimate of the impact of the SRM policy on the reduction of the small ruminant BSE infectious load that would enter the food chain at the EU level but to limit the assessment to the different SRM policy scenarios impact to individual sheep and goats.

6.2. Simulation of the impact of the SRM policy modification on BSE exposure risk

Sheep can be considered as a worst case scenario for goats. Indeed, preliminary data seem to indicate that BSE agent accumulation in the lymphoreticular system (LRS) of BSE infected goats might be significantly lower in goat than in sheep. However, due to the lack of definitive data on tissue distribution in infected goats, it is not possible at the moment to provide a separate simulation of the impact of SRM policy modification options for BSE in goats.

In this assessment, different scenarios were explored:

- the first two correspond to non retrieval of the SRM and to the retrieval of the current SRM list;
- the next four explored the impact of the non retrieval of the ileum, spleen, brain and spinal cord separately;
- the final one simulated the option in which only the dressed carcasses[18] would be commercialised.

18 The carcass of an animal after slaughter excluding head and the spinal cord.

Table 28 allows the estimation of the impact of the different SRM policy options on the infectious load potentially entering into the food chain for each age category. The reader should also consider the infectivity load as expressed in IC ID_{50} in C57Bl6 mice since the total amount of infectivity associated with an infected animal varies with the considered age. The developed calculation model applied here for the presented scenarios could be applied to explore the impact of other policy options. At the current stage of knowledge the biological significance of the infectivity load as measured by mouse bioassay cannot be inferred to oral BSE transmission risk in humans or farmed animals. Currently there are no data that allow the estimation of the minimal infectious dose of goat or sheep adapted BSE that would be sufficient to orally contaminate a human being.

Table 28: Simulation of the impact of the SRM policy modifications on the remaining BSE exposure risk by age category as a % of the total infectivity load (which is provided within brackets) expressed in IC ID_{50} in C57Bl6 mice. Please note that the table has to be read by columns and that the IC ID_{50} in C57Bl6 mice cannot be related to any quantifiable dietary transmission risk in farmed animals or humans.

Policy options	Below 3 months		3 – 12 months		above 12 months	
	Low estimate	High estimate	Low estimate	High estimate	Low estimate	High estimate
No SRM	(0)	100% ($3.6\ 10^4$)	100% ($1.6\ 10^4$)	100% ($1.8\ 10^7$)	100% ($1.8\ 10^7$)	100% ($1.4\ 10^8$)
Current SRM list	(0)	1.5% ($5.3\ 10^2$)	1% ($1.5\ 10^2$)	29.7% ($5.3\ 10^6$)	35% ($1.8\ 10^6$)	6.4% ($8.9\ 10^6$)
SRM with no removal of ileum	(0)	94% ($3.4\ 10^4$)	86% ($1.4\ 10^4$)	47% ($8.3\ 10^6$)	40% ($2.1\ 10^6$)	6.4% ($8.9\ 10^6$)
SRM with no removal of spleen	(0)	7% ($2.7\ 10^3$)	15% ($2.3\ 10^3$)	83% ($1.5\ 10^7$)	95% ($5\ 10^6$)	13% ($1.7\ 10^7$)
SRM with no removal of CNS	(0)	1.5% ($5.3\ 10^2$)	1% ($1.5\ 10^2$)	29.7% ($5.3\ 10^6$)	35% ($1.8\ 10^6$)	92% ($1.3\ 10^8$)
SRM with no removal of spinal cord	(0)	1.5% ($5.3\ 10^2$)	1% ($1.5\ 10^2$)	29.7% ($5.3\ 10^6$)	35% ($1.8\ 10^6$)	35%- ($4.8\ 10^7$)
Dressed carcass*	(0)	0% (0)	0% (0)	5% ($9.5\ 10^5$)	6% ($3.2\ 10^5$)	3% ($3.4\ 10^6$)

* The carcass of an animal after slaughter excluding head and the spinal cord.

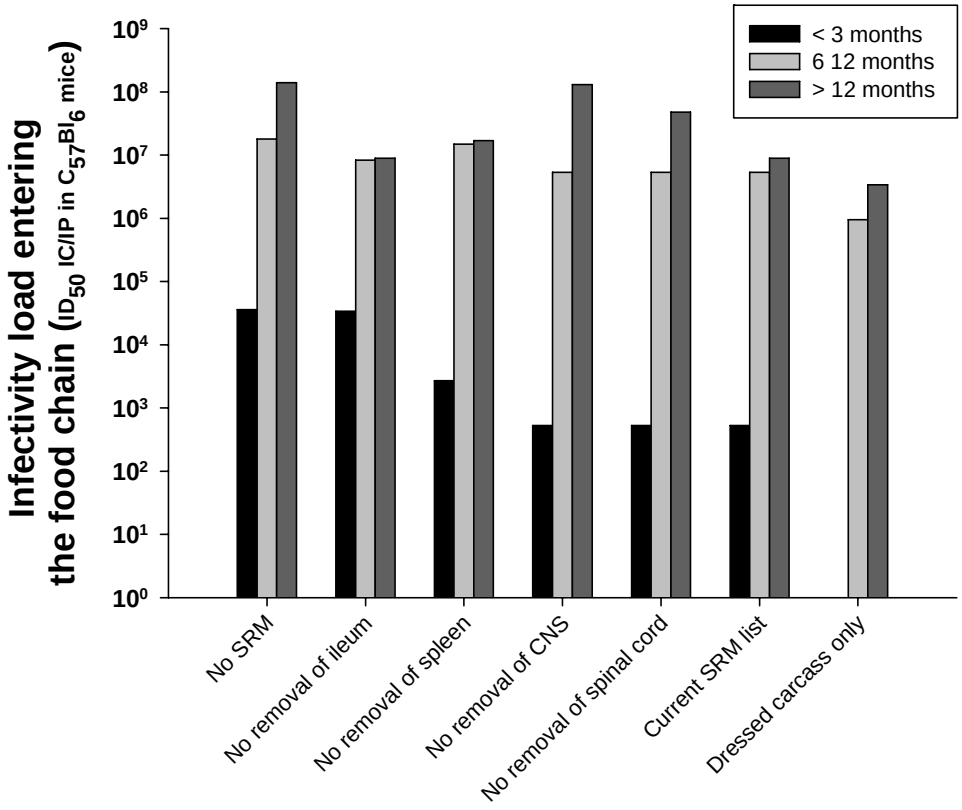

Figure 5: Impact of different SRM policy options on the BSE exposure risk by age category expressed as $\log_{10}$ IC ID_{50} in C57Bl6 mice. Please note that the IC ID_{50} in C57Bl6 mice cannot be related to any quantifiable dietary transmission risk in farmed animals or humans.

6.3. Conclusions

- According to the age of the considered individual, the current SRM list allows a relative reduction ranging from 65% to 99% of the BSE infectivity load that might be associated with an infected small ruminant entering into the food chain.

- In comparison to the current SRM policy, among the explored scenarios, the use of the dressed carcass is the only option allowing a more efficient reduction of the potential BSE infectious load associated with an infected small ruminant entering the food chain (reduction of 97% to 100% according to the considered age class).

- The elimination of the ileum has a major impact on the relative reduction of the BSE infectivity load that might enter in the food chain in animal aged below 12 months

- The CNS removal is the most efficient measure to reduce the relative infectivity load associated with a BSE infected small ruminant older than 12 months entering into the food chain.

- A modification of the SRM list driven only by consideration about BSE will also impact on the dietary exposure to Classical scrapie and Atypical scrapie agents.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- This assessment required several assumptions notably with regards to the pathogenesis of the disease, the infectious load and tissues mass in animals, prevalence estimates, number and age distribution of small ruminants slaughtered for human consumption in the EU Member States.

- The estimates of the infectious load are based on a simple approach using computations based on a low and a high estimate of each of the parameters. This provides order of magnitude estimates of the infectious load of TSE agents entering into the food chain at EU 27 level.

- The present approach could be replaced by a probabilistic model to provide more insight into the uncertainties. However, due to time and resources constraints it was not possible for the BIOHAZ Panel to develop and validate such a probabilistic model within the framework of this mandate.

- The infectivity load as expressed in this assessment (IC ID_{50} in C57Bl6 mice) cannot be related to any quantifiable dietary transmission risk in farmed animals or humans.

Classical scrapie in sheep

- According to the calculations carried out in the present model the current SRM removal allows a reduction of the infectivity associated to the carcass of an infected individual:

    - by a range between 85% and 100% in animals below 3 months of age;

    - by a range between 76% and 84% in animals from 3 months till less than 6 months of age;

    - by a range between 65% and 70% in animals from 6 months till less than 12 months of age;

    - by a range between 65% and 94% in animals from 12 months of age;

- Considering the limitations of the used calculation approach the effect of SRM removal at individual level for an animal aged below 3 months, between 3 and 6 months, between 6 and 12 months and above 12 months can be considered to range around 1 $\log_{10}$ reduction (infectious load as expressed in IC ID_{50} in C57Bl6 mice).

- It can be estimated that there is, after SRM removal, the same amount of infectivity in one animal aged above 12 months as in:

    - 10^2 to 10^7 infected lambs aged below 3 months (from which SRM would have been removed);

    - up to 1,200 infected lambs aged between 3 and 6 months (from which SRM would have been removed);

    - up to 5 infected animals aged between 6 and 12 months (from which SRM would have been removed).

- On the basis of data collected between 2007 and 2009, the total number of Classical scrapie infected sheep that could enter yearly into the food chain was estimated to approximately range between 16 and 67 thousand (most probable estimate 29 thousand) for the EU27 as a whole.

- Classical scrapie is present in a majority of EU member states. However because differences in the prevalence of the disease, population size and production system (age at slaughter), there are significant differences between certain member states with regards to sheep Classical scrapie infectivity load that may enter the food chain.

- This heterogeneity and the differences in consumption pattern between countries and regions mean that the dietary exposure to sheep Classical scrapie cannot be considered to be homogeneous in the EU27.

- At the EU27 level, the current SRM policy in force allows a global reduction of the potential exposure to sheep Classical scrapie in a proportion which can be estimated to be around 1 $\log_{10}$ (infectious load as expressed in IC ID_{50} in C57Bl6 mice).

Classical scrapie in goats

- According to the currently available knowledge, goat kids below 3 months of age even coming from Classical scrapie infected herds represent a negligible source of infectivity for the food chain.

- According to the calculations carried out in the present model the current SRM retrieval allows a reduction of the infectivity associated to the carcass of an infected individual:

    - by a range between 99% and 100% in animals from 3 months till less than 6 months of age;

    - by a range between 41% and 78% in animals from 6 months till less than 12 months of age;

    - by a range between 37% and 97% in animals from 12 months of age;

- Considering the limitations of the used calculation approach the effect of SRM removal at individual level for an animal aged between 3 and 6 months, between 6 and 12 months and above 12 months can be considered to range around 1 $\log_{10}$ reduction (infectious load as expressed in IC ID_{50} in C57Bl6 mice).

- It can be estimated that there is, after SRM removal, the same amount of infectivity in one animal aged above 12 months as in:

    - 10^2 to 10^7 infected goat kids aged between 3 and 6 months (from which SRM would have been removed);

    - up to 10^4 infected animals aged between 6 and 12 months (from which SRM would have been removed).

- On the basis of data collected between 2007 and 2009, the total number of Classical scrapie infected goats that could enter yearly into the food chain was estimated to approximately range between 10,000 and 34,000 (most probable estimate 13,000) for the EU27 as a whole.

- There is a heterogeneous distribution of the goat population in Europe and significant differences of the Classical scrapie prevalence in this species between countries. Therefore, Classical scrapie infected goats that enter into the food chain will originate almost exclusively from some member states.

- At the EU27 level, the current SRM policy in force allows a global reduction of the potential exposure to goat Classical scrapie which can be estimated to be around 1 $\log_{10}$ (infectious load as expressed in IC ID_{50} in C57Bl6 mice).

BSE in sheep

- With 95% confidence the number of BSE cases in the EU27 sheep population is equal to or below 4.2 per million sheep with a most probable value of 0, (under the assumption of a 50% sensitivity of the screening test). This estimate argues against any current widespread BSE epidemic within the EU sheep population.

- According to this estimate the most likely number of BSE cases in sheep that could enter yearly into the food chain in the EU is 0 and it is equal to or below 240 with 95% confidence.

- According to the calculations carried out in the present model the current SRM removal allows a reduction of the infectivity associated to the carcass of an infected individual:

    - by a range between 98% and 100% in animals below 3 months of age;

    - by a range between 86% and 99% in animals from 3 months till less than 6 months of age;

    - by a range between 58% and 70% in animals from 6 months till less than 12 months of age;

    - by a range between 65% and 94% in animals from 12 months of age;

- Considering the limitations of the used calculation approach the effect of SRM removal at individual level for an animal aged below 3 months, between 3 and 6 months, between 6 and 12 months and above 12 months can be considered to range around 1 $\log_{10}$ reduction (infectious load as expressed in IC ID_{50} in C57Bl6 mice).

- It can be estimated that there is, after SRM removal, the same amount of infectivity in one animal aged above 12 months as in:

    - 10^3 to 10^7 infected lambs aged below 3 months (from which SRM would have been removed);

    - 1 to 10^5 infected lambs aged between 3 and 6 months (from which SRM would have been removed);

    - up to 25 infected animals aged between 6 and 12 months (from which SRM would have been removed).

BSE in goats

- With 95% confidence the number of BSE cases in the EU27 goat population is equal to or below 53.4 per million goats (under the assumption of a 50% sensitivity of the screening test). This estimate argues against any current widespread BSE epidemic within the EU goat population.

- According to this estimate the number of BSE cases in goats that could enter yearly into the food chain in the EU is equal to or below 381 with 95% confidence.

- Preliminary biochemical and immunohistochemical data in goats suggest that there might be no major involvement of the lymphoid tissues in preclinical and clinical phase of the disease after oral experimental challenge. These data need to be completed and validated by bioassay.

- Before more complete information becomes available it is not possible to provide reliable specific estimates of the impact of SRM removal measures on the BSE exposure that would be associated with an infected goat entering into the food chain.

- In this context the estimates of the impact of SRM removal measures on the BSE exposure provided for BSE in sheep could be considered as a worst case scenario for BSE in goats.

Atypical scrapie in sheep and goats

- Low levels of infectivity can be present in peripheral tissues (lymphoid tissues, nerves, skeletal muscle) in preclinical and clinical cases of Atypical scrapie harbouring various genotypes. Consequently SRM measures cannot be assumed to prevent the entry of the Atypical scrapie agent into the food chain.

- There is currently no data on the kinetics of distribution of the Atypical scrapie agent into peripheral tissues of incubating small ruminants.

- There are uncertainties on the Atypical scrapie pathogenesis and its true prevalence in the EU small ruminant population. Therefore, the Panel is not in position to provide an assessment of the current Atypical scrapie infectious load entering into the food chain.

Answer to the first Term of Reference

- The TSE tissue infectivity distribution in small ruminants was revised and updated information is provided within the body of the text (section 2, tables 1 to 12).

Answer to the second Term of Reference

- The current SRM list allows a relative reduction of the BSE infectivity load that might be associated with an infected small ruminant entering into the food chain that can be considered to range around 1 $\log_{10}$ reduction (infectious load as expressed in IC ID_{50} in C57Bl6 mice).

- According to some simulations of the impact of different SRM policy modifications, the use of the dressed carcass only would allow a greater reduction of the BSE exposure risk than the current SRM policy measures.

- The elimination of the ileum has a major impact on the relative reduction of the BSE infectivity load that might enter in the food chain from an animal aged below 12 months

- The central nervous system removal is the most efficient measure to reduce the relative infectivity load associated with a BSE infected small ruminant older than 12 months entering into the food chain.

- A modification of the SRM list driven only by consideration about BSE will also impact on the dietary exposure to Classical scrapie and Atypical scrapie agents.

RECOMMENDATIONS

- It is recommended to update this assessment once data from ongoing experiments (BSE in goats pathogenesis study and infectivity load measurement in tissues, Atypical scrapie pathogenesis study) will become available.

- In order to provide more precise estimates of the impact of SRM removal on the infectious load of TSE agents entering into the food chain at EU 27 level it is recommended to develop a specific probabilistic model.

- It is recommended to improve the quality of the data collected on the small ruminant population (e.g. age category and destination of the animal).

- It is recommended to expand the current data collected in the context of the TSE surveillance activities by recording the tested animal age category and the type of rapid test used.

DOCUMENTATION PROVIDED TO EFSA

1. Letter (ref. n. SANCO E.2/ZH/bo-D(2009)520783 dated 24/12/2009) from the European Commission with a request for a scientific opinion on the review and update of the scientific opinion on BSE/TSE infectivity in small ruminant tissues.

2. SSC (Scientific Steering Committee), 2002. Update of the Opinion on TSE Infectivity distribution in ruminant tissues.

3. SSC (Scientific Steering Committee), 1998. Listing of Specified Risk Materials: a scheme for assessing relative risks to man - Opinion of the Scientific Steering Committee adopted on 9 December 1997 (Re-edited version adopted by the Scientific Steering Committee during its Third Plenary Session of 22-23 January 1998).

4. EFSA (European Food Safety Authority), 2005. Opinion on a quantitative assessment of risk posed to humans by tissues of small ruminants in case BSE is present in these animal populations. The EFSA Journal, 227, 1 - 11.

5. EFSA (European Food Safety Authority), 2007. Opinion on the quantitative risk assessment on the residual BSE risk in sheep meat and meat products. The EFSA Journal, 442, 1 - 44.

6. EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Panel on Biological Hazards on certain aspects related to the risk of Transmissible Spongiform Encephalopathies (TSEs) in ovine and caprine animals. The EFSA Journal, 466, 1 - 10.

7. EFSA (European Food Safety Authority), 2008. TSE risk assessment from carcasses of ovine and caprine animals below 6 months of age from TSE infected flocks intended for human consumption. 719, 1 - 27.

REFERENCES

Andreoletti O, Berthon P, Marc D, Sarradin P, Grosclaude J, van Keulen L, Schelcher F, Elsen JM and Lantier F, 2000. Early accumulation of PrP(Sc) in gut-associated lymphoid and nervous tissues of susceptible sheep from a Romanov flock with natural scrapie. J Gen Virol, 81, 3115-3126.

Andreoletti O, Lacroux C, Chabert A, Monnereau L, Tabouret G, Lantier F, Berthon P, Eychenne F, Lafond-Benestad S, Elsen JM and Schelcher F, 2002. PrP(Sc) accumulation in placentas of ewes exposed to natural scrapie: influence of foetal PrP genotype and effect on ewe-to-lamb transmission. J Gen Virol, 83, 2607-2616.

Andreoletti O, Morel N, Lacroux C, Rouillon V, Barc C, Tabouret G, Sarradin P, Berthon P, Bernardet P, Mathey J, Lugan S, Costes P, Corbiere F, Espinosa JC, Torres JM, Grassi J, Schelcher F and Lantier F, 2006. Bovine spongiform encephalopathy agent in spleen from an ARR/ARR orally exposed sheep. J Gen Virol, 87, 1043-1046.

Andreoletti O, Simon S, Lacroux C, Morel N, Tabouret G, Chabert A, Lugan S, Corbiere F, Ferre P, Foucras G, Laude H, Eychenne F, Grassi J and Schelcher F, 2004. PrPSc accumulation in myocytes from sheep incubating natural scrapie. Nat Med, 10, 591-593.

Barillet F, Mariat D, Amigues Y, Faugeras R, Caillat H, Moazami-Goudarzi K, Rupp R, Babilliot JM, Lacroux C, Lugan S, Schelcher F, Chartier C, Corbiere F, Andreoletti O and Perrin-Chauvineau C, 2009. Identification of seven haplotypes of the caprine PrP gene at codons 127, 142, 154, 211, 222 and 240 in French Alpine and Saanen breeds and their association with classical scrapie. J Gen Virol, 90, 769-776.

Bellworthy SJ, Dexter G, Stack M, Chaplin M, Hawkins SA, Simmons MM, Jeffrey M, Martin S, Gonzalez L and Hill P, 2005a. Natural transmission of BSE between sheep within an experimental flock. Vet Rec, 157, 206.

Bellworthy SJ, Dexter G, Stack M, Chaplin M, Hawkins SA, Simmons MM, Jeffrey M, Martin S, Gonzalez L and Hill P, 2008. Oral transmission of BSE to VRQ/VRQ sheep in an experimental flock. Vet Rec, 162, 130-131.

Bellworthy SJ, Hawkins SA, Green RB, Blamire I, Dexter G, Dexter I, Lockey R, Jeffrey M, Ryder S, Berthelin-Baker C and Simmons MM, 2005b. Tissue distribution of bovine spongiform encephalopathy infectivity in Romney sheep up to the onset of clinical disease after oral challenge. Vet Rec, 156, 197-202.

Benestad SL, Arsac JN, Goldmann W and Noremark M, 2008. Atypical/Nor98 scrapie: properties of the agent, genetics, and epidemiology. Vet Res, 39, 19.

Benestad SL, Sarradin P, Thu B, Schonheit J, Tranulis MA and Bratberg B, 2003. Cases of scrapie with unusual features in Norway and designation of a new type, Nor98. Vet Rec, 153, 202-208.

Buschmann A, Biacabe AG, Ziegler U, Bencsik A, Madec JY, Erhardt G, Luhken G, Baron T and Groschup MH, 2004a. Atypical scrapie cases in Germany and France are identified by discrepant reaction patterns in BSE rapid tests. J Virol Methods, 117, 27-36.

Buschmann A, Luhken G, Schultz J, Erhardt G and Groschup MH, 2004b. Neuronal accumulation of abnormal prion protein in sheep carrying a scrapie-resistant genotype (PrPARR/ARR). J Gen Virol, 85, 2727-2733.

Caplazi P, O'Rourke K, Wolf C, Shaw D and Baszler TV, 2004. Biology of PrPsc accumulation in two natural scrapie-infected sheep flocks. J Vet Diagn Invest, 16, 489-496.

Clouscard C, Beaudry P, Elsen JM, Milan D, Dussaucy M, Bounneau C, Schelcher F, Chatelain J, Launay JM and Laplanche JL, 1995. Different allelic effects of the codons 136 and 171 of the prion protein gene in sheep with natural scrapie. J Gen Virol, 76 (Pt 8), 2097-2101.

De Bosschere H, Roels S, Benestad SL and Vanopdenbosch E, 2004. Scrapie case similar to Nor98 diagnosed in Belgium via active surveillance. Vet Rec, 155, 707-708.

Del Rio Vilas VJ, Hopp P, Nunes T, Ru G, Sivam K and Ortiz-Pelaez A, 2007. Explaining the heterogeneous scrapie surveillance figures across Europe: a meta-regression approach. BMC Vet Res, 3, 13.

Detwiler LA, 1992. Scrapie. Rev Sci Tech, 11, 491-537.

Dexter G, Tongue SC, Heasman L, Bellworthy SJ, Davis A, Moore SJ, Simmons MM, Sayers AR, Simmons HA and Matthews D, 2009. The evaluation of exposure risks for natural transmission of scrapie within an infected flock. BMC Vet Res, 5, 38.

Dickinson AG and Fraser H, 1969. Genetical control of the concentration of ME7 scrapie agent in mouse spleen. J Comp Pathol, 79, 363-366.

Dickinson AG, Meikle VM and Fraser H, 1969. Genetical control of the concentration of ME7 scrapie agent in the brain of mice. J Comp Pathol, 79, 15-22.

EFSA (European Food Safety Authority), 2005a. Opinion on a quantitative assessment of risk posed to humans by tissues of small ruminants in case BSE is present in these animal populations. The EFSA Journal, 227, 1 - 11.

EFSA (European Food Safety Authority), 2005b. Scientific Report of the European Food Safety Authority on the Evaluation of Rapid post mortem TSE Tests intended for Small Ruminants. The EFSA Journal, 31, 1 - 17.

EFSA (European Food Safety Authority), 2007a. Opinion of the Scientific Panel on Biological Hazards on certain aspects related to the risk of Transmissible Spongiform Encephalopathies (TSEs) in ovine and caprine animals. The EFSA Journal, 466, 1 - 10.

EFSA (European Food Safety Authority), 2007b. Opinion on the quantitative risk assessment on the residual BSE risk in sheep meat and meat products. The EFSA Journal, 442, 1 - 44.

EFSA (European Food Safety Authority), 2008. TSE risk assessment from carcasses of ovine and caprine animals below 6 months of age from TSE infected flocks intended for human consumption. 719, 1 - 27.

EFSA (European Food Safety Authority), 2009. Scientific Opinion of the Panel on Biological Hazards on a request from the European Commission on genetic TSE resistance in goats. The EFSA Journal, (2009) 995, 1-25,

EFSA Panel on Biological Hazards, 2005. Opinion of the Scientific Panel on Biological Hazards on the request from the European Commission on classification of Atypical Transmissible Spongiform Encephalopathy (TSE) cases in small ruminants. The EFSA Journal, 276, 1 - 30.

EFSA Panel on Biological Hazards (BIOHAZ), 2006. Opinion of the Scientific Panel on biological hazards (BIOHAZ) on the Breeding programme for TSE resistance in sheep. The EFSA Journal, 382, 1 - 46.

EFSA Panel on Biological Hazards (European Food Safety Authority), 2008. Scientific and technical clarification in the interpretation and consideration of some facets of the conclusions of its Opinion of 8 March 2007 on certain aspects related to the risk of Transmissible Spongiform Encephalopathies (TSEs) in ovine and caprine animals. The EFSA Journal, 626, 1 - 11.

EFSA Panel on Biological Hazards (BIOHAZ), 2009. Scientific Opinion on genetic TSE resistance in goats in all European Union Member States. EFSA Journal, 7(11):1371, 42 pp.

Eloit M, Adjou K, Coulpier M, Fontaine JJ, Hamel R, Lilin T, Messiaen S, Andreoletti O, Baron T, Bencsik A, Biacabe AG, Beringue V, Laude H, Le Dur A, Vilotte JL, Comoy E, Deslys JP, Grassi J, Simon S, Lantier F and Sarradin P, 2005. BSE agent signatures in a goat. Vet Rec, 156, 523-524.

Elsen JM, Amigues Y, Schelcher F, Ducrocq V, Andreoletti O, Eychenne F, Khang JV, Poivey JP, Lantier F and Laplanche JL, 1999. Genetic susceptibility and transmission factors in scrapie: detailed analysis of an epidemic in a closed flock of Romanov. Arch Virol, 144, 431-445.

Ersdal C, Ulvund MJ, Benestad SL and Tranulis MA, 2003. Accumulation of pathogenic prion protein (PrPSc) in nervous and lymphoid tissues of sheep with subclinical scrapie. Vet Pathol, 40, 164-174.

Espinosa JC, Andreoletti O, Castilla J, Herva ME, Morales M, Alamillo E, San-Segundo FD, Lacroux C, Lugan S, Salguero FJ, Langeveld J and Torres JM, 2007. Sheep-passaged bovine spongiform encephalopathy agent exhibits altered pathobiological properties in bovine-PrP transgenic mice. J Virol, 81, 835-843.

Espinosa JC, Herva ME, Andreoletti O, Padilla D, Lacroux C, Cassard H, Lantier I, Castilla J and Torres JM, 2009. Transgenic mice expressing porcine prion protein resistant to classical scrapie but susceptible to sheep bovine spongiform encephalopathy and atypical scrapie. Emerg Infect Dis, 15, 1214-1221.

Everest S, Ramsay A, Hwthorn J, Chaplin M, Everitt S, Stack M, Jeffrey M, Moore J, Bellworthy S and Terry L, 2009. Detection and localisation of abnormal prion protein in the liver of TSE-affected sheep. Porto Carras, Chalkidiki Greece. 109.

Fediaevsky A, Maurella C, Noremark M, Ingravalle F, Thorgeirsdottir S, Orge L, Poizat R, Hautaniemi M, Liam B, Calavas D, Ru G and Hopp P, 2010. The prevalence of atypical scrapie in sheep from positive flocks is not higher than in the general sheep population in 11 European countries. BMC Vet Res, 6, 9.

Fediaevsky A, Morignat E, Ducrot C and Calavas D, 2009. A case-control study on the origin of atypical scrapie in sheep, France. Emerg Infect Dis, 15, 710-718.

Fediaevsky A, Tongue SC, Noremark M, Calavas D, Ru G and Hopp P, 2008. A descriptive study of the prevalence of atypical and classical scrapie in sheep in 20 European countries. BMC Vet Res, 4, 19.

Foster JD, Bruce M, McConnell I, Chree A and Fraser H, 1996. Detection of BSE infectivity in brain and spleen of experimentally infected sheep. Vet Rec, 138, 546-548.

Foster JD, Hope J and Fraser H, 1993. Transmission of bovine spongiform encephalopathy to sheep and goats. Vet Rec, 133, 339-341.

Foster JD, Parnham DW, Hunter N and Bruce M, 2001. Distribution of the prion protein in sheep terminally affected with BSE following experimental oral transmission. J Gen Virol, 82, 2319-2326.

Gavier-Widen D, Noremark M, Benestad S, Simmons M, Renstrom L, Bratberg B, Elvander M and af Segerstad CH, 2004. Recognition of the Nor98 variant of scrapie in the Swedish sheep population. J Vet Diagn Invest, 16, 562-567.

Georgsson G, Adolfsdottir JA, Palsdottir A, Jorundsson E, Sigurdarson S and Thorgeirsdottir S, 2008. High incidence of subclinical infection of lymphoid tissues in scrapie-affected sheep flocks. Arch Virol, 153, 637-644.

Georgsson G, Sigurdarson S and Brown P, 2006. Infectious agent of sheep scrapie may persist in the environment for at least 16 years. J Gen Virol, 87, 3737-3740.

Gomez N, Benedicto L, Geijo MV, Garrido JM, Garcia-Crespo D, Korkostegi JL, Hurtado A and Juste RA, 2007. Use of immunodiagnostic tests on an outbreak of scrapie in Latxa sheep: Pathogenetic and epidemiologic implications. Small Ruminant Research, 72, 141-148.

Gonzalez L, Martin S, Houston FE, Hunter N, Reid HW, Bellworthy SJ and Jeffrey M, 2005. Phenotype of disease-associated PrP accumulation in the brain of bovine spongiform encephalopathy experimentally infected sheep. J Gen Virol, 86, 827-838.

Gonzalez L, Martin S, Siso S, Konold T, Ortiz-Pelaez A, Phelan L, Goldmann W, Stewart P, Saunders G, Windl O, Jeffrey M, Hawkins SA, Dawson M and Hope J, 2009. High prevalence of scrapie in a dairy goat herd: tissue distribution of disease-associated PrP and effect of PRNP genotype and age. Vet Res, 40, 65.

Green DM, Del Rio Vilas VJ, Birch CP, Johnson J, Kiss IZ, McCarthy ND and Kao RR, 2007. Demographic risk factors for classical and atypical scrapie in Great Britain. J Gen Virol, 88, 3486-3492.

Groschup MH, Lacroux C, Buschmann A, Luhken G, Mathey J, Eiden M, Lugan S, Hoffmann C, Espinosa JC, Baron T, Torres JM, Erhardt G and Andreoletti O, 2007. Classic scrapie in sheep with the ARR/ARR prion genotype in Germany and France. Emerg Infect Dis, 13, 1201-1207.

Hadlow WJ, Kennedy RC and Race RE, 1982. Natural infection of Suffolk sheep with scrapie virus. J Infect Dis, 146, 657-664.

Hadlow WJ, Kennedy RC, Race RE and Eklund CM, 1980. Virologic and neurohistologic findings in dairy goats affected with natural scrapie. Vet Pathol, 17, 187-199.

Heggebo R, Press CM, Gunnes G, Lie KI, Tranulis MA, Ulvund M, Groschup MH and Landsverk T, 2000. Distribution of prion protein in the ileal Peyer's patch of scrapie-free lambs and lambs naturally and experimentally exposed to the scrapie agent. J Gen Virol, 81, 2327-2337.

Heikenwalder M, Federau C, Boehmer L, Schwarz P, Wagner M, Zeller N, Haybaeck J, Prinz M, Becher B and Aguzzi A, 2007. Germinal center B cells are dispensable in prion transport and neuroinvasion. J Neuroimmunol, 192, 113-123.

Hoinville LJ, Hoek A, Gravenor MB and McLean AR, 2000. Descriptive epidemiology of scrapie in Great Britain: results of a postal survey. Vet Rec, 146, 455-461.

Hourrigan J, Klingsporn A, Clark WW and Camp Md, 1979. Epidemiology of scrapie in the United States. In: Slow transmissible diseases of the nervous system. Volume 1. SB Prusiner, WJ Hadlow. Academic Press, New York, 331-356.

Hourrigan JL, 1988. Epidemiology of scrapie in the United States. Proceedings of the United States Animal Health Association, 92, 386-401.

Hunter N, Foster JD, Goldmann W, Stear MJ, Hope J and Bostock C, 1996. Natural scrapie in a closed flock of Cheviot sheep occurs only in specific PrP genotypes. Arch Virol, 141, 809-824.

Hunter N, Moore L, Hosie BD, Dingwall WS and Greig A, 1997. Association between natural scrapie and PrP genotype in a flock of Suffolk sheep in Scotland. Vet Rec, 140, 59-63.

Jeffrey M, Begara-McGorum I, Clark S, Martin S, Clark J, Chaplin M and Gonzalez L, 2002. Occurrence and distribution of infection-specific PrP in tissues of clinical scrapie cases and cull sheep from scrapie-affected farms in Shetland. J Comp Pathol, 127, 264-273.

Jeffrey M, Martin S, Thomson JR, Dingwall WS, Begara-McGorum I and Gonzalez L, 2001a. Onset and distribution of tissue prp accumulation in scrapie-affected suffolk sheep as demonstrated by sequential necropsies and tonsillar biopsies. J Comp Pathol, 125, 48-57.

Jeffrey M, Ryder S, Martin S, Hawkins SA, Terry L, Berthelin-Baker C and Bellworthy SJ, 2001b. Oral inoculation of sheep with the agent of bovine spongiform encephalopathy (BSE). 1. Onset and distribution of disease-specific PrP accumulation in brain and viscera. J Comp Pathol, 124, 280-289.

Kimberlin RH and Wilesmith JW, 1994. Bovine spongiform encephalopathy. Epidemiology, low dose exposure and risks. Ann N Y Acad Sci, 724, 210-220.

Kittelberger R, Chaplin MJ, Simmons MM, Ramirez-Villaescusa A, McIntyre L, MacDiarmid SC, Hannah MJ, Jenner J, Bueno R, Bayliss D, Black H, Pigott CJ and O'Keefe JS, In press. Atypical scrapie/Nor98 in a sheep from New Zealand. Journal of Veterinary Diagnostic Investigation, 22,

Konold T, Bone G, Simmons MM, Dexter G, Moore SJ and Pettitt RG, 2007a. Scrapie in goats. Vet Rec, 161, 395-396.

Konold T, Davis A, Bone G, Bracegirdle J, Everitt S, Chaplin M, Saunders GC, Cawthraw S and Simmons MM, 2007b. Clinical findings in two cases of atypical scrapie in sheep: a case report. BMC Vet Res, 3, 2.

Lacroux C, Orge L, Benestad S, Beringue V, Litaise C, Simon S, Simmons H, Lugan S, Corbiere F, Costes P, Morel N, Schelcher F and Andreoletti O, 2010. Atypical/Nor98 Scrapie Infectivity in Sheep Perpheral Tissues. 149.

Lacroux C, Simon S, Benestad SL, Maillet S, Mathey J, Lugan S, Corbiere F, Cassard H, Costes P, Bergonier D, Weisbecker JL, Moldal T, Simmons H, Lantier F, Feraudet-Tarisse C, Morel N, Schelcher F, Grassi J and Andreoletti O, 2008. Prions in milk from ewes incubating natural scrapie. PLoS Pathog, 4, e1000238.

Langeveld JP, Jacobs JG, Erkens JH, Bossers A, van Zijderveld FG and van Keulen LJ, 2006. Rapid and discriminatory diagnosis of scrapie and BSE in retro-pharyngeal lymph nodes of sheep. BMC Vet Res, 2, 19.

Lantier F, Berthon P, Lantier I, Barc C, Rossignol C, Leroux H, Bernardet P, Simmons H, Grassi J, Simon S and Andreoletti O, 2008. BSE transmission to lambs born from infected or contact ewes. Madrid. 83.

Lantier I, Berthon P, Leroux H, Rossignol C, Lacroux C, Torres JM, Simon S, Andreoletti O and Lantier F, 2009. Ovine and bovine PRNP transgenic mice allows discrimination between Scrapie and BSE in co-infected mice and sheep. Porrto Carras. 97.

Le Dur A, Beringue V, Andreoletti O, Reine F, Lai TL, Baron T, Bratberg B, Vilotte JL, Sarradin P, Benestad SL and Laude H, 2005. A newly identified type of scrapie agent can naturally infect sheep with resistant PrP genotypes. Proc Natl Acad Sci U S A, 102, 16031-16036.

Ligios C, Cancedda MG, Madau L, Santucciu C, Maestrale C, Agrimi U, Ru G and Di Guardo G, 2006. PrP(Sc) deposition in nervous tissues without lymphoid tissue involvement is frequently found in ARQ/ARQ Sarda breed sheep preclinically affected with natural scrapie. Arch Virol, 151, 2007-2020.

Ligios C, Sigurdson CJ, Santucciu C, Carcassola G, Manco G, Basagni M, Maestrale C, Cancedda MG, Madau L and Aguzzi A, 2005. PrPSc in mammary glands of sheep affected by scrapie and mastitis. Nat Med, 11, 1137-1138.

Loiacono CM, Thomsen BV, Hall SM, Kiupel M, Sutton D, O'Rourke K, Barr B, Anthenill L and Keane D, 2009. Nor98 scrapie identified in the United States. J Vet Diagn Invest, 21, 454-463.

McIntyre KM, del Rio Vilas VJ and Gubbins S, 2008. No temporal trends in the prevalence of atypical scrapie in British sheep, 2002-2006. BMC Vet Res, 4, 13.

McKinley MP, Bolton DC and Prusiner SB, 1983. A protease-resistant protein is a structural component of the scrapie prion. Cell, 35, 57-62.

Mitchell GB, O'Rourke KI, Harrington NP, Soutyrine A, Simmons MM, Dudas S, Zhuang D, Laude H and Balachandran A, Identification of atypical scrapie in Canadian sheep. J Vet Diagn Invest, 22, 408-411.

Nentwig A, Oevermann A, Heim D, Botteron C, Zellweger K, Drogemuller C, Zurbriggen A and Seuberlich T, 2007. Diversity in neuroanatomical distribution of abnormal prion protein in atypical scrapie. PLoS Pathog, 3, e82.

Onnasch H, Gunn HM, Bradshaw BJ, Benestad SL and Bassett HF, 2004. Two Irish cases of scrapie resembling Nor98. Vet Rec, 155, 636-637.

Onodera A, Ikeda T, Horiuchi M, Ishiguro N, Onuma M, Hirano N, Mikami T, Honda E, Hirai K, Kai K and et al., 1994. Survey of natural scrapie in Japan: analysis of RFLP types of the PrP gene and detection of PrPSc mainly in Suffolk sheep. J Vet Med Sci, 56, 627-632.

Orge L, Galo A, Machado C, Lima C, Ochoa C, Silva J, Ramos M and Simas JP, 2004. Identification of putative atypical scrapie in sheep in Portugal. J Gen Virol, 85, 3487-3491.

Orge L, Oliveira A, Machado C, Lima C, Ochoa C, Silva J, Carvalho R, Tavares P, Almeida P, Ramos M, Pinto MJ and Simas JP, 2010. Putative emergence of classical scrapie in a background of enzootic atypical scrapie. J Gen Virol, 91, 1646-1650.

Pattison IH and Millson GC, 1961. Further experimental observations on scrapie. J Comp Pathol, 71, 350-359.

Plinstone C, Hunter N, Foster J, Hart P, Manson JC and Barron R, 2010. Assessing the Risk of Sheep BSE Transmission ti Humans. Salzburg. 145.

Prusiner SB, 1982. Novel proteinaceous infectious particles cause scrapie. Science, 216, 136-144.

Prusiner SB, Cochran SP, Groth DF, Downey DE, Bowman KA and Martinez HM, 1982. Measurement of the scrapie agent using an incubation time interval assay. Ann Neurol, 11, 353-358.

Race R, Jenny A and Sutton D, 1998. Scrapie infectivity and proteinase K-resistant prion protein in sheep placenta, brain, spleen, and lymph node: implications for transmission and antemortem diagnosis. J Infect Dis, 178, 949-953.

Race R, Raines A, Raymond GJ, Caughey B and Chesebro B, 2001. Long-term subclinical carrier state precedes scrapie replication and adaptation in a resistant species: analogies to bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease in humans. J Virol, 75, 10106-10112.

Reckzeh C, Hoffmann C, Buschmann A, Buda S, Budras KD, Reckling KF, Bellmann S, Knobloch H, Erhardt G, Fries R and Groschup MH, 2007. Rapid testing leads to the underestimation of the scrapie prevalence in an affected sheep and goat flock. Vet Microbiol, 123, 320-327.

Roels S, Vanopdenbosch E, Langeveld JP and Schreuder BE, 1999. Immunohistochemical evaluation of tonsillar tissue for preclinical screening of scrapie based on surveillance in Belgium. Vet Rec, 145, 524-525.

Ryder S, Dexter G, Bellworthy S and Tongue S, 2004. Demonstration of lateral transmission of scrapie between sheep kept under natural conditions using lymphoid tissue biopsy. Res Vet Sci, 76, 211-217.

Ryder SJ, Dexter GE, Heasman L, Warner R and Moore SJ, 2009. Accumulation and dissemination of prion protein in experimental sheep scrapie in the natural host. BMC Vet Res, 5, 9.

Simmons MM, Konold T, Simmons HA, Spencer YI, Lockey R, Spiropoulos J, Everitt S and Clifford D, 2007. Experimental transmission of atypical scrapie to sheep. BMC Vet Res, 3, 20.

Simmons MM, Konold T, Thurston L, Bellworthy SJ, Chaplin MJ and Moore SJ, 2010a. The natural atypical scrapie phenotype is preserved on experimental transmission and sub-passage in PRNP homologous sheep. BMC Vet Res, 6, 14.

Simmons MM, Moore SJ, Terry L, Thorne L, Thurston L, Hawkins S, Konold T and Spiropoulos J, 2010b. Successful oral transmission of atypical scrapie in sheep. Salzburg. 14.

Siso S, Gonzalez L, Jeffrey M, Martin S, Chianini F and Steele P, 2006. Prion protein in kidneys of scrapie-infected sheep. Vet Rec, 159, 327-328.

SSC (Scientific Steering Committee), 2002. Update of the Opinion on TSE Infectivity distribution in ruminant tissues.

Stack M, Jeffrey M, Gubbins S, Grimmer S, Gonzalez L, Martin S, Chaplin M, Webb P, Simmons M, Spencer Y, Bellerby P, Hope J, Wilesmith J and Matthews D, 2006. Monitoring for bovine spongiform encephalopathy in sheep in Great Britain, 1998-2004. J Gen Virol, 87, 2099-2107.

Tabouret G, Lacroux C, Lugan S, Costes P, Corbiere F, Weisbecker JL, Schelcher F and Andreoletti O, 2010. Relevance of oral experimental challenge with classical scrapie in sheep. J Gen Virol, 91, 2139-2144.

Thorgeirsdottir S, Georgsson G, Reynisson E, Sigurdarson S and Palsdottir A, 2002. Search for healthy carriers of scrapie: an assessment of subclinical infection of sheep in an Icelandic scrapie flock by three diagnostic methods and correlation with PrP genotypes. Arch Virol, 147, 709-722.

Tixador P, Herzog L, Reine F, Jaumain E, Chapuis J, Le Dur A, Laude H and Beringue V, 2010. The physical relationship between infectivity and prion protein aggregates is strain-dependent. PLoS Pathog, 6, e1000859.

Tongue SC, Pfeiffer DU and Wilesmith JW, 2006. Descriptive spatial analysis of scrapie-affected flocks in Great Britain between January 1993 and December 2002. Vet Rec, 159, 165-170.

Tongue SC, Wilesmith JW, Nash J, Kossaibati M and Ryan J, 2008. The importance of the PrP genotype in active surveillance for ovine scrapie. Epidemiol Infect, 136, 703-712.

Tuo W, O'Rourke KI, Zhuang D, Cheevers WP, Spraker TR and Knowles DP, 2002. Pregnancy status and fetal prion genetics determine PrPSc accumulation in placentomes of scrapie-infected sheep. Proc Natl Acad Sci U S A, 99, 6310-6315.

Vaccari G, Di Bari MA, Morelli L, Nonno R, Chiappini B, Antonucci G, Marcon S, Esposito E, Fazzi P, Palazzini N, Troiano P, Petrella A, Di Guardo G and Agrimi U, 2006. Identification of an allelic variant of the goat PrP gene associated with resistance to scrapie. J Gen Virol, 87, 1395-1402.

van Keulen LJ, Bossers A and van Zijderveld F, 2008a. TSE pathogenesis in cattle and sheep. Vet Res, 39, 24.

van Keulen LJ, Schreuder BE, Vromans ME, Langeveld JP and Smits MA, 2000. Pathogenesis of natural scrapie in sheep. Arch Virol Suppl, 57-71.

van Keulen LJ, Vromans ME, Dolstra CH, Bossers A and van Zijderveld FG, 2008b. Pathogenesis of bovine spongiform encephalopathy in sheep. Arch Virol, 153, 445-453.

van Keulen LJ, Vromans ME and van Zijderveld FG, 2002. Early and late pathogenesis of natural scrapie infection in sheep. APMIS, 110, 23-32.

Vascellari M, Aufiero GM, Nonno R, Agrimi U, Vaccari G, Basilicata L, Falcaro C, Mancin M, Marcon S and Mutinelli F, 2005. Diagnosis and PrP genotype target of scrapie in clinically healthy sheep of Massese breed in the framework of a scrapie eradication programme. Arch Virol, 150, 1959-1976.

Vidal E, Tortosa R, Costa C, Benavides J, Francino O, Sanchez-Robert E, Perez V and Pumarola M, 2008. Lack of PrP(sc) immunostaining in intracranial ectopic lymphoid follicles in a sheep with concomitant non-suppurative encephalitis and Nor98-like atypical scrapie: a case report. Vet J, 177, 283-288.

Wells GA, Konold T, Arnold ME, Austin AR, Hawkins SA, Stack M, Simmons MM, Lee YH, Gavier-Widen D, Dawson M and Wilesmith JW, 2007. Bovine spongiform encephalopathy: the effect of oral exposure dose on attack rate and incubation period in cattle. J Gen Virol, 88, 1363-1373.

Westaway D, Zuliani V, Cooper CM, Da Costa M, Neuman S, Jenny AL, Detwiler L and Prusiner SB, 1994. Homozygosity for prion protein alleles encoding glutamine-171 renders sheep susceptible to natural scrapie. Genes Dev, 8, 959-969.

WHO (World Health Organisation), 2010. WHO Tables on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies. Available online at: www.who.int/bloodproducts/tablestissueinfectivity.pdf.

A. APPENDIX I: ATYPICAL SCRAPIE

Field Atypical/Nor98 scrapie cases (n=14) collected in 4 different countries (Portugal, Norway, France and UK) were investigated for the presence of PrP^{Sc} and infectivity in lymphoid tissues and central nervous system. Sheep harboured various PRP genotypes including those associated with high susceptibility to Atypical/Nor98 scrapie (homozygote or heterozygote $A_{136}F_{141}R_{154}Q_{171}$– AHQ) or to resistance (homozygote and heterozygote ARR) to Classical scrapie or BSE (Elsen et al., 1999). Amongst the fourteen cases six were identified by the active surveillance program either at rendering plant or slaughter house and eight through the passive surveillance network (clinical suspects). Three of these cases were identified in apparently healthy animals collected in three independent flocks where an Atypical/Nor98 scrapie cases had previously been identified (secondary cases - Portugal). Tissues were collected and handled in order to avoid cross contamination.

In each of these natural Atypical/Nor98 cases, PrP^{Sc} accumulation could be detected in the different brains by Western Blot (WB) and Immunohistochemistry (IHC). Conversely, no abnormal PrP deposits or infectivity were evidenced in any of the investigated lymphoid organs. Lymphoid tissues (spleen and/or mesenteric LN or retropharyngeal LN) from the seven UK sheep (six of which were clinical suspects) all gave negative results in bioassay in Tg338 mice.

Table 29: End-point titration of 10% (weight/volume) brain homogenates from five Atypical/Nor98 scrapie affected sheep in Tg338 mice.

Dilution	AHQ/AHQ Atypical/Nor98 scrapie (case 14)		ARR/ARR Atypical/Nor98 scrapie (case 15)		AFRQ/AFRQ Atypical/Nor98 scrapie (Case 1)		AFRQ/ARQ Atypical/Nor98 scrapie (Case 8)		AHQ/AHQ Atypical/Nor98 scrapie (Case 9)	
	Positive mice	Incubation period	Positive mice	Incubation period	Positive mice	Incubation period	Positive mice	Incubation period	Positive mice	Incubation period
neat	7/7	224+/-10	10/10	184+/-4	6/6	209+/-12	6/6	204+/-5	6/6	219+/-4
10^{-1}	ND		ND		ND		ND		ND	
10^{-2}	ND		ND		ND		ND		ND	
10^{-3}	ND		ND		ND		ND		ND	
10^{-4}	6/6	258+/-18	6/6	272+/-23	ND		ND		ND	
10^{-5}	6/6	294+/-41	6/6	300+/-17	6/6	308+/-34	6/6	321+/-28	6/6	315+/-51
10^{-6}	6/6	329+/-34	5/6	311+/-43	5/6	353+/-75	3/6	334+/-77	6/6	345+/-55
10^{-7}	2/6	360-412*	1/6	392*	1/6	451*	0/6	>550	2/6	368-532*
10^{-8}	1/6	392*	0/6	>720	0/6	>650	0/6	>550	0/6>	>620
Infectious titre (ID_{50} IC tg338/g)	$10^{9.5}$		$10^{9.1}$		$10^{9.1}$		$10^{8.7}$		$10^{9.5}$	

Table 30: Infectivity in central nervous system, lympho-reticular system of natural Atypical/Nor98 scrapie incubating or affected animals bearing various genotypes at codons 136, 141, 154 and 171 of the PRP gene.

Case	Genotype	Origin	Age	Category	Tissues	PrP^{Sc}	Tg338 transmission		Estimated infectious titre ($IC\ ID_{50}$ in Tg338/g)
							Positive mice	Incubation period in days (mean +/-SD)*	
1	AFRQ/AFRQ	FR	6 years	clinical	Brainstem	pos	6/6	256+/-14	$10^{5.5}$
					Prescapular LN	neg	3/5	334+/-10	$10^{3.4}$
2	ALRQ/ARR	PT	6 years	fallen stock*	Cortex	pos	6/6	231+/-17	$10^{6.7}$
					Retropharyngeal LN	neg	5/6	349+/-76	$10^{2.9}$
3	ARR/ARR	PT	8 years	fallen stock*	Cortex	pos	6/6	221+/-19	$10^{6.7}$
					Retropharyngeal LN	neg	4/6	348+-37	$10^{2.9}$
4	AFRQ/VRQ	PT	9 years	fallen stock*	Cortex	pos	6/6	239+/-28	10^{6}
					Retropharyngeal LN	neg	0/6	>650	
5	AHQ/AHQ	NO	3.5 years	clinical	Cerebellum	pos	5/5	224+/-21	$10^{6.7}$
					Parotid LN	neg	2/2	285-298*	$10^{4.4}$
6	AHQ/AHQ	NO	7.5 years	slaughter	Cerebellum	pos	5/5	248+/-15	$10^{5.8}$
					Popliteal LN	neg	1/10	401*	$\leq 10^{1.4}$
7	ARR/ARR	NO	7 years	slaughter	Cerebellum	pos	5/5	279+/-46	?
					Thoracic spinal cord	neg	5/5	346+/-85	$10^{2.9}$
					Prescapular LN	neg	0/6	>650 d	

* Additional case in a flock where a first Atypical case had already been identified

Table 31: Infectivity in peripheral tissues of experimentally challenged (intracerebral route) Atypical scrapie cases as assessed in mice over-expressing the ovine VRQ allele (Tg338)

TSE isolate	Case	Case origin	Genotype	Tissue	Bioassay results		
					Positive mice	Incubation period in days (mean +/-SD)	Estimated infectious titre (IC ID_{50} in Tg338/g)
Atypical/Nor98 scrapie	1	Experimental intracerebral	AFRQ/ARQ	Cerebellum	6/6	204+/-5	$10^{8.5}$
				Ileal LN	0/6	-	
				Prescapular LN	1/5	445*	$\leq 10^{1.4}$
				Brachial nerve	3/5	369+/-20	$10^{2.5}$
				External ocular muscle	5/6	409+/-66	$10^{2.3}$
	2	Experimental intracerebral	AHQ/AHQ	Cerebral cortex	6/6	219+/-4	$10^{8.3}$
				Ileal LN	0/6		
				Sciatic nerve	3/6	516+/-133	$10^{2.7}$
				External ocular muscle	2/6	370-450*	$\leq 10^{2.3}$

B. APPENDIX II: EPIDEMIOLOGY OF SCRAPIE

1. CLASSICAL SCRAPIE

1.1. Description of the prevalences

In sheep, the overall apparent prevalence of TSE cases compatible with Classical scrapie, excluding Cyprus and Slovenia which presented very specific situations, decreased from 2002 to 2009 (Figure 3). This apparent decrease should be mitigated for some countries since Atypical scrapie could have been merged with Classical scrapie in the first years of surveillance in some countries and the segregation of the two types of scrapie could result in an apparent reduction of prevalence of Classical scrapie. However, the trend was also confirmed at least in France where all historical samples that could have been Atypical scrapie were retested for classification (Fediaevsky et al., 2008).

Although this trend may not be similar in all the member states (Del Rio Vilas et al., 2007; Fediaevsky et al., 2008), it indicates that exposure to infected animals should be assessed only with recent data.

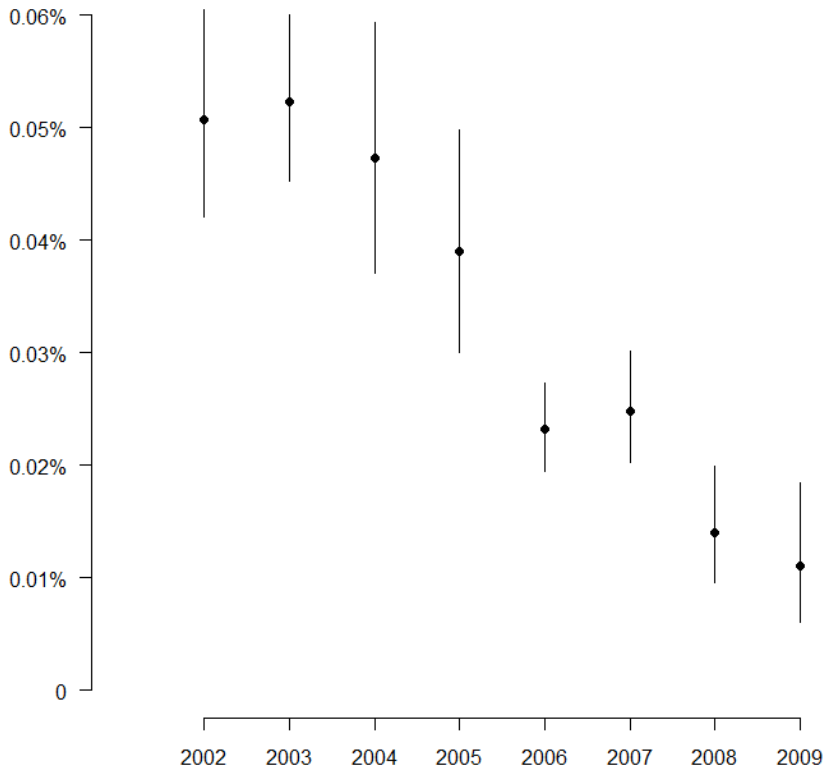

Figure 6: Apparent prevalence of Classical scrapie in healthy slaughter sheep in Member states (except Cyprus and Slovenia) from 2002 to 2009 (Source: European Commission), dots represent the prevalence rates and bars represent the 95% confidence intervals

From 2007 to 2009, 22 countries detected TSE cases in sheep compatible with Classical scrapie[19] for a total number of 3920/1,671,064 cases (0.23%, 95%CI: 0.227 – 0.242) among them 481/735,061 cases (0.07%, 95%CI: 0.060 – 0.071) were detected in sheep slaughtered for human consumption including Cyprus and 145/721,892 excluding Cyprus because of unclassified TSE cases (0.02%, 95%CI: 0.017 – 0.024). From 2007 to 2009, the apparent prevalence rates of confirmed CS in sheep slaughtered for human consumption varied widely and for countries with detected cases it ranged from 0.003% (4/153,490) in Portugal to 3.5% (12/343) in Slovenia. The high prevalence in Slovenia raises questions about the specific situation that prevails in that country or the origin of the reported data which could cover some animals culled from known infected flocks, the prevalence rate in Cyprus is also very high compared to other countries.

During the same period, 11 countries detected TSE cases compatible with Classical scrapie in goats[20] for a total number of 2,341 /551,677 (0.42% 95%CI: 0.40 – 0.44) cases out of which 358/302,344 (0.12% 95%CI: 0.11 – 0.13) were detected at slaughterhouse. Most of the cases (90%) were detected in Cyprus and no case was detected in 2009 at slaughterhouse.

Because the number of sheep tested in Malta and of goats tested in many countries[21] is very small, the prevalence rates can hardly be interpreted (wide confidence intervals) in other terms than presence or absence of detected cases.

The active surveillance in slaughter house should be based on random selection of animals, however many biases could occur. At slaughterhouse the main biases include over or under representation of some geographical regions, non random timing of sampling such as systematic same week days for sampling, sampling rate related to flock size or commercial pattern, biases on animal age or general appearance (Del Rio Vilas et al., 2007; Fediaevsky et al., 2009; Tongue et al., 2008).

Caution should be taken to compare prevalence rates between countries as differences in the genetic and age structure of the slaughtered population could bias the estimated prevalence rates.

The age and the genotype of the tested animals were unknown but these demographic characteristics were available for most of the cases.

19 Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

20 Cyprus, Finland, France, Greece, Italy, Norway, Portugal, Romania, Slovenia, Spain, United Kingdom

21 Austria,Czech Republic, Estonia, Finland, Ireland, Latvia, Malta, Poland, Slovakia, Slovenia, Sweden

1.2. Demographic characteristics of the cases

Tested animals should be over 18 months which, in practice, is verified through dentition examination but the distribution of the dentition of the tested population, which could be used as a proxy for age, was unavailable from the data reported to the European commission.

The age of the cases was missing for 38 sheep cases. The mean age of the cases detected at slaughter house was 52 months (4 years and 3 months). 7% of the cases were 12 to 24 months old, 22% of the cases were 24 to 36 months old and 71% of the cases were older than 36 months (maximum = 71 months).

The mean age of the cases increased from the period 2002 to 2006 to the period 2007-2009

The genotype was undetermined for 98 cases. Out of the 202 cases confirmed as Classical scrapie with a known genotype, the ARQ/ARQ represented 56% of the genotypes, 12% of the cases were ARQ/VRQ, 13% of the cases were ARR/VRQ and 5% of the cases were ARR/ARQ. The VRQ/VRQ genotype represented only 1.5% of the cases.

Because of the potential confusion between Atypical scrapie and Classical scrapie before 2007, the demographic characteristics of the cases are only considered for 2007 and onwards.

1.3. Sensitivity of the detection method

All the rapid tests approved for screening presence of Classical scrapie gave satisfactory evaluation test results (EFSA, 2005b). Differences in sensitivity could exist but they are usually not reported in epidemiological studies excepting one indication of a lower sensitivity of the Biorad test in VRQ carriers (Tongue et al., 2008) which has not been confirmed elsewhere.

Although the capacity of rapid tests to detect PrP^{Sc} is excellent for clinical cases, different studies suggest that the sensitivity of this method in pre-clinically affected animals is poor because of the poor levels of PrP^{Sc} in the obex at this stage the disease (Gomez et al., 2007; Reckzeh et al., 2007).

In a recent study, the sensitivity of the diagnostic method based on testing brainstem with rapid tests was estimated among 11 goats commercial flocks in France infected with Classical scrapie and monitored during 3 years[22]. The sensitivity of the method compared with IHC/ELISA on different samples varied according to the age of the animals and was lower when clinical cases were excluded, which should be the case for animals at slaughterhouse (Table 32).

Table 32: Sensitivity of rapid tests applied to brainstems of animals of different age categories

	Age					
	1 to 2 years (n=22/17)		2 to 3 years (n=59/49)		>=3 years (n=73/55)	
	Se	95 % CI	Se	95 % CI	Se	95 % CI
Including clinical cases	22.73	[7.82 - 45.37]	37.29	[25.04 - 50.85]	57.53	[45.41 - 69.03]
Excluding clinical cases	0.00	[0.00 – 10.51]	24.49	[13.34 – 38.87]	43.64	[30.30 – 57.68]

In goats, the sensitivity of the detection method on brainstem sample varied with the age of the animal, in association with the duration of the incubation period. In sheep, the length of the incubation period is strongly, but not only, determined by the genotype. Therefore assessment of the rapid tests

22 Andreoletti et al., PrioNet conference 2009, Banf, Canada. Available on-line at: www.neuroprion.org/en/home.html

sensitivities should be estimated for different genotypes at different stages of the incubation period. Such results are not currently available.

Animals should be tested from 18 months old. The distribution of animals between 18 months and 24 months old and over 24 months old is unknown but since animals slaughtered over 12 months are usually culled animals, one could expect that most of the tested animals are over 24 months, in France this age category represents less than 5% of the tested animals (Fediaevsky, unpublished data).

Since no accurate estimate is available, a general approximation of the sensitivity of the rapid tests is chosen at 50% for both sheep and goats.

1.4. Heterogeneity of Classical scrapie prevalence

Classical scrapie is a contagious disease that naturally transmits within infected flocks and that spreads between flocks mostly because of introduction of infected animals. As a result, the distributions of the prevalence rates are not homogenous within countries and cluster in flocks and in some areas (Green et al., 2007; Hoinville et al., 2000; Tongue et al., 2006). The within flock prevalence in infected flocks can vary widely and frequently reach more than 10% of the animals.

The prevalence of clinically and sub-clinically affected animals in Classical scrapie-affected flocks has been investigated by different research groups for several flocks in different European countries (Caplazi et al., 2004; Ersdal et al., 2003; Georgsson et al., 2008; Jeffrey et al., 2002; Langeveld et al., 2006; Ligios et al., 2006; Reckzeh et al., 2007; Roels et al., 1999; Thorgeirsdottir et al., 2002; Tongue et al., 2006; Vascellari et al., 2005). For these flocks, the prevalence varied between 3% (Jeffrey et al., 2002) and 41% (Ligios et al., 2006). Evidence for similar prevalence differences were obtained from affected goat herds (Gonzalez et al., 2009).

These variations depend on many different factors such as the genetic structure of the population at flock and country level, the time since the index case was occurred, the size of the flock, the farming practices, and the strain of scrapie. This heterogeneity of distribution is also reflected at the geographical level with areas of higher risk within countries and between countries as reflected by the different prevalence rates of Classical scrapie estimated by active surveillance.

2. ATYPICAL SCRAPIE

2.1. Description of the prevalences

Based on rapid tests recommended for detection of Atypical scrapie in brainstem samples, the apparent prevalence of Atypical scrapie at slaughterhouse from 2007 to 2009, ranged from 0.005 (1/18,321) in Greece to 0.23 (32/13,706) in Hungary (see Table 33) in sheep and from 0.005 (2/38,161) in France to 0.015 (3/17,205) in Portugal in goats. The overall prevalence of Atypical scrapie was estimated to 8 per 10,000 tested sheep.

Table 33: Apparent prevalence of Atypical scrapie at slaughter house from 2007 to 2009 in sheep and in goats (Source: European Commission)

Member State	Number of cases reported as Atypical scrapie	Number of rapid tests recommended for the detection of Atypical scrapie on brainstem samples	Prevalence rate (per 10,000 tests)	95% confidance interval
Sheep				
Belgium	8	6757	11.8	5.1 ; 23.3
Bulgaria	2	26154	0.8	0.1 ; 2.8
France	46	88213	5.2	3.8 ; 7.0
Greece	1	18321	0.5	0.0 ; 3.0
Hungary	32	13706	23.3	16.0 ; 32.9
Italy	21	62750	3.3	2.1 ; 5.1
Norway	14	26794	5.2	2.9 ; 8.8
Portugal	145	153490	9.4	8.0 ; 11.1
Sweden	4	7146	5.6	1.5 ; 14.3
United Kingdom	69	49838	14.0	10.9 ; 17.7
Goats				
France	2	38161	0.5	0.1 ; 1.9
Italy	2	14069	1.4	0.2 ; 5.1
Portugal	3	17205	1.7	0.4 ; 5.1

A recent study has shown that both peripheral (lymphoid organs, nerves and muscles) tissues from Atypical scrapie tested negative for PrP^{Sc} (as assessed by OIE validated WB method, immunohistochemistry and EU validated rapid tests) could contain significant level of infectivity according to Tg338 bioassay (up to 10^6 infectious units) (Lacroux et al., 2010).

These results strongly support the contention that PrP^{Sc} based diagnostic assays have limited performance for identifying Atypical/Nor98 scrapie cases. It is consequently highly probable that a significant number of Atypical/Nor98 cases remains undetected, leading to an underestimation of Atypical/Nor98 scrapie incidence in small ruminant population.

It is however not possible on the sole basis of these elements to evaluate the magnitude of this underestimation.

There are an important number of caveats to consider in the estimation of the epidemiological situation of Atypical scrapie in Europe.

First, the European TSE surveillance program in small ruminants has not been designed for Atypical scrapie surveillance. On one hand, the clinical surveillance is impaired by the clinical features, less obvious than in Classical scrapie (Konold et al., 2007b), and generally described in old animals (Benestad et al., 2008) which reduce the probability for clinical observations. On the other hand, the active surveillance system is based on screening brainstem sample with rapid tests for the detection of PrP^{Sc} whereas i) brainstem sample is inconstantly associated with the detection of PrP^{Sc} in infected animals (Benestad et al., 2008), ii) not all the approved rapid tests are recommended for the detection of Atypical scrapie in brainstem sample (EFSA, 2005b), iii) some recent results by Lacroux et al. (Lacroux et al., 2010) indicate that PrP^{Sc} could remain undetected in infectious tissues suggesting that any diagnostic method based on PrP^{Sc} detection could result in false negative results.

Second, reporting of Atypical scrapie is incomplete. The classification has been issued in 2005 (EFSA Panel on Biological Hazards, 2005), and Atypical scrapie has been considered in EU regulation only since 2007[23]. The reporting format of surveillance data to the Commission by the different Members States is not completely standardized and some of the data reported by some countries present inconsistencies such as different rapid tests reported for positive cases and surveillance figures or unknown case type even after 2006. In addition, the different demographic structures of sheep and goats populations in terms of genetic and age at sampling make difficult comparison between countries.

Third, because the active surveillance of TSE in small ruminants presents known numerous selection biases which are not specific to Atypical scrapie. At slaughterhouse the main biases include over or under representation of some geographical regions, non random timing of sampling such as systematic same week days for sampling, sampling rate related to flock size or commercial pattern, biases on animal age or general appearance.

The first group of limits to estimate epidemiological situation of Atypical scrapie conduct to underestimate the prevalence. The second and third groups of limits can generate biases in both ways.

Therefore because of these important limits, the results of the TSE surveillance give indications on the epidemiology of Atypical scrapie but cannot be used to derive an accurate level of prevalence in animals slaughtered for human consumption.

Some studies have described the apparent prevalence of Atypical scrapie in sheep slaughtered for human consumption (healthy slaughter) or collected as fallen stock, based only on tests recommended for the detection of Atypical scrapie on brainstem samples. The underestimated apparent prevalence of Atypical scrapie in sheep did not present important variations between countries (Fediaevsky et al., 2008) nor in time (Fediaevsky et al., 2008; McIntyre et al., 2008). Assuming that the bias conditions remain comparable in space and time, the distribution of the prevalence of Atypical scrapie appeared relatively homogenous especially compared with the prevalence of Classical scrapie. In a study including 11 European countries that reported Atypical scrapie between 2002 and 2007, the mean prevalence of Atypical scrapie was 5.5 (5.0-6.0) cases per ten thousand in abattoir surveillance and 8.1 (7.3-9.0) cases per ten thousand in fallen stock (Fediaevsky et al., 2010).

According to the data transmitted to the European Commission, to date, 18 European countries have reported Atypical scrapie in sheep[24] and only 4 in goats[25]. As in previous studies, the detection of Atypical scrapie cases was strongly associated with the use of rapid tests recommended for detection of Atypical scrapie in brainstem samples. Since 2007 among the 27 Member States, in sheep, only 12 cases / 393,014 tests were associated with tests not recommended for detection of Atypical scrapie in brainstem samples, while 680 cases / 1,030,545 tests were associated with tests recommended for detection of Atypical scrapie in brainstem samples.

In addition, from 2007 to March 2010, the apparent prevalence of Atypical scrapie in healthy slaughtered sheep was similar or higher than the prevalence of Classical scrapie in healthy slaughtered sheep in 8 countries[26] suggesting that Atypical scrapie represents a significant proportion of TSE infected small ruminants that enter into the EU food chain.

23 Commission Regulation (EC) No 727/2007 of 26 June 2007 amending Annexes I, III, VII and X to Regulation (EC) No 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible spongiform Encephalopathies. OJL 165/8, pages 1 – 13.

24 Belgium, Bulgaria, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Netherlands, Norway, Poland, Portugal, Slovakia, Spain, Sweden, United Kingdom

25 France, Italy, Portugal, Spain

26 Bulgaria, France, Hungary, Italy, Norway, Portugal, Sweden, United Kingdom

Some demographic data were considered for Atypical scrapie cases detected from 2007 to March 2010.

The Atypical scrapie sheep cases were in average 76 months old and 50% of the cases were detected between 49 and 98 months old. However, it is not possible to estimate apparent prevalence according to age categories since age of tested animals is not available.

The data on genotype were missing for more than 55% of the cases and had no information on the 141 codon for 95% of the 88 cases carrying an ARQ allele. The information on the codon 141 is also missing in the genotype from random sampling for genotyping.