

SCIENTIFIC OPINION

Opinion of the Scientific Panel on Biological Hazards on the human and animal exposure risk related to Transmissible Spongiform Encephalopathies (TSEs) from milk and milk products derived from small ruminants¹

(Question No EFSA-Q-2008-310)

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SUMMARY

Following a request from the European Commission (EC), the Panel on Biological Hazards (BIOHAZ) was asked to deliver a scientific opinion on the Human and animal exposure risk related to Transmissible Spongiform Encephalopathies (TSEs) from milk and milk products derived from small ruminants.

In a recent scientific article from Konold *et al.*, published on 8 April 2008 in BMC Veterinary Research, on "Evidence of scrapie transmission via milk" it is concluded that: "...*there is a risk of the transmission of scrapie from ewe to lamb via milk or colostrum. Infection of lambs via milk may result in shedding of the infectious agent into the environment...*".

The BIOHAZ Panel was invited to provide an opinion on the conclusions from the article of Konold *et al.* (2008), and if considered necessary, based on any additional available scientific data, to update the current risk assessments on the human and animal exposure related to Transmissible Spongiform Encephalopathies (TSEs) from milk and milk products derived from small ruminants.

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When approaching the mandate the BIOHAZ Panel did not consider the zoonotic potential of small ruminant TSE agents. This aspect is considered in detail in previous EFSA documents^{2,3}. The TSE agents considered in the assessment were Classical scrapie, Atypical scrapie and BSE. Moreover, the assessment was performed employing mainly data from TSE in sheep, which were considered valid also for TSE in goats due to the lack of more specific data in that species.

The Panel considered valid the conclusion of the article of Konold *et al.* (2008). Expanding the article of Konold *et al.* (2008), another study from Lacroux *et al.* (2008) independently demonstrated that Classical scrapie can be transmitted from susceptible ewe to transgenic mice via colostrum and milk. It was emphasized that both studies were designed to achieve the highest possibility of transmission success and that this could differ from the field situation. The Panel noted that in both studies, milk from asymptomatic donor ewes transmitted disease, indicating that clinically healthy, Classical scrapie-incubating sheep may shed the causal agents of these TSEs in milk. Moreover, the level of prion infectivity in small ruminant milk could become higher during the course of mastitis but the somatic cell count was considered as an unreliable indicator for presence or absence of TSE infectivity in small ruminant milk.

The Panel concluded that the use of milk and milk products from a flock with Classical scrapie may carry a TSE exposure risk for humans and animals. Furthermore, the use of milk and milk products from the general small ruminant population may carry a TSE exposure risk for humans and animals due to the presence of undetected affected flocks in that population. However, because of the difference in scrapie prevalence between affected flocks and the general small ruminant population, the risk of exposure for humans and animals associated with milk and milk products from the general small ruminant population will be lower than the risk from detected scrapie affected flocks.

The Panel also concluded that the exposure to a Classical scrapie agent via milk of an infected animal can be estimated to be 4 to 5 \log_{10} lower than the infectivity found in the same weight of brainstem from a terminally affected animal, and 2 to 3 \log_{10} lower than infectivity found in the same weight of lymphoid tissues from an animal incubating scrapie or from a clinically affected animal.

The BIOHAZ Panel further noted that no information is available concerning the presence of infectivity or PrP^{Sc} in colostrum or milk from small ruminants affected by Atypical scrapie or BSE. However, the Panel emphasized that due to the early and progressive peripheral tissue dissemination of the BSE agent in experimentally infected susceptible sheep, the occurrence of infectivity in colostrum and milk of BSE infected susceptible small ruminants would be likely. On the other hand, the apparent restricted dissemination of the agent of Atypical scrapie in affected individuals could limit its transmissibility through milk.

As there is large variation between MS in prevalence of scrapie and production of small ruminant milk, the human and animal exposure associated with small ruminant dairy products varies greatly between MS.

² 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* (2007) 466, 1-10

³ 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* (2008) 626, 1-11

The Panel further concluded that breeding of sheep for relative resistance to Classical scrapie according to the previous EFSA opinion⁴ can be expected to reduce human and animal exposure associated with small ruminant dairy products.

The Panel recommended to perform research in order to characterise the exposure risk via milk especially in Atypical scrapie and BSE in small ruminants, to investigate on the stability of prion infectivity in milk during further processing, and to obtain more data to confirm and expand the preliminary information available on the quantitation of infectivity levels in small ruminant milk fractions.

Key words: TSE, milk, small ruminants, exposure risk

⁴ Opinion of the Scientific Panel on Biological Hazards on “the breeding programme for TSE resistance in sheep”, *The EFSA Journal* (2006), 382, 1-46
www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620775678.htm

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Current scientific advice on TSE infectivity in milk or milk products derived from small ruminants

The TSE infectivity in milk or milk products from small ruminants has been addressed in different scientific opinions and statements from EFSA, the last update being the “Opinion from EFSA of 25 January 2007 on a quantitative risk assessment on the residual BSE risk in sheep meat and meat products”. The main conclusion from the referred scientific opinions and statements were that there are indications that infectivity in milk from small ruminants cannot be totally excluded, however, in the light of current scientific knowledge and irrespective of their geographical origin, milk and milk products derived from small ruminants are unlikely to present any risk of TSE contamination provided that milk is sourced from clinically healthy animals.

Recent results of scientific research

In a recent scientific article from Konold *et al.*, published on 8 April 2008 in BMC Veterinary Research, on "Evidence of scrapie transmission via milk" it is concluded that scrapie can be transmitted to genetically susceptible lambs via milk. The results of the study indicate that there is a risk of the transmission of scrapie from ewe to lamb via milk or colostrum. It is also concluded that infection of lambs via milk may result in shedding of the infectious agent into the environment.

Evaluation of the overall situation by the Commission

In view of the conclusions of the mentioned article and considering its potential implications for the policies for the prevention, control and eradication of TSE in small ruminants, it seems appropriate to request the European Food Safety Authority to update the current risk assessments on the subject.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Food Safety Authority (EFSA) is invited to provide an opinion on the conclusions from the article of Konold *et al.*, and if considered necessary, based on any additional available scientific data, to update the current risk assessments on the human and animal exposure related to Transmissible Spongiform Encephalopathies (TSEs) from milk and milk products derived from small ruminants.

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ASSESSMENT

1. INTRODUCTION

1.1 Regulatory background

According to Annex VII, Chapter A, point 2.3, letter (a) of the Regulation (EC) No 999/2001 as amended (TSE Regulation) (EC, 2001) all ovine and caprine animals originating from a flock in which BSE cannot be excluded shall be killed and completely destroyed. By default, no milk and milk products of sheep and goat can be sourced from a flock where BSE cannot be excluded.

Under the current European legislative framework:

- Milk from Scrapie affected flocks is not excluded from human consumption.
- Milk from Scrapie affected flocks is not excluded from animal feeding.

1.2 Previous SSC and EFSA assessments

The previous SSC risk assessments on TSE infectivity in milk (in cattle and small ruminants) are summarised in the “*Overview of the BSE risk assessments of the European Commission’s Scientific Steering Committee (SSC) and its TSE/BSE ad hoc Group*” (SSC, 2003). The conclusions of this document as regards to milk infectivity are reported here below:

“The evidence available to date does not point at milk or colostrum representing a possible TSE risk.

However, the SSC supports the recommendation that for precautionary reasons the milk, colostrum or milk products from suspect BSE cases should not be offered for consumption.

Should BSE become probable or be found in small ruminants then a reassessment has to be undertaken.”

The main conclusion of a previous EFSA statement on the safety of goat milk and milk product is:

“...milk and milk derivatives (eg lactoferrin, lactose) from small ruminants are unlikely to present any risk of TSE contamination provided that milk is sourced from clinically healthy animals.” (EFSA, 2004).

This conclusion was considered valid in subsequent EFSA Statements and Opinions (EFSA, 2005a; EFSA, 2005d; EFSA, 2007a).

1.3 Approach to the mandate

The zoonotic potential of small ruminant TSE agents is not considered in this assessment. This aspect is considered in detail in the EFSA 2007 Opinion on “*Certain aspects related to the risk of Transmissible Spongiform Encephalopathies (TSEs) in ovine and caprine animals*” and in the EFSA 2008 Report on “*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, 2007b; EFSA, 2008a).

The TSE agents considered in this assessment are: Classical scrapie, Atypical scrapie and BSE. Scrapie is a disease of ovine and caprine animals caused by a variety of TSE agents harbouring different biological properties that are still incompletely characterised, rather than by one specific transmissible entity. 'Classical scrapie' and 'Atypical scrapie' are operational rather than purely biological terms (EFSA, 2005c; Saegerman *et al.*, 2007; Benestad *et al.*, 2008; EFSA, 2008a).

Due to the lack of more specific data on goats, this risk assessment was performed employing mainly data from TSE in sheep. However, this was considered valid also for TSE in goats.

2. DESCRIPTION OF THE ARTICLE OF KONOLD *ET AL.* (2008)

The scientific publication "*Evidence of scrapie transmission via milk*" (Konold *et al.*, 2008) describes a study performed to investigate the possible transmission of scrapie infection from ewe to lamb through milk or colostrum.

In that study 18 genetically highly susceptible (VRQ/VRQ) lambs were fed milk from 12 VRQ/VRQ ewes naturally infected by the scrapie agent, with 15 VRQ/VRQ scrapie-free lambs used as controls.

The ewes were kept in a flock with high incidence of scrapie and the TSE status of each ewe was determined by immunohistochemical examination of a sample of recto-anal mucosa associated lymphoid tissue (RAMALT) prior to milking (except for two ewes that had already shown signs of scrapie at that time). Three ewes had already signs of scrapie at the start of lactation, whereas 5 ewes became symptomatic at mid-lactation, and another 4 after lactation⁵.

All ewes were culled after developing definite clinical signs of scrapie. The disease was then confirmed through post-mortem laboratory examination.

Recipient lambs were born from ewes that were the offspring of ewes imported from New Zealand (NZ), where no Classical scrapie has been detected, and that were kept in a flock that had never had any cases of Classical scrapie. The TSE-free status of the dams was confirmed by post-mortem laboratory examination.

The recipient lambs were housed in medium security accommodation that had never housed any TSE affected animals before and that was decontaminated before housing these animals.

A weekly sample of the milk, after the first week of lactation, was collected for Somatic Cells Count (SCC) determination. Nine of the twelve ewes had high somatic cell counts (> 100,000 cells per ml) at some stage during lactation.

The milk and colostrum from a single ewe was used to feed one or two lambs depending on the amount available. Thus, it is not possible to know whether colostrum or milk transmitted disease. Each 'set' of lambs was housed in a separate pen. After all milk had been consumed, the lamb sets were mixed and received milk replacer not containing any animal-derived product except for bovine whey protein. They were weaned at approximately six weeks of age, when they were provided with water and hay *ad libitum* and a daily ration of concentrate feed.

Ten VRQ/VRQ lambs that were born from ewes from the same NZ-derived flock and that were housed with their dams in the same building at around the same time as the recipient

⁵ Clarification provided by Dr. Timm Konold on 10 September 2008

lambs but in separate pens were used to check for environmental contamination (building controls).

Moreover, in order to investigate whether lateral transmission could have occurred after mixing, 5 lambs from the NZ-derived flock were introduced to the weaned recipient lambs (lateral transmission controls). Due to late availability of these lambs, they were mixed between one and three months after mixing of the milk recipient lambs.

As a result of intercurrent diseases, three recipient lambs were culled at 43, 44 and 105 days of age and one building control lamb was culled at 38 days of age. The immunohistochemical examination of the distal ileum of the two recipient lambs culled at 43 and 44 days of age revealed PrP^{Sc} accumulation in lymphoid follicles.

At approximately 7 months of age, all remaining recipient lambs were positive for PrP^{Sc} presence by immunohistochemical examination of RAMALT. None of the control lambs had detectable PrP^{Sc} presence in the RAMALT at the same time.

A second RAMALT immunohistochemical examination conducted in the control lambs from a biopsy taken approximately three months later showed PrP^{Sc} presence in 2 out of the 5 lateral transmission controls, whereas the building controls remained PrP^{Sc}-negative.

The authors concluded that:

“These results indicate that there is a risk of the transmission of scrapie from ewe to lamb via milk or colostrum. Infection of lambs via milk may result in shedding of the infectious agent into the environment as we also observed infection of other lambs raised using scrapie-free ewes and then mixed in with the scrapie milk recipients. The study continues with the remaining lambs kept alive to assess if clinical disease develops, and further milk collection and feeding is planned to improve our estimate of the risk of transmission.”

The complete article is freely available at: www.biomedcentral.com/1746-6148/4/14

Konold (2008) has recently reported the confirmation of these transmission results by replication of this study without mixing milk recipients. Seven pairs of VRQ/VRQ lambs were fed milk from each of seven dams incubating Classical scrapie as before but housed separately during feeding of milk and colostrum, and after weaning. RAMALT biopsy identified one or both animals of each pair positive for PrP^{Sc} at 4-5 months of age while building controls remained PrP^{Sc} negative.

2.1 Host factors/TSE agents interactions

The study of Konold *et al.* (2008) was performed using:

- VRQ/VRQ ewes as milk donors for VRQ/VRQ lambs;
- Classical scrapie only.

In sheep the VRQ allele is considered to be associated with high susceptibility to most of the currently circulating Classical scrapie agents (Thorgeirsdottir *et al.*, 1999; Tranulis *et al.*, 1999; Acin *et al.*, 2004; Billinis *et al.*, 2004). Consequently, the situation investigated here (dietary exposure of VRQ/VRQ lambs to milk/colostrum collected from VRQ/VRQ sheep, which were close from clinical scrapie onset) could be considered as an unfavourable situation.

In this study, all samples were collected from a single flock, with the implication that investigated ewes were exposed to a limited range of (if not a single) TSE agents. Interaction between the host genotype and TSE agent are known to impact on the kinetics of prion dissemination in ewes. Such interaction could influence dramatically the dynamics of infectivity shedding in mammary secretions (colostrum and milk). Consequently, caution should be taken before inferring those observations to other situations (host genotype / other TSE agent).

In the case of Classical scrapie the ARR allele carriers are considered less susceptible to infection than other genotypes (Hunter *et al.*, 1997; Elsen *et al.*, 1999; EFSA, 2006). Moreover, in case of infection those animals do not accumulate consistent levels of PrP^{Sc} in their peripheral tissues (van Keulen *et al.*, 1996; Jeffrey *et al.*, 2002; Langeveld *et al.*, 2006) and seem less likely to spread TSE agents at lambing (Andreoletti *et al.*, 2002; Lacroux *et al.*, 2007).

Detailed information on the distribution of the prion protein allele frequency in the main sheep breeds in the EU can be found in pages 80 – 84 of the “*Report on the monitoring and testing of ruminants for the presence of Transmissible Spongiform Encephalopathy (TSE) in the EU in 2003, including the results of the survey of prion protein genotypes in sheep breeds.*” (EC, 2004).

2.2 Conclusions on the article of Konold *et al.* (2008)

- The study demonstrates that Classical scrapie can be naturally transmitted from susceptible ewe to susceptible lamb via colostrum and/or milk.
- It is not possible to conclude whether colostrum or milk transmitted disease.
- The majority of donor ewes were asymptomatic at the start of lactation.
- Some donor ewes developed clinical symptoms after lactation.
- There is no apparent correlation between somatic cell counts at any stage during lactation and Classical scrapie transmission.
- Some additional lateral transmission between milk recipient lambs may have occurred and possibly contributed to the complete attack rate.

3. OTHER STUDIES ON SCRAPIE TRANSMISSION THROUGH MILK

Apart from the Konold study another source of data related to PrP^{Sc} and infectivity presence in milk was recently made available. This study was carried out in France in a naturally scrapie infected flock of Langlade (Lacroux *et al.*, 2008) (this work has been accepted for publication in Plos Pathogens - www.plospathogens.org/home.action).

In this study, PrP^{Sc} was identified in lacteal ducts and mammary acini lumen in sheep incubating Classical scrapie and harbouring ectopic lymphoid follicles typical of Maedi-Visna virus (MVV) infection; all donor ewes were positive for MVV by PCR. Such PrP^{Sc} deposits were observed in ewes bearing different PrP genotypes (ARQ/ARQ, ARQ/VRQ and VRQ/VRQ).

No PrP^{Sc} deposits were observed in mammary glands of:

- Controls ewes (New Zealand VRQ/VRQ TSE sheep).
- Ewes infected with MVV but not having developed ectopic mammary follicles
- Ewes bearing ARR/xxx and ARQ/ARQ genotypes when no PrP^{Sc} deposits were detected in lymphoreticular system, even when ectopic lymphoid follicles consecutive to MVV infection were observed in udder.

These observations confirm and expand a previous study, which reported presence of PrP^{Sc} in mammary gland parenchyma from sheep incubating natural scrapie and harbouring ectopic lymphoid follicles (Ligios *et al.*, 2005).

In order to characterise presence of infectivity in colostrum and milk, samples were collected in 13 individuals and tested by intra-cerebral bioassay in tg338 mice (which over express the VRQ ovine PrP variant). Ewes were either ARQ/VRQ or VRQ/VRQ scrapie incubating (INRA Langlade flock). The bioassay was carried out after fractionation of the milk into three different components: cell pellet, casein whey and cream. The animals were all sampled during their first lactation (age at lactation start 13-15 months). In the Langlade flock, scrapie clinical onset in VRQ/VRQ animals is observed between 19 and 22 months old, while in ARQ/VRQ it is between 26 and 36 months old.

This experiment, which is still incomplete, has already allowed confirmation of the presence of infectivity in both colostrum and milk. To date, transmission was observed with samples of milk and colostrum collected in 10 of the animals (including ewes bearing both ARQ/VRQ and VRQ/VRQ genotypes). Milk was fractionated in cell pellet, casein whey and cream. All three fractions were found positive. The considered milk samples had low somatic cell counts (<10⁴/ml).

Despite the lack of detectable PrP^{Sc} in mammary glands of ewes that did not harbour ectopic lymphoid follicles in this tissue, infectivity was detected in both colostrum and milk collected in 3 ewes with histologically healthy mammary glands.

3.1 Quantitation of infectivity

The incubation period observed in tg338 mice inoculated with samples of milk and colostrum was compared to that corresponding to a 1/10 serial dilution (end titration 10^{6.8} ID50/g) of a brainstem homogenate from a Langlade flock scrapie affected animal at terminal stage of the disease. This comparison allowed a provisional estimate of the infectious dose (ID50) of these samples. The range of infectious titre in one ml of milk from ewes without MVV mammary lesions (sterile milk and a somatic cell count < 10⁴ Cells/ml) was equivalent to that observed

from 0.2 to 6 µg of brainstem from a terminally affected scrapie sheep (this corresponds to 1.3 to about 40 IC tg338 ID50/ml as compared to 10^{6.8} IC tg338 ID50/g in the reference brainstem homogenate). Due to the low number of samples for which definitive results are available at the moment, these values should be considered with caution.

To put this information into perspective, the infectivity found in a litre of sheep milk would be comparable to the infectivity that can be found in about:

- 0.01 – 0.1 grams of brainstem material from a terminally affected sheep.
- 0.5 g of lymphoid tissue from 5 months and older Classical scrapie incubating sheep.

3.2 Conclusions on the study by Lacroux *et al.* (2008)

- The study confirms and expands the findings of Konold *et al.* (2008) study that Classical scrapie can be experimentally transmitted from susceptible ewe to transgenic mice via colostrum and milk.
- Both colostrum and milk, and three components of the latter (cell pellet, casein whey and cream), are able to transmit disease.
- The donor ewes were asymptomatic during lactation (clinical onset up to 20 months after the start of first lactation).
- The somatic cell count was low in samples that transmitted disease.
- While all donor ewes had detectable PrP^{Sc} in mammary tissue only when ectopic lymphoid follicles of MVV infection were present, infectivity of milk was also found in ewes with histologically healthy mammary glands.
- The level of infectivity per litre of milk was equivalent to that found in 0.01 – 0.1 grams of brainstem material from a terminally affected sheep. Moreover, the infectivity found in a litre of sheep milk would be comparable to the infectivity that can be found in about 0.5 g of lymphoid tissue from 5 months and older TSE incubating sheep.
- In agreement with the authors, since no major involvement of lymphoreticular system is usually observed in heterozygous ARR animals affected by Classical scrapie, ewes bearing such genotype could be considered less at risk to shed prions in milk. Therefore, breeding of sheep for relative resistance to Classical scrapie according to the previous EFSA opinion (EFSA, 2006) can be expected to reduce human and animal exposure associated with small ruminants dairy products.

3.3 Epidemiological studies on the role of milk replacers

Only one published study examined the risk of contamination of flocks by scrapie associated with milk replacer use in sheep flocks (Philippe *et al.*, 2005). This case-control study was conducted in France between 1999 and 2000. Ninety-four scrapie affected and 350 control flocks were matched by location and main breed. A statistically significantly higher risk of being a case flock was associated with the use of milk replacers of unknown composition. However, a potential confounding effect of concentrate feeds and the contamination of fat used in milk replacers could not be excluded. Moreover, the exact composition of milk

replacers in terms of species of origin is unclear.⁶ Because epidemiological studies on the subject remain limited, caution should be taken in drawing conclusion from this single study.

Available information received from FEFAC and EDA⁷ indicate that:

- There is no data on the batch sizes.
- The goat milk whey is almost exclusively used for direct pig feeding whereas the ewes' milk whey can also be used in the formulation of milk replacers intended for mammalian farm animals (including ruminants).
- The overall production of milk replacers from ewe's milk whey powder seems to be very low. A rough estimate provided by FEFAC experts would indicate that less than 5,000 tons of whey powder from ewe milk is produced in the EU and used by the EU milk replacers industry, compared to approximately 700,000 tons of whey powder from cow milk used every year.
- There is no information about the use of milk sourced from scrapie affected flocks. Since there are no legal restrictions on its use in the EU, it can be assumed that it is used.

4. GENERAL CONSIDERATIONS ABOUT TSE INFECTIVITY IN MILK AND MILK PRODUCTS DERIVED FROM SMALL RUMINANTS

4.1 Role of mastitis

Mastitis is a clinical or sub-clinical inflammation disorder of the mammary gland due to different causes, such as bacteria, viruses, fungi and endotoxins.

The major types of bacteria involved in subclinical ovine mastitis are coagulase-negative staphylococci (Haenlein, 2002; Bergonier and Berthelot, 2003; Bergonier *et al.*, 2003; Leitner *et al.*, 2004), which are found on the skin of the udder and in the contacting environment.

Among viruses, the lentivirus Maedi-Visna Virus (MVV) is known to cause lymphoproliferative changes with diffuse interstitial infiltrate and/or peri-ductal follicle-like aggregations in the mammary gland. Such infections have a subclinical course with secretion of apparently normal milk (Anderson *et al.*, 1985; Molen *et al.*, 1985).

Most cases of subclinical mastitis cannot be diagnosed grossly, therefore their condition can be determined only by bacteriological testing, and/or indirect tests, such as SCC, the California Mastitis Test (CMT), or N-Acetyl- β -D-Glucosaminidase (NAGase) activity (Bergonier *et al.*, 2003; Leitner *et al.*, 2004).

SCC mainly considers the leukocytes in milk since the proportion of epithelial cells is less than 2-3% in bacteriologically negative sheep milk (Bergonier and Berthelot, 2003; Bergonier *et al.*, 2003).

The different sub-populations of leukocytes constituting SCC in bacteriologically negative milk are neutrophils (10-35%), macrophages (45-85%) and lymphocytes (10-17%) (Bergonier *et al.*, 2003). The leukocytic composition of milk from infected udder varies, and neutrophils become the major cell type having a high correlation with the SCC (Gonzalo and Gaudioso,

⁶ Letter of FEFAC to EFSA received on 26 September 2008.

⁷ Letter of FEFAC to EFSA received on 26 September 2008. Communications received from EDA on 7 and 10 October 2008.

1985; Moroni and Cuccuru, 2001). Hence, the increase of SCC in bacterial mastitis is mainly due to a high migration rate of neutrophils into the udder (Bergonier *et al.*, 2003; Leitner *et al.*, 2004).

Sheep milk SCC varies in a number of different situations. SCC rises during the first 3 weeks and at the end of the lactation (Bergonier *et al.*, 2003; Rosati *et al.*, 2005); also daily fluctuations of cell count have been described (Gonzalo *et al.*, 1994). Moreover, SCC is influenced by other several factors such as intra-flock factors and breed (Gonzalo *et al.*, 2005). Considering that exhaustive individual SCCs are rarely available, the analysis of bulk milk SCCs could be an easy way to estimate the whole flock mammary infectious status.

There are data available with regard to the threshold by which it can be possible to calculate a presumed prevalence of mastitis within the flocks. For instance in French flocks an increase of 100,000 cells per ml in bulk milk was associated with a rise of mastitis prevalence of 2.5% (Bergonier *et al.*, 2003). In Spain, flocks with 250,000 and 1 million cells per ml of milk had a mastitis prevalence of 16% and 35%, respectively (Romeo *et al.*, 1998). Thus, in contrast to cattle milk, there is no strict correlation between SCC and bacterial mastitis in small ruminants.

In ovine mammary gland affected by sub-clinical mastitis, SCC has been reported as efficient in diagnosing bacteriological infection (Gonzalez-Rodriguez *et al.*, 1995; Bergonier and Berthelot, 2003), although another study of 492 milk samples from ewes during a period of 10 weeks of lactation did not demonstrate any valid association between positivity for bacteria by culture and SCC (Hariharan *et al.*, 2004).

Given that a high number of non-pathological factors could potentially influence the SCC in sheep, different threshold values for discriminating between infected and uninfected udders have been reported. In French flocks the cut-off is considered to range from 100,000 to 250,000 cells/ml of milk, while in Croatian, Spanish and Italian flocks it was established at 250,000 cells/ml (Pengov, 2001), 300,000 cells/ml (Gonzalez-Rodriguez *et al.*, 1995) and 265,000 cells/ml (Rosati *et al.*, 2005), respectively.

In the former Italian survey (Rosati *et al.*, 2005) it was reported that the SCC mean in bulk milk, from different sheep farms, was 1,333,000 cells/ml and the threshold of 265,000 cell/ml, which was capable to discriminate between infected and uninfected udders, had a sensitivity of 30% and a specificity of 86%. These data raise doubts on the validity of this indirect diagnostic tool.

So far, studies have focused on the correlation between SCC and bacteriological examination, whereas no data are available with regard to the characterization of histological damage in the mammary gland parenchyma and its correlation with SCC values.

4.1.1 Usefulness of SCC as mastitis indicator in respect to TSE risk

Monocytes, macrophages and dendritic cells are considered as cells that can potentially carry PrP^{Sc} (Manuelidis *et al.*, 2000; Aucouturier *et al.*, 2001; Mabbott and Bruce, 2001; Shlomchik *et al.*, 2001; Huang *et al.*, 2002). In a study in sheep naturally co-infected by both scrapie and lympho-follicular mastitis due to Maedi-Visna, PrP^{Sc} accumulation was detected in lymphoid follicles adjacent to milk ducts (Ligios *et al.*, 2005). Therefore, at least in natural scrapie in sheep, mastitis may lead to prion accumulation in the mammary gland. Moreover, during subclinical or clinical mastitis the blood-milk barrier could be damaged and TSE infectivity in small ruminants could pass directly from blood to milk (EFSA, 2005a).

As consequence of the increasing presence in milk of these above mentioned inflammatory cells or/and the formation of peri-ductal lympho-follicular structures in which PrP^{Sc} may replicate, it is reasonable to suspect that the risk of prion infectivity in small ruminant milk potentially can become higher during the course of mastitis.

Since increase of the SCC is believed to indicate bacteriological infections (Bergonier *et al.*, 2003; Leitner *et al.*, 2004), mastitis with high SCC does not seem necessary for having prion infectivity in milk from scrapie-affected sheep. No studies have been made so far in order to define severity and characteristics of the histopathological changes that potentially can be present in these infected mammary glands. Such clarification is very important since only monocytes, macrophages and dendritic cells seem to be able to carry PrP^{Sc}, as well as only lymphoproliferative mastitis leads to intra-mammary PrP^{Sc} accumulation. Finally, no clear evidence is available for influence of MVV mastitis on SCC at a large scale; in MVV seropositive Sarda breed sheep it was reported that SCC was not influenced (Legrottaglie *et al.*, 1999).

In a previous EFSA opinion it was concluded that “...SCC cannot be relied on neither as a specific indicator for TSE nor as an indicator of udder health.” (EFSA, 2005a) in goats.

Therefore, on the basis of all the above mentioned knowledge and on the experimental data currently available, the SCC is an unlikely indicator in small ruminant milk for TSE risk.

4.2 Dynamics of the infection and milking career

Data available clearly established that prion infectivity shedding in small ruminants milk can be observed as early as in the first lactation period. This is of fundamental importance for TSE risk assessment, since all sheep in a flock that are incubating scrapie can potentially spread prion infectivity by milk during lactation.

A risk assessment of milk infectivity at flock level should take into account.

- Prevalence of scrapie within a flock.
- Length of the incubation period of scrapie.
- Number of lactations during the incubation period of scrapie.
- The age and genotype structure of a flock.
- Duration of the lactation and production level.
- Management of the flock.

In Classical scrapie infection usually occurs around birth and clinical scrapie manifests in sheep most frequently between 2 and 5 years of age (Detwiler and Baylis, 2003). Ewes usually have the first lamb at 15-18 months old, and thus can shed prion infectivity by milk for more than one lactation.

Cases of scrapie in animals less than 18 months of age are not common, whereas cases of scrapie can occur in animals older than 60 months (Detwiler and Baylis, 2003).

The majority of animals in a dairy flock are between 2 to 5 years old, since at these ages they have the highest milk production performance. A quantitative assessment of the infectivity that would enter into the food chain associated with milk would have to take into account the fact that the 2 to 5 years of age group has the highest prevalence of subclinical and clinical scrapie.

The EU active surveillance programme for TSE in small ruminants is based on the testing of a limited number of sheep and goats randomly selected at rendering plants and slaughterhouses. This programme was implemented to provide a basis for evaluating the evolution of the TSE prevalence over the years. Tests that are carried out are based on the detection of PrP^{Sc}, the currently only known biochemical marker for TSE, in the posterior brainstem of animals older than 18 months. Due to the pathogenesis of the infection (early contamination with involvement of lymphoid tissues and late neuroinvasion phase) a scrapie incubating animal can be found negative with such test.

The combination of :

- the apparent low prevalence of TSE in the general population;
- the number of tests which are carried out versus the total number of small ruminants;
- the limits of the tests for establishing the true infectious status of an individual

results in the lack of detection of a part of the TSE affected flocks. According to some models, about 10% of the affected flocks would be detected (Hopp *et al.*, 2003).

The present active surveillance by *post-mortem* screening of the obex using rapid testing for PrP^{Sc} contributes to the identification of the presence of a TSE in a given flock and of the assessment of the TSE status of the general population but is intrinsically unable to detect all affected flocks (see the two previous paragraphs). Hence, small ruminants could potentially excrete prion infectivity by milk during more than one lactation before becoming positive in the obex.

4.3 Infectivity of sheep milk in respect to distinct TSE agents

4.3.1 Classical scrapie

The Lacroux *et al.* (2008) and Konold *et al.* (2008) studies were carried out using colostrum and milk from two independent naturally Classical scrapie affected flocks. No comparison between the TSE agents involved was performed. As scrapie is a disease of ovine and caprine animals caused by a variety of TSE agents harbouring different biological properties that are still incompletely characterised, rather than by one specific transmissible entity (EFSA, 2008a), it is unknown whether other Classical scrapie agents would behave in the same way.

4.3.2 Atypical scrapie

No information is available concerning the presence of infectivity or PrP^{Sc} in colostrum or milk from sheep affected by Atypical scrapie. This lack of data limits the possibility to produce, at this stage, a risk assessment for this particular TSE agent.

However, some specific epidemiological and biological elements differentiating Atypical scrapie from Classical scrapie agents should be considered.

In Classical scrapie affected flocks, consistent numbers of secondary cases are usually identified and the prevalence of the infection can range from 3% to more than 40% (EFSA, 2008b), while in Atypical scrapie affected flocks, identification of secondary cases remains rare (see section 4.5). These observations support the notion of a limited (if any) inter-individual transmission in Atypical scrapie affected flocks.

Moreover, while in Classical scrapie affected sheep preclinical involvement of peripheral tissue is widespread, the apparent restricted dissemination of the Atypical scrapie agent in affected individuals could limit its transmissibility through milk.

However, in the current state of knowledge the existence of Atypical scrapie infectivity spreading through colostrum or milk, with all inferring consequences in term of animal health and human exposure, cannot be ruled out.

A current project in Europe funded by the Food Standard Agency (FSA M03058, Infectivity and abnormal PrP in tissues from sheep exposed to Atypical scrapie) addresses the question of presence of infectivity in colostrum and milk from Atypical scrapie contaminated sheep. However, considering the probable duration of such experiment, no specific results are expected to become available before several years.

4.3.3 BSE

The specific question of presence of infectivity in milk and colostrum of BSE infected sheep is currently investigated in the QLK-CT-01-309 EU funded project (BSE in sheep). Colostrum and Milk samples were collected from ARQ/ARQ ewes orally infected with BSE. This particular genotype is supposed to be more susceptible to BSE than VRQ allele carriers (Foster *et al.*, 1993). Samples were fractionated and inoculated into tg mice models (tg sheep XI, which over-express the ARQ ovine allele, and tg110 mice, which over-express the Bovine PrP gene). At this stage of the project, all colostrum and milk fractions were inoculated into the mice. Results from this experiment should become available within the next one or two years.

Experimental BSE in sheep has a similar pathogenesis to that of Classical scrapie. In both cases an early and progressive peripheral tissue dissemination of the infectious agent is observed in the affected individuals. Moreover, under experimental conditions inter-individual transmission of BSE in sheep has been reported (Bellworthy *et al.*, 2005).

To date, only a single case of natural BSE has been identified in a goat and none in sheep.

Considering the early and progressive peripheral tissue dissemination of the BSE agent in experimentally infected susceptible sheep, the occurrence of infectivity in colostrum and milk of BSE infected susceptible small ruminants would be likely.

4.4 TSE infectivity of milk in respect to goats

Currently data available with regard to goat milk and colostrum are extremely limited.

Milk samples from two scrapie incubating goats (on the basis of tonsil biopsy) were collected in a field flock affected by Classical scrapie in France (Lacroux *et al.*, 2008). Shortly after milk sampling both animals were killed, and a low PrP^{Sc} amount was detected in obex (by both IHC and ELISA) of one of the two animals, which suggests that these individuals were around or below the first half of the incubation period of the disease. Except the biochemical PrP^{Sc} profile (classical three band profile / exclusion of BSE cases based on the discriminating assays), no data is currently available concerning the nature of the involved TSE agent. Milk fractions (cell pellet, casein whey and cream) were inoculated into tg338 mice (n=6 per fraction). Mice were monitored for 650 days post inoculation and killed without showing any TSE clinical signs. In the brain and spleen from one of the six mice inoculated with the cell fraction prepared from one of the two goats, PrP^{Sc} was detected by western blot. No other mouse was found positive for PrP^{Sc} presence.

This experiment provides a proof of concept for the presence of infectivity in goat milk at the preclinical stage of Classical scrapie. However, because of its imperfect design (short duration of mice observation, likely low infectious titre and low number of investigated samples) no conclusion should be drawn at this stage concerning (i) the level of infectivity, (ii) the frequency of infectivity shedding, and (iii) the absence of infectivity in other milk fraction than cellular pellet.

Since these first results were obtained, two new experimental projects were designed to specifically address infectivity presence in colostrum and milk in goats. The projects started respectively at the end of 2006 and in early 2007. No result should be expected before the year 2010.

The first experiment deals with Classical scrapie and is conducted in France (INRA/AFSSA funded project 'Genetics and pathogenesis of scrapie in goats'). Goats bearing different polymorphisms (chosen because they could potentially influence susceptibility to TSE in this species) were orally challenged with natural goat scrapie isolate. Presence of TSE infectivity in colostrum and milk will be investigated by bioassay in the most appropriate mouse model for the chosen isolate.

The second experiment is conducted in the framework of the EU funded project 'Goat BSE' FOOD-CT-2006-36353. In this project presence of infectivity in colostrum and milk collected from goats orally challenged with BSE (first and second passage) will be assessed by bioassay.

4.5 Prevalence of TSEs in flocks

It is not possible to predict the prevalence of Classical scrapie in an affected flock but observation carried out in naturally infected flocks indicate that prevalence can range from 3% to more than 40% (EFSA, 2008b).

According to surveillance data the TSE prevalence at animal level in the general EU small ruminant population is estimated to be 0.1% in healthy slaughtered and 0.17% in fallen stock sheep, and 0.05% in healthy slaughtered and 0.14% in fallen stock goats (data received from the European Commission on 5 September 2008).

The information about the prevalence of Atypical scrapie in an affected flock is scarce. In the flocks submitted to extensive TSE examination in the context of culling, one or two additional cases are occasionally observed (Benestad *et al.*, 2003; Onnasch *et al.*, 2004; Konold *et al.*, 2007; Luhken *et al.*, 2007). These epidemiological evidences suggest that either Atypical scrapie is not transmissible by direct contact between sheep, or that the transmission rate under natural conditions is low (Fediaevsky *et al.*, 2008). It was recently reported that the prevalence of Atypical scrapie in Europe is remarkably homogeneous as compared to Classical scrapie, and that the annual prevalence estimates of Atypical scrapie vary from 0.1‰ in healthy slaughter stream in 2004 in Switzerland to 2.5‰ in fallen stock in 2003 in Great Britain (Fediaevsky *et al.*, 2008).

As regards to BSE only one case in a goat has been identified so far in small ruminants. The case was reported in 2005 (EC, 2005a; Eloit *et al.*, 2005) and identified by the retrospective examination of a sample taken from a dead goat in 2002, which was born before the European total feed ban of 2001 (EFSA, 2005b). There is currently a BSE suspect in a Scottish goat under examination (CRL_STEG, 2008b). Also this suspect was detected through a retrospective examination of a sample taken from a dead goat in 1990, born in 1987 before the first ruminant feed ban was implemented in the UK.

During examination of goat tissues from animals in a goat herd culled following confirmation of scrapie in Great Britain, another suspect case was recently identified by discriminatory IHC, Western Blot and ELISA tests where BSE could not be excluded. This case has been referred for strain typing bioassay as required by EU regulation 36/2005 (EC, 2005b) and new information on its characteristics should be available in two years (CRL_STEG, 2008a).

4.6 Classical scrapie infectivity level in milk from the general EU small ruminant population

Using data received from the European Commission (EC) on 5th September 2008 on TSE surveillance in healthy slaughtered sheep and goats in the EU Member States (MS) during the period 01/01/2002 – 27/08/2008 the upper 95% confidence level of the overall scrapie prevalence was calculated by species and EU MS.

The data obtained does not allow discriminating between Classical and Atypical scrapie. For that reason the data for scrapie in the EU, when used as indicator for Classical scrapie, could be seen as an overestimation.

On the other hand, an underestimation of the Classical scrapie prevalence in healthy slaughtered small ruminants is expected when screening the brainstem of these animals using a rapid test for PrP^{Sc}, because it is considered as a poor indicator for the absence of TSE infection in small ruminants peripheral tissues (EFSA, 2008b).

In order to get a rough estimate of scrapie prevalence across the EU despite of the limitations mentioned, the scrapie prevalence level was calculated through the data provided by the EC and was assumed to be the Classical scrapie prevalence in healthy slaughtered sheep and goats by EU MS (Table 1).

Table 1. Estimated scrapie prevalence, as upper 95% confidence level, in healthy slaughtered sheep and goats by MS (period 2002 – to August 2008; some MS may not have data for the complete period)

MS	Healthy slaughtered sheep			Healthy slaughtered goats		
	N° of Tests	TSE cases	Prevalence as upper 95% CI	N° of Tests	TSE cases	Prevalence as upper 95% CI
Austria*	7,476	0	0.00%	1,209	0	0.00%
Belgium*	18,605	5	0.05%	2,145	0	0.00%
Bulgaria [§]	28,768	2	0.02%	6,390	0	0.00%
Cyprus [†]	13,065	708	5.81%	12,273	325	2.93%
Czech republic [°]	2,764	2	0.17%	171	0	0.00%
Denmark*	5,444	1	0.05%	1,058	0	0.00%
Estonia [†]	5,736	0	0.00%	95	0	0.00%
Finland*	9,666	1	0.03%	791	0	0.00%
France*	440,142	279	0.07%	358,626	9	0.00%
Germany*	99,617	31	0.04%	8,531	0	0.00%
Greece*	70,874	150	0.25%	29,289	6	0.04%
Hungary [†]	16,240	6	0.07%	409	0	0.00%
Ireland*	204,493	43	0.03%	23	0	0.00%
Italy*	217,854	142	0.08%	82,630	26	0.04%
Latvia [†]	2,283	0	0.00%	48	0	0.00%
Lithuania [†]	6,705	0	0.00%	168	0	0.00%
Luxembourg*	1,498	0	0.00%	1,216	0	0.00%
Malta [†]	115	0	0.00%	5	0	0.00%
Netherlands*	96,837	127	0.15%	65,534	0	0.00%
Poland [†]	7,022	0	0.00%	31	0	0.00%
Portugal*	255,474	205	0.09%	27,993	0	0.00%
Romania [‡]	25,999	8	0.05%	457	0	0.00%
Slovakia [°]	13,454	19	0.20%	14	0	0.00%
Slovenia [§]	900	3	0.71%	73	0	0.00%
Spain*	197,303	97	0.06%	108,834	9	0.01%
Sweden*	22,154	8	0.06%	285	0	0.00%
United Kingdom*	206,920	190	0.10%	5,589	1	0.05%

* start of surveillance 01/01/2002

§ start of surveillance during 2002

° start of surveillance during 2003

† start of surveillance during 2004

‡ start of surveillance during 2006

Information related to ewes' and goats' milk produced on farm in EU were obtained from Eurostat on 22/08/2008. These data do not include milk directly suckled by lambs or kids at farm level. The figures related to 2006 (the most complete) are reported in Table 2. To support the reliability of the Eurostat figures, data has been obtained also from FAO (<http://faostat.fao.org>): the results from these two sources were very similar.

Table 2. **Sheep and goats milk production in thousands of tons by MS in 2006 (source Eurostat: http://epp.eurostat.ec.europa.eu/portal/page?_pageid=1090_30070682,1090_33076576&_dad=portal&_schema=PORTAL)**

MS	Quantity of milk produced (thousands of tons) in 2006			Percentage of the overall EU small ruminant milk production
	Sheep	Goats	Total amount small ruminants	
Austria	8.2	13.7	21.9	0.5%
Belgium	nd	nd	nd	na
Bulgaria	107.5	102.3	209.8	4.4%
Cyprus	15.5	23.7	39.2	0.8%
Czech republic	nd	nd	nd	na
Denmark	0	0	0.0	0.0%
Estonia	nd	0.5	*0.5	*0.0%
Finland	0	nd	*0.0	*0.0%
France	270.3	593.5	863.8	18.1%
Germany	nd	35	*35.0	*0.7%
Greece	662.7	427	1,089.7	22.8%
Hungary	2.6	4.4	7.0	0.1%
Ireland	nd	nd	nd	na
Italy	548.3	53.0	601.3	12.6%
Latvia	nd	3	*3.0	*0.1%
Lithuania	nd	6.7	*6.7	*0.1%
Luxembourg	nd	0.8	*0.8	*0.0%
Malta	1.8	1.3	3.2	0.1%
Netherlands	nd	140.5	*140.5	*2.9%
Poland	1	20	21.0	0.4%
Portugal	99.8	29.5	129.3	2.7%
Romania	436.0	215	651.0	13.6%
Slovakia	8.7	0.1	8.8	0.2%
Slovenia	0.4	1.4	1.8	0.0%
Spain	438.7	503.7	942.4	19.7%
Sweden	0.0	0.0	0.0	0.0%
United Kingdom	0.0	0.0	0.0	0.0%
Total	2,601.6	2,175.1	4,776.6	100.0%

nd: not declared, na: not applicable

* this figure could be higher as “not declared” data does not necessarily imply zero production

The Eurostat figures show large differences by country: about 87% of the production is concentrated in Greece, Spain, France, Romania and Italy, and another 10% is produced in Bulgaria, the Netherlands and Portugal.

For further estimation, the following assumptions were that:

- the prevalence of scrapie does not differ between dairy animals and the general small ruminants adult population;
- the milk production does not differ between scrapie incubating animals and the general small ruminant adult population.

The Classical scrapie infected quantity of small ruminant milk produced by MS were estimated with these assumptions based on the data provided in Tables 1 and 2. This quantity is equivalent to the total amount of small ruminant milk produced times the estimated Classical scrapie prevalence by species and MS. This estimate is reported in Table 3.

Table 3. Estimated quantity (in thousands of tons) of milk produced by scrapie-infected animals by MS in 2006

MS	Estimated quantity of Classical scrapie infected milk produced (thousands of tons) in 2006			Percentage of the overall EU small ruminant Classical scrapie infected milk production
	Sheep	Goats	Total amount small ruminants	
Austria	0.00	0.00	0.00	0.0%
Belgium	na	na	na	na
Bulgaria	0.02	0.00	0.02	0.4%
Cyprus	0.90	0.69	1.59	33.8%
Czech republic	na	na	na	na
Denmark	0.00	0.00	0.00	0.0%
Estonia	na	0.00	*0.00	*0.0%
Finland	0.00	na	*0.00	*0.0%
France	0.19	0.02	0.22	4.6%
Germany	na	0.00	*0.00	*0.0%
Greece	1.63	0.16	1.78	37.8%
Hungary	0.00	0.00	0.00	0.0%
Ireland	na	na	na	na
Italy	0.42	0.02	0.44	9.3%
Latvia	na	0.00	*0.00	*0.0%
Lithuania	na	0.00	*0.00	*0.0%
Luxembourg	na	0.00	*0.00	*0.0%
Malta	0.00	0.00	0.00	0.0%
Netherlands	na	0.00	*0.00	*0.0%
Poland	0.00	0.00	0.00	0.0%
Portugal	0.09	0.00	0.09	1.9%
Romania	0.23	0.00	0.23	4.8%
Slovakia	0.02	0.00	0.02	0.4%
Slovenia	§0.00	0.00	§0.00	0.1%
Spain	0.26	0.07	0.33	6.9%
Sweden	0.00	0.00	0.00	0.0%
United Kingdom	0.00	0.00	0.00	0.0%
Total	3.75	0.97	4.72	100.0%

na: not applicable

* this figure could be higher as “not declared” data related to sheep or goat milk does not necessarily imply zero production (see Table 2)

§ the estimated quantity of Classical scrapie infected milk produced was in reality about 0.003 thousands of tons. However, this value has been rounded off to the second decimal place for consistency within the table.

Using above data for further estimation, the following assumptions were that:

- after collection the Classical scrapie infected milk is homogeneously mixed with the non-infected small ruminant milk produced at MS level;
- the level of Classical scrapie infectivity in milk is the same in sheep and goats;
- according to the preliminary data provided in section 3.1 (Quantitation of infectivity) the possible level of Classical scrapie infectivity in small ruminant milk from clinically healthy animals was considered to possibly range between:
 - Low: 1.3 IC tg338 ID50/ml of milk;
 - High: 40 IC tg338 ID50/ml of milk.

The total number of Classical scrapie infectious doses in the total small ruminant milk production by species and MS was estimated by multiplying the estimated quantity of Classical scrapie infected small ruminant milk produced (as provided in Table 3) by the level of Classical scrapie infectivity in small ruminant milk. Then, the Classical scrapie infectious doses per kg of small ruminant milk by species and MS can be estimated, dividing the total number of Classical scrapie infectious doses in the total small ruminant milk production by the total kg of small ruminant milk produced.

This estimate is provided in Table 4, which reports the Classical scrapie infectivity level per kg of milk as IC tg338 ID50 and considers two different scenarios to take into account the assumed range of Classical scrapie infectivity in small ruminant milk.

Table 4. **Estimated Classical scrapie infectious doses per kg of small ruminant milk by species and MS.**

MS	Estimated infectious doses per kg of sheep milk (IC tg338 ID50 /kg)		Estimated infectious doses per kg of goat milk (IC tg338 ID50 /kg)	
	Scenario I:	Scenario II:	Scenario I:	Scenario II:
	low infectivity level	high infectivity level	low infectivity level	high infectivity level
Austria	0.0	0.0	0.0	0.0
Belgium	na	na	na	na
Bulgaria	0.2	6.6	0.0	0.0
Cyprus	75.5	2,322.9	38.1	1,172.9
Czech republic	na	na	na	na
Denmark	*0.7	*21.7	0.0	0.0
Estonia	na	na	0.0	0.0
Finland	*0.4	*12.2	na	na
France	0.9	28.3	0.1	1.7
Germany	na	na	0.0	0.0
Greece	3.2	98.2	0.5	14.8
Hungary	0.9	26.6	0.0	0.0
Ireland	na	na	na	na
Italy	1.0	30.4	0.6	17.4
Latvia	na	na	0.0	0.0
Lithuania	na	na	0.0	0.0
Luxembourg	na	na	0.0	0.0
Malta	0.0	0.0	0.0	0.0
Netherlands	na	na	0.0	0.0
Poland	0.0	0.0	0.0	0.0
Portugal	1.2	36.5	0.0	0.0
Romania	0.7	20.8	0.0	0.0
Slovakia	2.7	81.9	0.0	0.0
Slovenia	9.2	284.0	0.0	0.0
Spain	0.8	23.6	0.2	5.5
Sweden	*0.8	*24.5	0.0	0.0
United Kingdom	*1.4	*41.9	*0.7	*21.2

na: not applicable

* please note for these countries that the infected milk is only directly suckled by lambs or kids at farm level since there is no production of sheep or goat milk for other purposes (see Table 2)

When reading Table 4 the previously outlined assumptions and the following pitfalls potentially affect both validity and usefulness of the estimates obtained:

- In EU goat and sheep milk production and transformation (cheese production) are strongly regionalised.
- While a large part of the milk production is collected and transformed by dairy factories, a part of, or in some cases, the total milk production of some flocks can enter into on-farm processing.
- In case of the presence of an infected flock in an area, the cooperative dairy collection and transformation will introduce a dilution of the infectivity contained in milk. However, no

estimation can be provided on the reduction factor that such dilution could provide. Indeed the size of the milk batches is depending on the type of production and on specific characteristics of the process that will be applied in each factory.

As previously mentioned the total output of Classical scrapie infectious doses in the total small ruminant milk production by MS can be estimated multiplying the estimated quantity of Classical scrapie infected small ruminant milk produced (as provided in Table 3) by the level of Classical scrapie infectivity in small ruminant milk.

This estimate is provided in Table 5, which reports the estimated total load of Classical scrapie infectious doses in the overall small ruminant milk production by MS as \log_{10} IC tg338 ID50. As the previous Table 4, it considers two different scenarios to take into account the assumed range of Classical scrapie infectivity in small ruminant milk.

To put this information into perspective, Table 5 also provides the equivalent of the estimated Classical scrapie infectious doses in terms of grams of brainstem material from a terminally Classical scrapie affected sheep (infectious titer $10^{6.8}$ IC tg338 ID50/g as reported in section 3.1).

Table 5. Estimated total output of Classical scrapie infectious doses in the small ruminant milk production by MS.

MS	Estimated total output Classical scrapie infectious doses in the overall small ruminant milk production (\log_{10} IC tg338 ID50)		Equivalent in grams of brainstem material from a terminally Classical scrapie affected sheep	
	Scenario I: low infectivity level	Scenario II: high infectivity level	Scenario I: low infectivity level	Scenario II: high infectivity level
	Austria	na	na	0
Belgium	na	na	na	na
Bulgaria	7.4	8.9	4	113
Cyprus	9.3	10.8	328	10,106
Czech republic	na	na	na	na
Denmark	na	na	0	0
Estonia	na	na	*0	*0
Finland	na	na	*0	*0
France	8.4	9.9	45	1,370
Germany	na	na	*0	*0
Greece	9.4	10.9	368	11,311
Hungary	6.3	7.8	0	11
Ireland	na	na	na	na
Italy	8.8	10.2	90	2,785
Latvia	na	na	*0	*0
Lithuania	na	na	*0	*0
Luxembourg	na	na	*0	*0
Malta	na	na	0	0
Netherlands	na	na	*0	*0
Poland	na	na	0	0
Portugal	8.1	9.6	19	577
Romania	8.5	10.0	47	1,440
Slovakia	7.4	8.9	4	112
Slovenia	6.6	8.1	1	18
Spain	8.6	10.1	67	2,076
Sweden	na	na	0	0
United Kingdom	na	na	0	0

na: not applicable

* this figure could be higher as “not declared” data related to sheep or goat milk does not necessarily imply zero production (see Table 2)

4.7 Classical scrapie infectivity level in milk from Classical scrapie affected flocks

In order to get a rough estimate of scrapie infectivity level in milk from Classical scrapie affected flocks, the following assumptions were made:

- according to the preliminary data provided in section 3.1 (Quantitation of infectivity) the possible levels of Classical scrapie infectivity in small ruminant milk from clinically healthy animals was considered to possibly range between:
 - Low: 1.3 IC tg338 ID50/ml of milk
 - High: 40 IC tg338 ID50/ml of milk
- according to the data provided in section 4.5 of the Opinion (Prevalence of TSEs in flocks) the possible levels of Classical scrapie prevalence in scrapie affected flocks can range between:
 - Low: 3%
 - High: 40%
- the level of Classical scrapie infectivity in milk is the same in sheep and goats;
- the Classical scrapie prevalence does not differ between lactating animals and the total animals in affected flocks.

The range of Classical scrapie infectivity in one kg of small ruminant milk from a Classical scrapie affected flock was estimated. This estimate is provided in Table 6, which reports the Classical scrapie infectivity level per kg of milk as IC tg338 ID50 and considers two different scenarios to take into account the assumed range of Classical scrapie infectivity in small ruminant milk and of Classical scrapie prevalence in affected flocks.

Table 6. **Estimated range of Classical scrapie infectivity in one kg of small ruminant milk from a scrapie affected flock**

	Scrapie prevalence	Infectious titer (IC tg338 ID50/ml milk)	Estimated infectious doses per kg of small ruminant milk (IC tg338 ID50/kg)
Scenario I: low infectivity level	3%	1.3	39
Scenario II: high Infectivity level	40%	40	16,000

4.8 General considerations about small ruminant dairy products

- The relative high cost, typical organoleptic characteristics and cultural eating habits strongly influence human consumption of small ruminant dairy products. Currently, the lack of data on the impact of such factors makes it impossible to establish human consumption patterns that would reflect the diversity of situations met in the population.
- Milk transformation processes could also impact on the infectivity content of the final product. While prion infectivity in tissues such as brain can survive high temperatures, behaviour of low titres in a fluid matrix is unknown. Moreover, while presence of prion infectivity in cream, casein whey and cellular pellet is established, no data are available on the possible concentration - elution of infectivity following processing. Specific investigations on the stability of prion infectivity in milk during further processing would be needed.

5. HUMAN AND ANIMAL EXPOSURE RISK RELATED TO TSEs FROM MILK AND MILK PRODUCTS DERIVED FROM SMALL RUMINANTS

5.1 Classical scrapie

5.1.1 Milk from the general small ruminant population

Section 4.6 details the Classical scrapie infectivity level in milk from the general EU small ruminant population, as an estimate with mentioned assumptions and potential pitfalls. With large variation between MS, the Classical scrapie infectivity level of small ruminant milk from the general small ruminant population was estimated to potentially vary between 0 and more than 2000 IC tg338 ID50 per kg.

5.1.2 Milk from infected flocks

Section 4.7 details the Classical scrapie infectivity level in milk from Classical scrapie affected flocks, as an estimate with mentioned assumptions. The Classical scrapie infectivity level of small ruminant milk from a scrapie affected flock was estimated to potentially vary between 39 and 16,000 IC tg338 ID50 per kg.

5.2 Atypical scrapie

No information is available concerning the presence of infectivity or PrP^{Sc} in colostrum or milk from small ruminants affected by Atypical scrapie. However, epidemiological observations support the notion of a limited (if any) inter-individual transmission in Atypical scrapie affected flocks. Moreover, the apparent restricted dissemination of the agent of Atypical scrapie in peripheral tissues of affected individuals could limit its transmissibility via milk.

5.3 BSE

To date, only a single case of natural BSE has been identified in a goat and none in sheep.

No information is available concerning the presence of infectivity or PrP^{Sc} in colostrum or milk from small ruminants affected by BSE. Because of the early and progressive peripheral tissue dissemination of the BSE agent in experimentally infected susceptible sheep, the occurrence of infectivity in colostrum and milk of BSE infected susceptible small ruminants would be likely.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- The BIOHAZ Panel considers the conclusions from the article of Konold *et al.* (2008) valid, namely that: “...*there is a risk of the transmission of scrapie from ewe to lamb via milk or colostrum. Infection of lambs via milk may result in shedding of the infectious agent into the environment...*”.
- Expanding the article of Konold *et al.* (2008), another study independently demonstrated that Classical scrapie can be transmitted from susceptible ewe to transgenic mice via colostrum and milk.
- Both studies were performed with donor ewes with a genotype of the highest susceptibility to Classical scrapie from two independent naturally affected flocks. This could be considered to reflect an unfavourable situation. Recipient animals were either lambs with a genotype of the highest susceptibility to Classical scrapie or transgenic mice over-expressing this ovine genotype.
- Thus both studies were designed to achieve the highest possibility of transmission success and that could differ from the field situation.
- Both colostrum and milk, and three components of the latter: cell pellet, casein whey and cream, were found to transmit a Classical scrapie TSE agent.
- In both studies, milk from asymptomatic donor ewes transmitted disease, indicating that clinically healthy, Classical scrapie-incubating sheep may shed the causal agents of these TSEs in milk.
- Milk taken from ewes with histologically healthy mammary glands was also shown to contain infectivity.
- The level of prion infectivity in small ruminant milk could become higher during the course of mastitis. However, the somatic cell count (SCC) is an unreliable indicator for presence or absence of TSE infectivity in small ruminant milk.
- The use of milk and milk products from a flock with Classical scrapie may carry a TSE exposure risk for humans and animals.
- The use of milk and milk products from the general small ruminant population may carry a TSE exposure risk for humans and animals due to the presence of undetected affected flocks in that population. However, because of the difference in scrapie prevalence between affected flocks and the general small ruminant population, the risk of exposure for humans and animals associated with milk and milk products from the general small ruminant population will be lower than the risk from detected scrapie affected flocks.
- The use in feed of milk from small ruminants incubating scrapie would give rise to both intra and interspecies dietary exposure of animals.
- Exposure to a Classical scrapie agent via milk of an infected animal can be estimated to be 4 to 5 \log_{10} lower than the infectivity found in the same weight of brainstem from a terminally affected animal and 2 to 3 \log_{10} lower than the infectivity found in the same weight of lymphoid tissues from an animal incubating scrapie or from a clinically affected animal.

- While currently data with regard to goat milk and colostrum are extremely limited, the same assumptions may apply as in sheep.
- No information is available concerning the presence of infectivity or PrP^{Sc} in colostrum or milk from small ruminants affected by Atypical scrapie or BSE.
 - Because of the early and progressive peripheral tissue dissemination of the BSE agent in experimentally infected susceptible sheep, the occurrence of infectivity in colostrum and milk of BSE infected susceptible small ruminants would be likely.
 - The apparent restricted dissemination of the agent of Atypical scrapie in affected individuals could limit its transmissibility through milk.
- As there is large variation between MS in prevalence of scrapie and production of small ruminant milk, the human and animal exposure associated with small ruminant dairy products varies greatly between MS.
- Breeding of sheep for relative resistance to Classical scrapie according to the previous EFSA opinion can be expected to reduce human and animal exposure associated with small ruminants dairy products.

RECOMMENDATIONS

- Research is needed in order to characterise the exposure risk via milk especially in Atypical scrapie and BSE in small ruminants, including the influence of the different PrP genotypes.
- Specific investigations on the stability of prion infectivity in milk during further processing are recommended.
- More data are needed to confirm and expand the preliminary information available on the quantitation of infectivity levels in small ruminant milk fractions.

DOCUMENTATION PROVIDED TO EFSA

Letter (ref. n. SANCO E.2/JOV/mfd/D(2008) 520255 dated 15/04/2008) from the European Commission with a request for a scientific opinion on the human and animal exposure risk related to Transmissible Spongiform Encephalopathies (TSEs) from milk and milk products derived from small ruminants.

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