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TAFS[1] position paper on the Safety of Milk and Milk Products with respect to prion diseases (TSEs) of domesticated ruminants

This position paper replaces two previous papers, respectively on bovine and small ruminant milk. The consolidation is intended to ensure consistency and clarity, and to eliminate perceived conflicts, especially in light of continuing research into the natural transmissibility of scrapie via milk.

The following currently available scientific evidence supports the view that Bovine Spongiform Encephalopathy (BSE) and other prion-related diseases, generally referred to as Transmissible Spongiform Encephalopathy (TSEs), are unlikely to be transmitted to humans through bovine or small ruminant milk. This position is in line with the current opinions of both the World Health Organization (WHO) and the European Commission's various scientific working groups.

As with any scientific evidence this conclusion does not absolutely ensure the absence of the TSE agent from milk, but if the agent were present in milk it would be at such a low level that the risk of transmitting the disease to human would be negligible. It is however important to remember that this paper only considers risks arising from milk and milk derived components of milk products. The latter may of course be complex mixtures, where specific risk management measures prevent the introduction of contaminated ingredients[(28)].

Historical evidence
Consideration of risks from bovine milk, in the face of the BSE epidemic, naturally took into account the results of specific research on bovine milk, along with historical research on the transmission of scrapie via milk produced by small ruminants (sheep and goats). This formed the basis of many other controls to protect consumers, most notably the designation of Specified Risk Materials[(28)].

Infection and pathogenesis with scrapie in sheep is different from BSE in cattle. There is extensive involvement of various systems and organs in small ruminants, while the distribution of infectivity in cattle is more limited[(28)]. Therefore, extrapolations from ovine TSE studies to BSE have limited validity. Nevertheless the following scientific evidence did not indicate the potential for ovine milk to

[1] TAFS is an international platform created by a group of scientists, food industry experts, animal health regulators, epidemiologists, diagnosticians, food producers, and consumers. Its purpose is to establish and maintain lines of communication for the dissemination of reliable information to the public that can maintain confidence in the safety of food with regard to Transmissible Animal Diseases (TAD).

be the route of transmission of TSE to other species. This route could however be valid for transmission of scrapie or other TSE forms to offspring (see below):

- Ovine colostrum and mammary gland from high-risk ewes in the preclinical phase of scrapie failed to show detectable infectivity in susceptible mice that were injected intra-cerebrally[14].
- Hourrigan[16, 17] failed to detect infectivity in mice by injecting by various routes milk and colostrum from sheep and goats naturally-infected with scrapie.
- It is well known, however, that the lymphatic system is involved in TSE pathogenesis in sheep, as it has been demonstrated by the detection of both PrP^{Sc} and infectivity during early preclinical diseases stages[1, 15, 23, 25, 31, 32, 27, 28]. This pathogenesis is different from cattle[11, 28, 34, 35].
- PrP^{Sc} was identified in mammary glands of sheep affected by scrapie and mastitis caused by a lentivirus. PrP^{Sc} localizes to macrophages and follicular dendritic cells[21]. The mastitis in this study was defined by histopathological changes and not by the conventional clinical or diagnostic assays.
- An EFSA[7] interpretation of available science at the time concluded, in a statement that "infectivity in the milk from small ruminants cannot be totally excluded", but that "milk and milk derivatives from small ruminants are unlikely to present any risk of TSE contamination provided that milk is sourced from clinically healthy animals".

Bovine milk and BSE

Research into the pathogenesis of BSE has been extensive, but due to the long time scales involved in both natural and experimental infections, progress was relatively slow. In common with research into other prion diseases, molecular markers were therefore occasionally used as surrogates for the presence of infectivity. The key molecular marker is a molecule referred to as PrP^{Sc}, an abnormally folded version of a normal protein called PrP^{C}[2]. The detection of PrP^{C} indicates no more than a potential to support the presence of PrP^{Sc}. The detection of PrP^{Sc} suggests the likely presence of infectivity. Recent scientific publication data indicated the presence of PrP^{C} in bovine milk[13, 22]. It is to be expected that PrP^{C} will be found in milk as it is a protein found in many tissues. Indeed, it would be a surprise if it had not been detected in milk.

[2] **The relationship of prions, PrP^{c} and PrP^{Sc}** :

- PrP^{C} is a naturally occurring protein in vertebrates. It is present in numerous tissues (brain, muscle, heart, etc) and fluids (urine, blood, milk, etc) and plays a physiological role. It is not linked to any disease.
- Upon infection with prions, the naturally occurring PrP^{C} is converted into a pathological form (referred to as PrP^{Sc}).
- PrP^{Sc} are infectious particles causing transmissible spongiform encephalopathies (TSE) like BSE, scrapie, or Creutzfeldt-Jakob disease (CJD).
- Specific available diagnostic techniques can distinguish between PrP^{C} and PrP^{Sc} and therefore between infected animals (containing PrP^{Sc}) and normal animals with PrP^{C}

The presence of PrP^{C} is not in any way an indicator of the disease. Thus, the finding of PrP^{C} in milk does not change the previous assessment of TSE risk in milk in any way. A finding of PrP^{Sc} in milk, however, would change that assessment, but the extent of the risk would likely depend upon the level of PrP^{Sc} presence.

Bovine milk has not been found as a route of transmission of BSE:

- Milk or colostrum from clinically BSE-affected cows was collected from various stages of lactation. The milk then was injected in combined intra-cerebral and intra-peritoneal injections into mice and in a separate study was also given orally to mice. Most mice survived 650 days – none developed spongiform encephalopathy or any other specific pathology[2, 24]
- Milk, mammary glands and draining lymph nodes (supra-mammary) from clinically BSE-affected cows were fed orally to mice. None developed spongiform encephalopathy or any other specific pathology[29].
- The study funded by UK Food Standards Agency and aimed at detecting PrP^{Sc} in milk of cattle infected with the BSE agent failed to detect it in the cell fraction of milk collected at different stages of lactation in experimentally infected cows. The study examined milk from approximately mid-incubation period up to and including clinical onset of BSE, and included samples with evidence of the presence of mastitis[12]. Considering the methods used and their detection limits, it means that if infectivity were present in bovine milk, it would be at a level below the detection limit of the assay methods.
- In cattle, infectivity in lymphoid tissue has only been found in the distal ileum [30, 34] and the palatine tonsil of the tongue[11, 35]. Extra-neural involvement in BSE is relatively low. This lessens the risk of infectivity in inflamed tissues with ectopic lymphoid follicles and its secretion in milk, as compared to the situation in small ruminants.
- Observational and epidemiological evidence do not indicate milk or its products to be a risk factor in transmitting the BSE or other TSE agent:
- A significant indication that milk does not transmit the disease was the beef suckler study[3, 36, 37], where no cases were reported although all of the calves received colostrum and milk from BSE-infected cows. If there is maternal transmission via milk or colostrum, we would expect a higher incidence of BSE cases in offspring in suckler herds than those in non-BSE dairy herds.
- Although cases of BSE have been detected in the offspring of BSE cases in the UK, these have been in smaller numbers than predicted by the above study, and have most probably been caused by exposure to contaminated feed. Outside the UK, no BSE has been observed so far in the offspring of BSE cows.
- A maternal cohort study in bovines provided statistical evidence of a maternal risk enhancement[4, 5], but did not provide evidence on the risk of transmission[6], even though critics of risk management approaches in the early 1990s argued that such transmission from cow to calf was inevitable[19].

Ovine milk and TSE

Building upon the historical research referred to above, more recently new evidence has been published in relation to the potential for the transmission of scrapie via milk[18, 20]. The results, although incomplete, have enabled a review of the implications by the European Food Safety Authority[10], and prompted initial regulatory action by the European Commission.

TAFS welcomes publication of these findings. They contribute to the understanding of the mechanisms of transmission of scrapie as a prion disease of sheep and goats that has been known for over 200 years. Scrapie is not a highly contagious disease. It does not spread easily, but it is difficult to eradicate. It is known to spread between sheep, both from ewe to lamb and to other unrelated sheep and goats. The exact route of transmission has not been determined so far. There are several possible routes, which include contact with placenta of infected ewes, or possibly before birth while the lamb is still in the womb. Transmission via milk and/or uterine fluids after birth are additional possibilities. In view of the significance of these scientific advances, we provide a brief summary of each below.

Ewe to lamb transmission[18]

This study attempted to assess the scope for transmission, under **a worst-case scenario**, by collecting milk from sheep of highly susceptible genetic makeup (high risk group), at a time when they were either about to die of scrapie, or when the first clinical signs were seen. Their milk was collected and fed to lambs that were born to uninfected mothers and kept in isolation while they received the milk. These lambs were also of the most susceptible genotype (VRQ/VRQ).

The lambs have been shown to be infected by the testing of tissue samples collected either while still alive, by biopsy, or from some that had died of other diseases. At the time of publication, none have yet reached the point of clinical disease themselves, and infectivity itself has not been demonstrated. Tests have revealed the presence of abnormal prion protein (PrP^{Sc}) that is normally recognised as a marker for the presence of infectivity.

The success of the study was dependent on having scrapie-free lambs to receive the milk. Despite having fully susceptible ewes and susceptible lambs, the ease with which the lambs were infected is a surprise. The experimental design anticipated transmission to smaller numbers of lambs. For this reason lambs received both milk and colostrum (the milk produced within the first 24-48 hours after lambing) from the same ewe in order to maximise the likelihood of transmission. As a result, the authors could not conclude whether transmission occurred via colostrum, milk or both. The study is therefore being repeated, but with lambs being fed with **either** colostrum or milk.

This is important for several reasons.

- Firstly, colostrum would not be used for human consumption.

- Secondly, colostrum is rich in protein and antibodies that help to protect the lamb in the early days of life before its own immune system is fully developed. For that reason farmers sometimes collect and freeze colostrum to feed to other lambs, sometimes pooling it to feed to several lambs. This practice could increase the potential for infection of lambs at their most vulnerable time of life.

Although the study described below confirms the presence of infectivity in colostrum, it is necessary to establish the status of colostrum from sheep that are unaffected by other pathogens. In other words, the result should be dependent solely on the presence of scrapie in an otherwise healthy sheep.

Ewe to mouse transmission[20]

This study also made use of a naturally infected experimental flock of sheep in France, but differed from the previous study in that the flock was infected with both scrapie and Maedi-Visna (MV). MV is a viral disease, that has previously been shown to potentially predispose to the transmission of scrapie via milk[21]. It causes a form of mastitis (lympho-proliferative chronic mastitis) that particularly involves the formation of discrete ectopic lymphoid follicles in the mammary tissue. These have previously been shown to stain heavily for abnormal prion protein in scrapie infected sheep[21].

In the French study, the researchers compensated for some of the difficulties normally presented by mouse inoculation studies, of not being able to inoculate sufficient material into a mouse to guarantee transmission, by concentrating their starting material (colostrum and milk). In addition, they used highly sensitive mice (Tg338)[33], that were genetically modified to produce sheep prion protein of the VRQ genotype[27].

The study was supported by post-mortem examination of many sheep for the presence of abnormal PrP in the udder, and for the presence or absence of mastitis. Although the biological assay study has not been completed, it has reached a point where it is possible to conclude conclusively that particular fractions of milk are infectious.

Preliminary findings include:

- Abnormal PrP was only detected in ewes that harboured ectopic lymphoid follicles, and correlated with the detection of abnormal PrP in other peripheral lymph nodes. **This therefore limited the positivity to sheep with the most susceptible genotypes that are predisposed to peripheral infection (VRQ/VRQ; VRQ/ARQ; ARQ/ARQ).**
- In addition, abnormal PrP was detected in lacteal ducts and mammary acini suggesting a high probability that it would be excreted in milk/colostrum.
- Nevertheless, infectivity studies detected scrapie infectivity in both colostrum (collected within 12 hours of lambing) and milk (collected 20 days after lambing). The infectivity was associated with cellular, cream and casein-whey fractions.
- Most critically, infectivity was even detected in fractions derived from sheep in which there was no visible clinical or pathological evidence of mastitic lesions.
- In all samples, preliminary estimations suggest that infectivity levels are very low, but potentially higher in mastitic milk or colostrum than from healthy udders.

EFSA BioHazards Panel Opinion[10]

The findings summarised above were supported by EFSA, but the BioHazards panel stressed that in both studies conditions had been maximised to facilitate detection of infectivity. In other words, **they were worst-case scenarios, and may not be fully representative of natural infection in farm animals.** Nevertheless, the conclusion that both animals and humans were clearly at risk of exposure to scrapie infectivity via milk could not be ignored.

Scrapie prevalence varies between countries, but is so low as to represent only a small risk to consumers from national populations of small ruminants. The risk relating to flocks/herds in which scrapie has been diagnosed was, however, greater and possibly warranted specific action to limit exposure risks. This is important as past assumptions that risk management measures could be limited to the exclusion of mastitic milk[7] from the human food chain have been demonstrated to be untenable, given the findings in the French study of infectivity in milk from sheep with apparently healthy udders .

EFSA stressed that its assessment of human health risk had not changed from its previous positions in 2007 and 2008[8, 9]. As suggested by the French researchers, a combination of low infectivity levels in milk, and low prevalence of scrapie, coupled with the historical absence of a definite link between scrapie and human disease, suggested that milk from the general population of small ruminants could be considered low risk.

The BioHazards Panel could not offer specific advice with respect to risk from milk of sheep infected with BSE or atypical scrapie[26, 27], but anticipated that the recognised peripheral distribution of infectivity in some small ruminants infected with BSE could lead to the presence of infectivity in milk. Atypical scrapie has not yet been identified in peripheral tissues of infected animals.

Risk Management Options for small ruminants

Further to the EFSA and AFSSA Opinions, the EU agreed to new controls on 26 November 2008 (SANCO/3660/2008) that will be applied when legislative changes are in force. These recognise the potential role of milk in spreading classical scrapie (or BSE) between small ruminants, and are primarily intended to protect animal health. Consequently:-

- While investigations into a case of suspect TSE in a sheep or goat continue, the use of milk and milk products derived from the animals in the flock/herd in question will be restricted to that holding until confirmatory results are available.
- After confirmation that the diagnosis is of classical scrapie, milk and milk products from the flock cannot be sold for feeding to ruminant species. Sale for feeding to non-ruminants will be confined to use within the borders of the Member State concerned. These measures apply until all susceptible animals have been culled.
- A derogation allowing the deferring of culling for up to 5 years in specific circumstances has been reduced to 18 months in dairy herds/flocks.

- Imports of milk and milk products into Member States, intended for feeding to ruminants, will need to be subject to additional certification relating to the scrapie status of the flock/herd of origin.

These represent only a part of the raft of regulatory actions that apply to classical scrapie affected flocks or herds in Europe. The culling of genetically susceptible sheep or all sheep or goats in the flock/herd, and voluntary programmes of breeding for resistance in sheep are also involved. If BSE cannot be excluded, the flock or the herd must be culled and any milk or milk products on the holding destroyed. The new measures do highlight however that the focus of attention is on the protection of animal health, by reducing opportunities for the spread of infection. Realistically, it is probable that the effect of the measures will vary significantly from country to country, depending on the prevalence of scrapie, the number of small ruminant dairy herds/flocks and the extent to which milk from sheep and goats is sold for animal feed. In many countries small ruminant milk production is only a minor component of industry objectives.

TAFS Position – overall risk from TSEs in milk

Scrapie is not recognised as a risk to humans, although this cannot be ruled out with certainty. The risk to humans from scrapie, and the scientific uncertainties that underpin any statement on risk, have been discussed at length in the EFSA Opinions cited. Since there is no established evidence to date that scrapie poses a risk to human health, the finding that infectivity is present in milk of scrapie-infected animals does not give any reason to change our view that ovine and caprine milk are safe for human consumption.

These results do not at the moment have any direct implications with respect to the risk from BSE in milk from cattle. Although an equivalent study has not been conducted in cattle, other studies attempting to find infectivity in bovine milk have not succeeded. Proving the total absence of infectivity is extremely difficult. The evidence for the absence of natural spread of BSE between cattle, from cow to calf or between unrelated cattle does however suggest that even in natural equivalent of this experiment, the feeding of calves on cows' milk, transmission has not occurred, or does so only rarely. Consequently, cows' milk is unlikely to carry BSE infectivity that might put consumers at risk. Furthermore, the control measures that have been put in place to eradicate BSE, and protect consumers in the interim, are succeeding in reducing numbers of infected cattle year by year, and the prevalence of BSE in cattle in all countries where BSE has been detected is extremely low.

In conclusion, the studies, and their interpretation by EFSA, help us to better understand the epidemiology of scrapie and exposure risks faced. Despite the precautionary impetus for additional measures to further strengthen animal health protection in regard to small ruminant TSEs, the question of the safety of products derived from bovine milk destined for human consumption remains unchanged.

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