



AGENCE FRANÇAISE  
DE SÉCURITÉ SANITAIRE  
DES ALIMENTS

THE DIRECTOR GENERAL

Maisons-Alfort, 8 October 2008

## OPINION

### of the French Food Safety Agency on the possible animal and public health consequences of new available scientific findings on the intra-species transmission of the classical scrapie agent by milk

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#### **Review of the request:**

In a letter dated 21 April 2008, the French Food Safety Agency consulted the TSE Scientific Panel about the work by Konold *et al.*<sup>1</sup> describing intra-species transmission of the sheep scrapie agent *via* milk. The Panel was asked to assess whether these works or other recent findings are liable to modify the Panel's previous recommendations both on the animal health measures towards small ruminants and the risks from human consumption of small ruminant milk.

#### **Context:**

The Panel has examined the question of the infectivity risk from small ruminant milk in several of its previous opinions (framework opinion 2001 updated in March 2005, note of 10 March 2006, opinion of 20 July 2006). The Panel stated that it was not possible to exclude infectivity in the milk of ewe's or goats suffering from TSE and predicted a low potential level of infectivity. It also described ongoing work designed to look for PrP<sup>Sc</sup> and the infectious agent in ewes' milk and where applicable to assess the corresponding levels of infectivity in ewe colostrum and milk.

Two information sources about these questions have since become available to the Panel. These are:

- studies published by Konold *et al.* describing intra-species transmission of the ovine scrapie agent *via* milk;
- work currently being published by the CEA and ENVT (Lacroux *et al.*)<sup>2</sup> teams describing (i) the presence of PrP<sup>Sc</sup> in the lactation duct lumens and mammary gland acini, (ii) the infectious nature of different colostrum and milk fractions (cell pellet, cream and complementary fraction (skimmed milk with cells removed)) in ewes incubating scrapie as a result of intracerebral inoculation in a transgenic ovine mouse.

#### **Expert assessment methods:**

The results of a study by Konold *et al.* were examined by the animal TSE epidemiological working group. The consequences on risk of transmission within affected flocks and between flocks were described in an analysis presented in the appended report of 26 June 2008.

The Panel also examined and discussed the results of the study by Konold *et al.*, together with those of the French study during its meetings on 27 May and 26 June 2008.

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<sup>1</sup> Konold T, Moore SJ, Bellworthy SJ, Simmons HA. Evidence of scrapie transmission via milk, BMC Vet. Res., 2008, 4:14.

<sup>2</sup> Data submitted for publication, presented on 8 October to the Prion 2008 Congress in Madrid (Lacroux *et al.*).

**Evidence:****1) Work by Konold *et al.***

Work conducted by Konold's team shows that there is sufficient infectivity in mammary secretions (colostrum and milk) to infect some of the exposed lambs. This new source of transmitting classical scrapie is in addition to those already identified (placenta, saliva, spread of prions in the environment, etc.). In view of the protocol used for this study, however, it is currently neither possible to provide a definitive conclusion about the effectiveness of this phenomenon nor on its relative contribution to the transmission of the disease under natural conditions.

**2) French studies<sup>2</sup>**

As could be predicted from the work by Ligios *et al.*<sup>3</sup>, recent work from two French teams has identified PrP<sup>Sc</sup> in the milk ducts and lumens of secreting acini in ewes incubating natural classical scrapie (INRA Langlade herd) with lymphoproliferative lesions (ectopic follicles) from a lentivirus infection (Maedi). This phenomenon was found in ewes with different sensitive genotypes (ARQ/ARQ, ARQ/VRQ and VRQ/VRQ).

It should be noted that no accumulation of PrP<sup>Sc</sup> could be detected in the mammary gland:

- in control animals (New Zealand genotype VRQ/VRQ ewes not infected with scrapie);
- in animals infected with the virus responsible for Maedi co-infected with scrapie but which had not developed lymphoproliferative lesions in the mammary gland;
- in ARR/xxx genotype animals and some ARQ/ARQ genotype animals in which no accumulation of PrP<sup>Sc</sup> was found in the lymphoreticular system despite the presence of numerous ectopic follicles in the mammary parenchyma.

In addition, in the same study, experimental inoculations (intracerebral route) in transgenic mice (Tg 338 over-expressing the ovine PrP VRQ allele) were performed using 13 samples of colostrum and milk obtained from ewes carrying the ARQ/VRQ and VRQ/VRQ genotypes and incubating natural classical scrapie (INRA Langlade herd). These bio-studies, some of which are still ongoing, have confirmed infectivity to be present in both types of secretions. At present the actual transmission of the disease has been seen for 10 of the 13 samples tested. These experiments were performed using three fractions of milk or colostrum: cream, the cell pellet and the complementary fraction (skimmed milk with cells removed). All three fractions were found to be infectious.

Despite the apparent absence of PrP<sup>Sc</sup> in the mammary parenchyma, infectivity was detected in colostrum and milk of ewes which did not exhibit Maedi mammary lesions at the time of their death (n=3). Within the limitations of the available data, however, the infectivity levels involved appear to be less in ewes with a healthy mammary gland than in ewes with Maedi lesions (n=6).

Quantitatively from the data currently available, the highest titre found in ewe milk (which furthermore complied with the current health criteria, with a somatic cell count of less than 10<sup>4</sup> C/ml) was estimated to be 10<sup>1.6</sup> IU50/ml (ic route). This should be compared to the 10<sup>6.6</sup> IU50 value per gram of obex (posterior cerebral trunk) found in a ewe in the terminal phase of classical scrapie (INRA Langlade herd).

<sup>3</sup> Ligios C, Sigurdson CJ, Santucci C, Carcassola G, Manco G, Basagni M, Maestrale C, Cancedda MG, Madau L, & Aguzzi A.(2005): PrP<sup>Sc</sup> in mammary glands of sheep affected by scrapie and mastitis. *Nature Med.* 11 : 1137-1138.

### **3) Considerations about caprine scrapie, BSE in small ruminants, atypical scrapie in small ruminants and classical or atypical BSE in cattle**

Little or no specific data are currently available about the presence of infectivity or pathological PrP in the milk of:

- i) goats suffering from classical scrapie;
- ii) small ruminants suffering from atypical scrapie;
- iii) small ruminants suffering from BSE;
- iv) cattle suffering from classical or atypical forms of BSE.

This lack of data clearly limits the ability to assess the specific risk of colostrum and milk from animals infected with these types of agents.

Some information on the biology of these forms of TSE can however be incorporated in the discussion:

- In terms of classical scrapie in goats or BSE in goats and sheep, the pathogenesis of the disease is similar to that seen in sheep infected with classical scrapie. As a result, the Panel considers that colostrum and milk from any small ruminants of sensitive genotype which are incubating classical scrapie or BSE is liable to contain infectivity.

- In terms of atypical scrapie in small ruminants, the limited number of secondary cases in affected herds<sup>4</sup> argues against actual dissemination of this TSE agent, particularly in milk.

- In terms of classical BSE, the low spread of the infectious agent in peripheral tissues in cattle in the incubation or clinical phase and the extremely limited number of secondary cases reported in affected herds<sup>5</sup> also suggests that the spread of the infectious agent through milk is very limited, if not zero.

- In terms of atypical BSE in cattle, in view of the almost complete absence of data on the pathogenesis of these agents in the species of interest, no conclusion can be drawn about potential presence of infectivity in the colostrum or milk of infected animals at this stage. It must be noted however, that these cases are extremely rare (in the region of one case per million animals tested in France).

### **4) Quantitative risk assessment of human exposure from milk and milk products**

Such an assessment requires quantified data on:

- the infectious load in milk;
- the effect of collection practices and milk processing technological procedures on the infectious load per metric unit of product;
- consumption of processed and unprocessed dairy products.

The French studies have identified infectivity to be present in the major fractions of milk (cream, skimmed milk with cells removed, cell pellet) and have produced quantitative data on the level of infectivity involved in each of these fractions.

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<sup>4</sup> Afssa Lyon data.

<sup>5</sup> In countries in which the prevalence of secondary cases can be estimated in the absence of total culling or culling of cohorts.

However,

- These data have only been obtained from a small number of samples, all from the same herd;
- No information is available about the impact of milk processing procedures on the levels of endogenous infectivity (the effect of heating on endogenous infectivity in the milk matrix, concentration/elution of infectivity during the different processing technological procedures).

Collective processing and collection practices for milk and the direct processing of the milk produced are liable to very considerably influence the infectious loads of milk entering the processing system where this is difficult to quantify.

Finally, quantifying exposure risks from milk or dairy products from small ruminants requires a reliable estimate of levels of consumption. Whilst data on the production flow of these products are known, there is no information available on individual consumption of these products. In view of the considerable regionalisation of the sector and the typical nature of the products, calculating an average consumption level per inhabitant would not appear appropriate.

In view of these limitations, the Panel considers that a quantitative assessment of the risk of human exposure to TSE agents from milk or dairy products cannot be performed appropriately as knowledge currently stands.

#### **Conclusions and recommendations:**

The combination of the works by Konold *et al.* and the French studies lead to an unambiguous conclusion that the infectious agent is present in colostrum and milk, which comply with all of the current health criteria, originating from ewes carrying the sensitive genotype and incubating classical scrapie. As a precaution and because of the similarities between the pathogenesis of classical sheep and goat scrapie, the Panel considers that the same health status should be applied to the colostrum and milk of goats infected with classical scrapie. The same applies in principle to colostrum or milk from small ruminants which could possibly be infected by the classical BSE agent.

There are no data available at present on the potential infectivity of milk or colostrum from small ruminants suffering from atypical scrapie or cattle suffering from atypical BSE, although the Panel does consider that the corresponding animals carry a lower risk of spread of an infectious agent responsible for TSE via milk (see chapter 5.3).

Finally, in terms of cattle suffering from classical BSE, data (see chapter 5.3) indicate that milk from these animals does not represent a source of infection to animals or humans.

According to the data from the active epidemiological surveillance programme, the prevalence of TSE in the general small ruminant population is in the region of 1 case per 1000 animals tested. High incidences have been reported in some flocks suffering from classical scrapie (up to 40% of numbers). Since its opinion of January 2007<sup>6</sup> the Panel has examined the question of increased risk of exposure, for human health, to classical scrapie agents from flocks of infected animals, compared with the general small ruminant population. The Panel considers that the conclusions of this opinion are applicable to the issue of the risk of exposure to TSE agents through colostrum and milk.

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<sup>6</sup> Afssa opinion of 15 January 2007 on the change in animal health measures in flocks of sheep and goats in which a case of classical or atypical scrapie has been detected.

As a result, the Panel distinguishes two situations in its risk assessment:

- (i) the so-called general small ruminant population;
- (ii) herds identified as being infected.

## **1) Animal health risks**

### **1.1 Herds of small ruminants not suspected of infection**

Colostrum and milk can represent an infection vector not only within flocks, but can also contribute to inter-herd transmission effects either through direct exchange of colostrum or milk or through the use of feed including milk derivatives obtained from herds of small ruminants infected with classical scrapie.

Furthermore, within current knowledge, the possibility of inter-species transmission (particularly between species of ruminants) of the classical scrapie agent through the use of milk or feed containing milk derivatives obtained from a flock of sheep suffering from classical scrapie (particularly milk replacements) cannot be excluded. The transmissibility of some classical scrapie isolates to cattle has been established through intra-cerebral inoculation, although the few available studies on the subject report no transmission of these agents after oral exposure in cattle<sup>7</sup>.

In view of the current limitations of the TSE screening systems and tools for small ruminants, only some of the affected herds are detected. As a result, even if animals from herds not suspected of infection carry a lesser theoretical risk, colostrum and milk from these herds may nevertheless represent a source of intra and inter-species exposure to a risk of TSE, particularly for ruminants in the absence of a species barrier.

In order to avoid any re-circulation of the infectious agent, the Panel recommends that the colostrum and milk from all herds of small ruminants be reserved for feeding animals from the herd and that inter-herd exchanges and marketing of milk, colostrum and their derivatives be excluded for ruminant feeds (although approved for monogastric animals).

### **1.2 Infected herds of small ruminants or those suspected of infection**

The infectivity of colostrum and milk from sheep suffering from classical scrapie is an established risk of intra-species transmission. Currently available data do not allow the extent of this source of infection to be quantified compared to the other identified sources of the infectious agent (placenta, saliva etc.), although this new finding must be considered whenever TSE is suspected in a herd.

As a result, for herds of small ruminants suspected of having TSE, the Panel recommends that :

- the colostrum and milk produced by the herd be reserved only for feeding lambs or kids from the herd.
- inter-herd exchanges and marketing of colostrum and milk obtained from these uses for feeding production animals (ruminants and monogastric animals) be suspended.
- these arrangements be continued until the result of the confirmatory test.

If infection is confirmed, the Panel recommends:

- for classical scrapie, that the measures be maintained until all of the genetically sensitive animals have been removed from the herd. After applying animal health measures, the status of the herd with regard to milk will be equivalent to that of a herd not suspected of having infection (see 1.1) ;
- for atypical scrapie, lifting the restriction measures. This final recommendation on atypical scrapie will be reconsidered depending on new knowledge about the specific form of TSE.

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<sup>7</sup> Cutlip RC (2001) Resistance of cattle to scrapie by the oral route. Can J Vet Res. 65(2):131-2.



**2) Risk of human exposure:****2.1) Herds of small ruminants not suspected of infection**

In view of:

- the low detected prevalence of scrapie (classical and atypical)<sup>8</sup> and the very low if not zero estimated prevalence of BSE<sup>9</sup> in the general small ruminant population,
- the modest levels of infectivity found until now in milk from small ruminants compared to other products liable to enter the food chain,

the Panel considers that milk from small ruminants from herds not suspected of TSE only contributes to a limited extent to the overall risk of consumer exposure to TSE agents.

It should also be noted that any transmission of TSE from small ruminants to humans is probably limited by a species barrier effect. As a result, the Panel does not recommend any restriction on the use or marketing of milk or colostrum from herds of small ruminants not suspected of TSE or for their processed products in human food.

**2.2) Infected herds of small ruminants or those suspected of infection**

In view of the high potential incidence of the disease in herds suffering from classical scrapie (up to 40% of numbers) the Panel considers that consumption of milk or milk products from these productions is liable to result in excess consumer exposure.

As a result, if TSE is suspected in a herd of small ruminants, the Panel recommends that:

- Marketing of the milk and milk products from the herd be prohibited for human foods;
- These arrangements be continued until the results of the confirmatory test.

If infection is confirmed, the Panel recommends for cases of classical scrapie that the measures be maintained until all of the genetically sensitive animals have been removed from the herd.

The Panel recommends that if atypical scrapie infection is confirmed, no restriction be made on the marketing of milk obtained from the affected herds. This position may be reassessed when new findings on the specific form TSE are available.

Finally, the Panel recalls that:

- whilst within current knowledge it is still impossible to guarantee the status of a herd in terms of classical scrapie with absolute certainty, continuation of the genetic selection policy in sheep should considerably reduce consumer exposure risks to classical TSE through this species.
- continuing a rational screening (and typing) programme for TSE agents remains essential to monitor the change in prevalence of these diseases, variations in which directly impact consumer exposure to the risk of TSE.

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<sup>8</sup>In 2007:

- Classical scrapie: at rendering plants:  $2.9 \cdot 10^{-4}$  in sheep, 0/60 000 tests in goats; slaughter:  $7.6 \cdot 10^{-5}$  in sheep,  $8.0 \cdot 10^{-6}$  in goats;
- Atypical scrapie: at rendering plants:  $7.9 \cdot 10^{-4}$  in sheep,  $6.8 \cdot 10^{-5}$  in goats; slaughter:  $5.1 \cdot 10^{-4}$  in sheep,  $1.1 \cdot 10^{-5}$  in goats (source Afssa Lyon report).

<sup>9</sup> Afssa opinion on the change in the TSE surveillance programme in small ruminants on the consumer risk of BSE of 20 July 2007.

**Main references:**

Konold T, Moore SJ, Bellworthy SJ, Simmons HA. Evidence of scrapie transmission via milk, BMC Vet. Res., 2008, 4:14.

Cutlip RC, Miller JM, Hamir AN, Peters J, Robinson MM, Jenny AL, Lehmkuhl HD, Taylor WD, Bisplinghoff FD (2001) Resistance of cattle to scrapie by the oral route. Can J Vet Res. 65(2):131-2.

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

Lacroux C1; Simon S2; Benestad S.L3; Maillet S2; Mathey J1; Lugan S1; Corbiere F1, Cassard H1; Costes P1; Bergonier D1; Weisbecker J.L4; Moldal T3; Simmons H5;Lantier F6; Feraudet C1; Morell N2; Schelcher F1, Grassi J2; Andreoletti O1 Prions in milk from ewes incubating natural scrapie Prion 2008 Madrid.

Afssa opinion of 25 March 2005 on the assessment of risk from TSE in the small ruminant sector, strengths and weaknesses of the current system (re-update of 2001 opinion).

Afssa opinion of 20 July 2006 on the assessment of risk from the potential presence of BSE in sheep.

Afssa opinion of 15 January 2007 on the change in health policing measures in herds of goats and sheep in which a case of classical or atypical scrapie has been detected.

Afssa opinion of 20 July 2007 on the change in the TSE surveillance programme in small ruminants on the consumer risk of BSE.

**Key words: TSE, milk, small ruminants**

**Report of the Animal TSE Epidemiology Working Group  
“intra-species transmission of the scrapie agent through milk”  
26 June 2008**

1. Request

In a letter dated 21 April 2008, the French Food Safety Agency drew the attention of the TSSE Scientific Panel to the works by Konold *et al.* describing intra-species transmission of the sheep scrapie agent in milk. The panel was asked to assess whether these works or other recent findings are liable to modify the panel's previous recommendations both on the animal health measures towards small ruminants and the risks from human consumption of small ruminant milk.

The TSE Epidemiological Working Group was requested to consider the epidemiological aspects of the question, focussing particularly on animal health.

This report was produced from the discussions which took place on 14 May and 11 June 2008.

2. Analysis of the results from the article by Konold *et al.*

The work presented in this article appears to have been correctly undertaken and the results appear to be valid. Three questions were nevertheless raised and posed to the authors of the article:

- Was the herd suffering from Maedi? There is no precise answer to this question as no specific in-depth follow up was conducted for the question: there was certainly no overt Maedi present in a clinical form in the herd
- Do the results concern the milk as such or were milk and colostrum mixed? Despite the fact that the test of the article stated that milk was only distributed from the second week of lactation, the authors stated that they had removed the colostrum: both milk and colostrum were administered to the lambs.
- What form of scrapie was involved? No typing was undertaken to determine whether this was classical or atypical scrapie. It is nevertheless very likely that this was classical scrapie, particularly as it had a high annual incidence in the herd (8.5%). It should be noted that the herd contained a high proportion of VRQ/VRQ sensitivity allele for research purposes.

In addition, the results only relate to VRQ/VRQ ewes, which is very unfortunate in view of the question. The current selection programme removes this type of animal although it allows animals with a resistance allele (ARR/XXX) and animal type ARQ/ARQ to be retained.

In this study, the working group noted the possibility or even ease of transmission of scrapie from colostrum and milk before the clinical phase of the disease in ewes and discussed the request from this perspective. An important question which remains unanswered is the possibility or otherwise of scrapie transmission through milk to genotypes other than the sensitive genohomozygotes.

3. Discussion of the request in terms of the “animal health” risk of transmission of (classical) scrapie in sheep

There is no previous Afssa opinion on this question.

The working group distinguished the risk of transmission within the herd from the risk of transmission between herds.

Transmission within infected herds

In terms of the risk of transmission within an infected herd it may be assumed firstly that transmission from milk is no greater than transmission from infected placentas around the time of birth and secondly that the current genetic selection programme in infected herds should reduce the risk of transmission from milk within the herd through the introduction of resistance genes: this would nevertheless assume that sensitive/resistant genotype ewes produce little/no PrPres in



milk. As a result, the working group does not see a need to change the current rules on the transmission of classical scrapie within herds of infected sheep.

The consequences on classical scrapie for which genetic determinants have not to date been identified (published) cannot be inferred from the results of the study.

#### Inter-herd transmission

In terms of inter-herd risk of transmission it appears that two findings from these new works are not currently taken into account in the regulations: the sales or removal of colostrum, milk and lactoserum from the farm and beyond this of all products from milk produced by the herd obtained from scrapie-infected herds for use by other farm animals. These two factors could represent a risk of transmitting infection outside of the herd. As a result, the working group considers that for farms suffering from scrapie, consideration be given to prohibiting all removal of colostrum or milk products to other farms or for animal feed purposes. As a precaution it proposes that this measure be applied to small domestic ruminant farms (sheep and goats) suffering from classical scrapie.

For dairy goat farming, from which in some situations surplus milk from the first month of lactation is not distributed to lambs (and not used for cheese-making), removing these in the same way as rearing waste (manure, straw, etc) does not appear to constitute a specific risk of the environment other than that which already exists from placentas and other sheep waste/excrement contained in manure, provided that the regulations on this subject are respected.

#### 4. Discussion of the request in terms of health safety, i.e. risk to humans from BSE and other TSE in small ruminants

Although this was not the role given to it, the working group also considered the question of the public health risk from human consumption of milk from small ruminants.

Three previous opinions and notes address the BSE question in small ruminants (2001 framework opinion re-updated in March 2005, note of 10 March 2006, opinion of 20 July 2006).

All of them considered the potential infectivity of milk from small ruminants with BSE. Recent results do not cast any fundamental doubt over the previous opinions: particularly as the surveillance/sampling system applied in slaughterhouses and quartering yards have shown that the prevalence of BSE in sheep, as it exists, is very low or zero.

In terms of the public health risk from scrapie, complete absence of risk to humans has never been demonstrated. In this context a precautionary rule was used in the previous opinions.

In reality, discussing the risk from infectivity of milk in humans involves comparing this risk to the risk from small ruminant meat (comparing infectivity levels) and with the safety measures taken for this risk (particularly, not consuming sheep carcasses which do not have resistance allele and come from farms subject to a prefectural order for being placed under surveillance because of classical scrapie).