Appendix IX (contd)

3. ~~in the case of a country, *zone* or *compartment* where there has been an indigenous *case*,~~ cattle selected for export were born after the date from which the ban on the feeding of ruminants with *meat-and-bone meal and greaves* derived from ruminants was effectively enforced.

Article 2.3.13.8.

When importing from a country, *zone* or *compartment* with an undetermined BSE risk, *Veterinary Administrations* should require:

for cattle

the presentation of an *international veterinary certificate* attesting that:

1. the feeding of ruminants with *meat-and-bone meal* and *greaves* derived from ruminants has been banned and the ban has been effectively enforced;

2. all BSE *cases*, as well as:

   a) all cattle which, during their first year of life, were reared with the BSE *cases* during their first year of life, and, which investigation showed consumed the same potentially contaminated feed during that period, or

   b) if the results of the investigation are inconclusive, all cattle born in the same herd as, and within 12 months of the birth of, the BSE *cases*,

   if alive in the country, *zone* or *compartment*, are permanently identified, and their movements controlled, and, when slaughtered or at death, are completely destroyed;

3. cattle selected for export:

   a) are identified by a permanent identification system enabling them to be traced back to the dam and herd of origin and are not the progeny of BSE suspect or confirmed females;

   b) were born at least 2 years after the date from which the ban on the feeding of ruminants with *meat-and-bone meal* and *greaves* derived from ruminants was effectively enforced.

Article 2.3.13.9.

When importing from a country, *zone* or *compartment* posing a negligible BSE risk, *Veterinary Administrations* should require:

for *fresh meat* and *meat products* from cattle (other than those listed in point 1 of Article 2.3.13.1.)

the presentation of an *international veterinary certificate* attesting that:

1. the country, *zone* or *compartment* complies with the conditions in Article 2.3.13.3.;

2. the cattle from which the *fresh meat* and *meat products* were derived passed ante-mortem and post-mortem inspections.

Article 2.3.13.10.

When importing from a country, *zone* or *compartment* with an undetermined BSE risk, *Veterinary Administrations* should require:

for *fresh meat* and *meat products* from cattle (other than those listed in point 1 of Article 2.3.13.1.)

the presentation of an *international veterinary certificate* attesting that:

1. the country, *zone* or *compartment* complies with the conditions referred to in Article 2.3.13.4.;

2. the cattle from which the *fresh meat* and *meat products* were derived passed ante-mortem and post-mortem inspections;

3. cattle from which *the fresh meat* and *meat products* destined for export were derived were not subjected to a stunning process, prior to slaughter, with a device injecting compressed air or gas into the cranial cavity, or to a pithing process;

4. the *fresh meat* and *meat products* were produced and handled in a manner which ensures that such products do not contain and are not contaminated with:

    a) the tissues listed in points 1 and 2 of Article 2.3.13.13.,

    b) mechanically separated meat from the skull and vertebral column from cattle over 30 months of age.

Article 2.3.13.11.

When importing from a country, *zone* or *compartment* with an undetermined BSE risk, *Veterinary Administrations* should require:

for *fresh meat* and *meat products* from cattle (other than those listed in point 1 of Article 2.3.13.1.)

the presentation of an *international veterinary certificate* attesting that:

1. the cattle from which the *fresh meat* and *meat products* originate:

    a) have not been fed *meat-and-bone meal* or *greaves* derived from ruminants;

    b) passed ante-mortem and post-mortem inspections;

    c) were not subjected to a stunning process, prior to slaughter, with a device injecting compressed air or gas into the cranial cavity, or to a pithing process;

2. the *fresh meat* and *meat products* were produced and handled in a manner which ensures that such products do not contain and are not contaminated with:

    a) the tissues listed in points 1 and 3 of Article 2.3.13.13.;

    b) nervous and lymphatic tissues exposed during the deboning process;

    c) mechanically separated meat from the skull and vertebral column from cattle over 12 months of age.

Appendix IX (contd)

Article 2.3.13.12.

1. Ruminant-derived *meat-and-bone meal* or *greaves*, or any *commodities* containing such products, which originate from a country, *zone* or *compartment* defined in Article 2.3.13.3. should not be traded if such products were derived from cattle born before the date from which the ban on the feeding of ruminants with *meat-and-bone meal* and *greaves* derived from ruminants had been enforced.

~~1.~~2. Ruminant-derived *meat-and-bone meal* or *greaves,* or any *commodities* containing such products, which originate from a country, *zone* or *compartment* defined in Articles 2.3.13.4. and 2.3.13.5. should not be traded between countries.

Article 2.3.13.13.

1. From cattle of any age originating from a country, *zone* or *compartment* defined in Articles 2.3.13.4. and 2.3.13.5., the following commodities, and any commodity contaminated by them, should not be traded for the preparation of food, feed, fertilisers, cosmetics, pharmaceuticals including biologicals, or medical devices: tonsils and distal ileum. Protein products, food, feed, fertilisers, cosmetics, pharmaceuticals or medical devices prepared using these commodities (unless covered by other Articles in this Chapter) should also not be traded.

2. From cattle that were at the time of slaughter over 30 months of age originating from a country, *zone* or *compartment* defined in Article 2.3.13.4., the following commodities, and any commodity contaminated by them, should not be traded for the preparation of food, feed, fertilisers, cosmetics, pharmaceuticals including biologicals, or medical devices: brains, eyes, spinal cord, skull and vertebral column. Protein products, food, feed, fertilisers, cosmetics, pharmaceuticals or medical devices prepared using these commodities (unless covered by other Articles in this Chapter) should also not be traded.

3. From cattle that were at the time of slaughter over 12 months of age originating from a country, *zone* or *compartment* defined in Article 2.3.13.5., the following commodities, and any commodity contaminated by them, should not be traded for the preparation of food, feed, fertilisers, cosmetics, pharmaceuticals including biologicals, or medical devices: brains, eyes, spinal cord, skull and vertebral column. Protein products, food, feed, fertilisers, cosmetics, pharmaceuticals or medical devices prepared using these commodities (unless covered by other Articles in this Chapter) should also not be traded.

Article 2.3.13.14.

Veterinary Administrations of *importing countries* should require:

for gelatine and collagen prepared from bones and intended for food or feed, cosmetics, pharmaceuticals including biologicals, or medical devices

the presentation of an *international veterinary certificate* attesting that:

1. the *commodities* came from a country, *zone* or *compartment* posing a negligible BSE risk;

OR

2. they originate from a country, *zone* or *compartment* posing a controlled or undetermined BSE risk and are derived from cattle which have passed ante-mortem and post-mortem inspections; and that

   ~~a) skulls from cattle over 30 months of age at the time of slaughter have been excluded ;~~

   ~~b)~~a) the bones have been subjected to a process which includes all of the following steps:

      i) pressure washing (degreasing),

      ii) acid demineralisation,

      iii) acid or alkaline treatment,

      iv) filtration,

      v) sterilisation at >138°C for a minimum of 4 seconds,

      or to an equivalent or better process in terms of infectivity reduction (such as high pressure heating)~~;~~.

~~OR~~

~~3. they originate from a country, *zone* or *compartment* posing an undetermined BSE risk and are derived from cattle which have passed ante-mortem and post-mortem inspections; and that~~

   ~~a) skulls and vertebrae (except tail vertebrae) from cattle over 12 months of age at the time of slaughter have been excluded;~~

   ~~b) the bones have been subjected to a process which includes all of the following steps:~~

      ~~i) pressure washing (degreasing),~~

      ~~ii) acid demineralisation,~~

      ~~iii) acid or alkaline treatment,~~

      ~~iv) filtration,~~

      ~~v) sterilisation at >138°C for a minimum of 4 seconds,~~

      ~~or to an equivalent or better process in terms of infectivity reduction (such as high pressure heating).~~

Article 2.3.13.15.

Veterinary Administrations of *importing countries* should require:

for tallow and dicalcium phosphate (other than as defined in Article 2.3.13.1.) intended for food, feed, fertilisers, cosmetics, pharmaceuticals including biologicals, or medical devices

the presentation of an *international veterinary certificate* attesting that:

Appendix IX (contd)

1. the *commodities* came from a country, *zone* or *compartment* posing a negligible BSE risk; or

2. they originate from a country, *zone* or *compartment* posing a controlled BSE risk, are derived from cattle which have passed ante-mortem and post-mortem inspections, and have not been prepared using the tissues listed in points 1 and 2 of Article 2.3.13.13.

Article 2.3.13.16.

Veterinary Administrations of *importing countries* should require:

for tallow derivatives (other than those made from protein-free tallow as defined in Article 2.3.13.1.) intended for food, feed, fertilisers, cosmetics, pharmaceuticals including biologicals, or medical devices

the presentation of an *international veterinary certificate* attesting that:

1. they originate from a country, *zone* or *compartment* posing a negligible BSE risk; or

2. they are derived from tallow meeting the conditions referred to in Article 2.3.13.15.; or

3. they have been produced by hydrolysis, saponification or transesterification using high temperature and pressure.

— text deleted

Appendix XXVIII

SUPPORTING DOCUMENT FOR CHAPTER 2.3.13. OF THE TERRESTRIAL ANIMAL HEALTH CODE ON BOVINE SPONGIFORM ENEPHALOPATHY

This document is provided in support of the recommendations in the present *Terrestrial Code* chapter. It deals only with the risks associated with the presence of the bovine spongiform encephalopathy (BSE) agent in cattle (Bos taurus and Bos indicus) although the BSE ad hoc committee acknowledged there may be a need in the future to address the subject of BSE in small ruminants.

The availability of experimental infectivity data has significantly increased in the recent years. During the same interval, extremely sensitive tests have been developed, including those employing highly sensitive transgenic mice strains and potentially more sensitive laboratory PrP detection methods. With the development of such highly sensitive methods, the probability of detection of PrP^{BSE} in tissues that are not currently listed as infectious is increasing. However, such findings need to be considered in context, and their relevance to establishing risk to consumers evaluated carefully when the quantity of PrP^{BSE} detected is potentially below the limit of detection of intracerebral (i.c.) cattle to cattle bioassay. By June 2006, 156 variant Creutzfeldt-Jakob-Disease (vCJD)-cases had been detected in the United Kingdom (UK), a country where most probably the majority of the population was exposed to the BSE-agent. The latest models of the vCJD epidemic estimate that the potential scale of the clinical epidemic arising from food-borne exposure is unlikely to exceed 400 future cases (Clarke and Ghani, 2005). The relatively low number of predicted vCJD cases in relation to the massive exposure to the BSE agent is suggested to be due mainly to a significant species barrier between cattle and humans (Comer and Huntly, 2004, Bishop et al, 2006).

Therefore, data from cattle to cattle transmission studies (intracerebral) are taken as the baseline for recommendations in the *Terrestrial Animal Health Code*.

Section 2.3.13.1

The list of tradable commodities is mainly based on insights from experimental transmission studies of BSE to cattle and from the epidemiological data relating to natural disease. Currently the formulation of this list does not take into account the BSE risk status of the cattle population in the *exporting country*, *zone* or *compartment* (Article 2.3.13.1*)* or the current conditions for trade in commodities according to the BSE status (Articles 2.3.13.8-2.3.13.16).

How the BSE agent behaves biologically in cattle was originally surmised from what was known about scrapie in small ruminants. However, subsequent data from examinations of tissues from field cases of BSE and from experimental pathogenesis studies of BSE in cattle indicate that the tissue distribution of BSE agent in cattle is more restricted than was originally inferred from the understanding of the pathogenesis of scrapie in sheep and goats.

The search for infectivity in tissues of BSE-infected cattle has examined material either from natural cases or from experimental, sequential kill, time course studies using orally challenged cattle. Tissues have also been examined for the detection of the disease-specific form of PrP. The majority of tissue infectivity assays have been conducted in inbred mouse strains (RIII or C57Bl), although there have been a limited number of studies in cattle and, most recently, in transgenic (Tg) mice over-expressing the bovine PrP gene (Tg bov XV). The current situation with regard to tissues examined for infectivity or PrP^{BSE} and their categorization according to level of infectivity, irrespective of the stage of disease is given in Table 1.[1]

[1] : **Adapted from: Report of WHO TSE Consultation: Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies, 14-16 September 2005.**

Appendix XXVIII (contd)

Infectivity/ PrP^{BSE} detected in natural clinical cases

In natural cases of clinically-affected cattle, BSE infectivity has been detected only in the brain, spinal cord and retina by assay in inbred mice (Fraser & Foster 1994; MAFF 1998; Buschmann & Groschup 2005). Assays of infectivity by intracerebral inoculation of cattle identified additionally infectivity in a pool of nictitating membranes, but not in pools of lymph nodes or spleens from natural cases (S.A.C. Hawkins, pers. comm). BSE infection has been transmitted to mice, by feeding affected brain ({Barlow & Middleton, 1990) but not by feeding extraneural tissues (Middleton & Barlow 1993).

Transgenic bovinised mice (Tgbov XV mice), over expressing the bovine PrP gene, have been constructed with a sensitivity for detecting BSE infectivity in cattle which exceeds that of RIII mice by at least 10,000-fold, and even that of cattle by approximately10-fold These mice were challenged with brain and spleen pools from clinical BSE cases sourced in the UK, and a comprehensive range of tissues and fluids from a single, late-stage pregnancy, German case showing clinical signs of BSE (Buschmann & Groschup 2005). Transmissions were obtained from the UK-sourced brain pool, but not the spleen pool. From the German BSE case infectivity was detected in brain, spinal cord, retina and optic nerve, distal ileum, and peripheral nerve. Less than 100% attack rates and prolonged incubation periods characterized the transmissions from peripheral nervous system tissue (facial and sciatic nerves) and the titers can be conservatively estimated to be ~ 10^4-10^5 less per g than that of the CNS (brain stem). Additionally, a single mouse (of 10) developed disease after 520 days when inoculated with semi-tendinosous muscle from the BSE-affected pregnant cow. The results support a conclusion that there is a limited distribution of BSE infectivity in bovine tissues (Buschmann and Groschup 2005).

PrP^{BSE} detection has been reported in the peripheral nerves of a case of BSE in Japan (Iwamaru et al., 2005). Additionally, three 80- to 95-month-old Holstein dairy cattle slaughtered at abattoirs in Japan were examined for the distribution of PrP^{BSE} by immunohistochemistry (IHC) and Western blot (WB) analyses. The cattle are reported to have shown no clinical signs relevant to BSE but were screened as positive by the Bio-Rad TeSeE test. These positive results were confirmed by IHC or WB in a specimen of the medulla oblongata. Histopathologically, these cattle showed no vacuolation in tissue sections from the central nervous system except for the medulla oblongata. Both IHC and WB analyses revealed PrP^{BSE} accumulation in the brain, spinal cord, satellite and ganglionic cells of the dorsal root ganglia, and the myenteric plexus of the distal ileum. In addition, small amounts of PrP^{BSE} were detected in the peripheral nerves of two of the cattle by WB. No PrP^{BSE} was demonstrated by either method in the Peyer's patches of the distal ileum, additional lymphoid tissues including the palatine tonsils, lymph nodes, and spleen, or other tissues. These Japanese researchers noted that the distribution of PrP^{BSE} accumulation in this naturally-occurring, preclinical stage was different from that reported for cattle inoculated experimentally with the BSE agent (Iwata et al., 2006), although the clinical signs recorded as being present at slaughter are considered consistent with some BSE cases identified in the UK (D Matthews, pers. comm.).

Infectivity/ PrP^{BSE} detected in cattle after experimental oral exposure to the BSE agent

To determine the temporal and spatial development of infectivity and pathological changes following oral exposure, pathogenesis studies of experimental BSE in cattle were initiated. In experimentally oral exposed cattle, BSE infectivity has been detected by inbred mouse assay in the distal ileum (through much of the disease course from six months post exposure) and in the CNS and in sensory ganglia (dorsal root ganglia) of the peripheral nervous system from late in the incubation period (Wells et al 1994; 1996; 1998). Infectivity has also been detected in sternal bone marrow in cattle experimentally exposed to BSE agent by the oral route (but only at a single time point [38 months] during clinical illness) (Wells et al 1999).

Due to the species barrier (cattle-mice), the bioassay of BSE infectivity in mice is less sensitive than bioassay in cattle. A comparative bioassay in which pooled brains from five confirmed cases of BSE were titrated in cattle and mice, showed that the titer measured is 500 fold higher when assayed in cattle than in mice (i.e. the bioassay in mice of bovine tissues infected with BSE agent is less sensitive than bioassay in cattle) (Hawkins et al 2000; SSC 2002b). Assays in cattle of selected tissues from this sequential time point oral exposure study confirmed

infectivity in distal ileum (from six through 18 months after exposure and during clinical disease) and in the CNS at the earliest time post-exposure detected by the inbred mouse assay, but not before, and has detected infectivity in palatine tonsil (at a single time point 10 months post exposure), which was not detected by the mouse assay (Wells et al 2005). Immunohistochemical examination of tonsil from the donor cattle killed sequentially failed to reveal PrP^{BSE} at any time in the incubation period or clinical phase (Wells et al 2005).

Bone marrow from experimentally exposed cattle in the clinical phase of disease has not transmitted by assay in cattle.

A wide range of other tissues (including most lymphoreticular tissues) from cattle with BSE, both naturally exposed and experimentally induced and from cattle in the incubation period after experimental exposure, have shown no detectable infectivity using conventional mouse bioassays or ongoing parallel bioassays in cattle to date (Wells et al 1996; 1998; 2005; SSC 2002b).

The localisation of PrP^{BSE} has been examined by immunohistochemistry (IHC) in the distal ileum of cattle up to 40 months after they had been exposed orally to the agent of BSE, and from an additional group of cattle six months after a similar exposure, and in naturally occurring clinical cases of BSE (Terry et al 2003). PrP^{BSE} was detected mainly in macrophages, in a small proportion of the follicles of the Peyer's patches in the distal ileum in the experimentally exposed cattle throughout much of the course of the disease. The earliest time point in the experimental disease at which PrP^{BSE} could be detected in Peyer's patches was at 6 months post inoculation, 26 months prior to visualisation of PrP^{BSE} in the CNS. The observations were in agreement with the infectivity data derived from mouse bioassays of the distal ileum (Wells et al 1998). In the later stages of the disease, the proportion of immunostained follicles increased as the total number of follicles decreased as a consequence of lymphoid tissue involution with age. In the additional experimental group of cattle, killed at six months post exposure, PrP^{BSE} was confined to the Peyer's patches of the distal ileum but no immunolabelling was detected in the lymphoid tissue of the duodenum, jejunum or colon. PrP^{BSE} could also not be detected in the distal ileum in naturally occurring clinical cases of BSE. In some of the cases, from all three groups of cattle tested, there was some sparse immunolabelling of the neurons of the distal ileal myenteric plexus.

It has to be noted that even with the greatly increased sensitivity of detection methods, the range of tissues in which infectivity had been found had not significantly changed (Table1).

In the recommendations, appropriate weight was given to the significant amount of data available as a result of the natural route of exposure of cattle and the outcomes of research involving cattle to cattle transmission studies where there is no species barrier.

2.3.13.1, 1a: Milk and milk products

Supporting evidence for the safety of milk and milk products with regard to BSE transmission:

Experimental data:

Inoculation of RIII mice with udder from a BSE affected cow did not detect infectivity (Foster and Fraser 1994). An experiment using milk derived from cattle with BSE in early, mid and late lactation and either inoculated or fed to susceptible mice has revealed no evidence of infectivity (Taylor et al. 1995).

Experiments in which mice were fed milk and mammary gland from clinically affected cows have failed to transmit the disease within the natural lifespan of the recipients (Middleton & Barlow 1993).

Furthermore, milk samples from cows orally challenged as calves with bovine brain from clinically affected cows and followed over four lactations were tested for the presence of PrP^{BSE} using a very sensitive ELISA and a Western blot test. The results of this study do not provide any evidence for the presence of PrP^{BSE} in the milk from cattle incubating BSE at levels defined by the limits of sensitivity of the two analytical methods used. (Everest et al. (2006), *Journal of General Virology*, in press)

In addition, colostrum from a clinical BSE case did not transmit infectivity when tested in highly sensitive transgenic mice (Buschmann & Groschup 2005).

Appendix XXVIII (contd)

Epidemiological data

Several studies have examined vertical/maternal transmission but none has shown evidence that transmission of the BSE agent occurs through milk (Wrathall *et al.*, 2002; Wilesmith and Ryan, 1997; Wilesmith *et al.*, 1997; SSC 2001).

In conclusion, experimental and epidemiological evidence do not indicate milk or its products to be a risk factor in transmitting the BSE agent.

2.3.13.1, 1b: semen and in vivo derived cattle embryos collected and handled in accordance with the recommendations of the International Embryo Transfer Society

Supporting evidence for the safety of semen and in vivo derived cattle embryos with regard to BSE transmission:

Experimental data

No detectable infectivity has been found in susceptible mice fed placenta from confirmed cases of BSE (Middleton & Barlow 1993, Barlow & Middleton 1990, Bradley 1990), nor in placenta, placental fluids, ovary or uterine caruncle following mouse inoculation (Fraser &Foster 1994; MAFF 1997, SSC 2000). Male reproductive tissues (testis, epididymis, prostate, semen, seminal vesicle) inoculated into mice showed no infectivity (Fraser & Foster 1994; MAFF 1999).

In another study to detect possible infectivity in the foetal membranes and placenta of cattle with clinical BSE, recipient cattle were dosed oro-nasally with a pooled tissue homogenate from BSE cattle. The recipients were killed at 24 and 84 months post infection (p.i.) with no evidence of disease (Bradley, 1996, SSC 2000).

In another study (Wrathall *et al.*, 2002) semen from 13 bulls, 8 with clinical BSE, was used for artificial insemination (AI) of 167 clinically affected cows in the terminal stages of BSE. The resultant embryos were treated according to the recommendation of the International Embryo Transfer Society (IETS). 587 viable embryos were transferred into 347 recipient heifers imported from NZ and 266 live offspring were born, of which 54,1% had a BSE positive sire as well as a BSE positive dam. The recipients and the offspring were monitored for 7 years after birth. All brains of recipients and offspring were examined for BSE by histopathology and the immunohistochemical detection of PrP^{BSE} with negative results. Additionally, one thousand and twenty non-viable embryos were inoculated i.c. into 48 susceptible mice, which were all negative at 700 days p.i. Additionally, uterine flush fluid samples from 41 cows were tested for BSE infectivity by i.c. and intraperitoneal inoculation of 946 mice. One of these mice had some vacuolar pathology, but its relevance proved difficult to determine as the putative incubation period was inconsistent with the survival of remaining mice in the group. All other mice with injections of flush fluids from the same cow were negative when finally killed and examined.

In another study, caruncles and amniotic fluid derived from a clinically affected cow inoculated into highly sensitive transgenic mice showed no infectivity (Buschmann & Groschup, 2005).

Epidemiological data

In a cohort study, 316 offspring of BSE confirmed cows (cases) and 316 offspring from cows over six years old and without BSE from the same farm and age cohort (controls) have been observed under controlled conditions over a seven-year period. The purpose of the study was to determine whether maternal transmission occurs, and the incidence if it does. There was a statistically significant risk difference between the two cohorts examined i.e. calves born to dams with BSE and calves born to healthy dams >6 years old. This difference was 9.7% with a relative risk of 3.2 for offspring of cows that developed clinical BSE. This enhanced risk for the offspring of BSE dams appeared to decline the later the offspring was born after the 1988 feed ban was in place but increased the closer that parturition was to the onset of clinical disease in the dam. The results cannot distinguish between a genetic component and true maternal transmission for which there is no other evidence. A combination of a genetic cause (i.e. increased susceptibility to feed exposure which could have occurred in any cattle in the study)

or genuine transmission fits the computer model of the epidemic best (Curnow *et al.*, 1997; Gore *et al.*, 1997; Donnelly *et al.*, 1997a,b,c,; Wilesmith *et al.*, 1997). Later studies by Donnelly *et al.* (2002) significantly reduced the estimated risk to offspring, although they recognized that the introduction of culling of offspring of confirmed cases made estimation of the risk impossible other than by back-calculation methods. The route for the hypothetical maternal transmission of BSE has not been established. Given that <1% of the offspring of affected cattle in the general epidemic may succumb to this means of exposure, it is likely to be difficult to determine the route.

Furthermore, Wilesmith and Ryan (1997) have found no cases of BSE in the offspring of beef suckler cows with BSE, suggesting that neither milk nor direct contact appear to be involved. However, all calves receive colostrum and beef calves are suckled for up to six months of age. Since there is little epidemiological evidence that maternal transmission occurs in BSE (the way that transmission from milk would be exhibited if it occurred), it can be concluded that bovine milk does not contain any infectivity. A specific analysis of data from the study on offspring of beef suckler cows with BSE which suckled their young for substantial periods showed no occurrence of BSE in the offspring (Wilesmith *et al.*, 1997), suggesting neither milk nor close contact was a factor.

No offspring of BSE cases have been reported with BSE outside the UK. Unfortunately relatively few offspring have been tested for BSE globally, and most are relatively young when culled and tested, making it difficult to conclusively rule out the possibility of transmission. Nevertheless, it seems clear that if vertical transmission is occurring, it is a rare event.

Scientific reviews, undertaken in 1999 and 2002 (SSC 1999 and 2002b) concluded it unlikely that bovine semen constitutes a risk factor for the transmission of BSE and that embryos need only be subjected to those measures prescribed by the International Embryo Transfer Society protocols.

In conclusion, experimental and epidemiological evidence do not indicate male and female reproductive tissues to be a risk factor in transmitting the BSE agent.

2.3.13.1, 1c: hides and skins and
2.3.13.1, 1d Gelatin and collagen prepared exclusively from hides and skins and
2.3.13.14 gelatin and collagen prepared from bones and intended for food or feed, cosmetics, pharmaceuticals including biologicals, or medical devices

Supporting evidence for the safety of hides, skins and bones and gelatin and collagen prepared from them with regard to BSE transmission:

Hides and skins

Experimental data

Hides and skin from natural cases of BSE contain no detectable infectivity when bioassayed in laboratory mice (Fraser and Foster, 1994) and intracerebrally in cattle (Wells *et al.*, 2005).

In conclusion, experimental evidence does not indicate hides and skins and gelatin and collagen prepared from them to be a risk factor in transmitting the BSE agent provided that slaughter procedures prevent contamination with CNS.

Bones

Experimental data

With respect to gelatin and collagen prepared from bones, studies of mice intracerebrally injected with bone marrow from cattle with naturally occurring clinical BSE have not demonstrated infectivity. These data for BSE are based on transmissions attempted from a very small number of animals but they are, in general, consistent

Appendix XXVIII (contd)

with those in studies of the pathogenesis of BSE in experimentally orally exposed cattle. In a single group of cattle challenged with 100g of BSE infected brain tissue (Wells *et al*, 1996, 1998) and killed sequentially at approximately 4 month intervals from 2-40 months post exposure, infectivity was detected, at a level close to the limit of detectability by mouse bioassay, in the sternal bone marrow from animals killed in the clinical phase of the disease at 38 months p.i. (but not before and not after) (Wells *et al.*, 1999).

The inconsistent result of the absence of detectable infectivity in bone marrow in this study at the later time point of 40 months p.i. has raised, amongst other alternative explanations, the possibility that the finding of infectivity at 38 months p.i. may have been the result of an accidental procedural contamination. Nevertheless, there is limited evidence from previous studies of other TSEs that infection of bone marrow, although not part of the general pathogenesis pattern, could be a rare event occurring late in the incubation period.

Risk assessment results

With respect to gelatin and collagen prepared from bones, a quantitative risk assessment (EFSA Journal 2006 312) of the residual risk in bone-derived gelatin, assuming sourcing of bones from animals which passed ante and post mortem inspection, but regardless of the country of origin, calculated different scenarios resulting in different risk levels. This risk assessment did not consider the risk of sourcing bones other than those fit for human consumption. The risk assessment indicates that the relevant exposures are regarded as very small compared to the historical exposure (1980-2001) of the UK human population due to meat and meat products in its diet. Based on these results, the risk of exposure appears to be much lower than previously thought. The removal of skull and vertebral column from the source materials results only in a very small risk reduction.

However, the input parameters to the supporting risk assessment model sourced animals only from the healthy slaughter sub-population and did not address the scenario where material was sourced from cattle not subject to ante- and post-mortem inspection.

Based on these calculations, the conditions recommended for gelatin derived from bones could be modified for countries, zones or compartments of undetermined or controlled BSE risk, while allowing for the anticipated problems associated with the practical implementation of the recommendations.

For both categories of status, the cattle must have passed ante- and post-mortem inspection and the commercial process for gelatin production must have been correctly carried out. For controlled risk countries, zones or compartments, the source of bones for gelatin production could be expanded to include vertebrae, and bones could be sourced from countries of undetermined risk provided that bones from the skull and vertebral column were excluded.

There is no specific information with regard to the production of collagen from bones, nor to the safety of such a process.

In conclusion, there are indications that gelatin and collagen prepared from bones are not risk factors in transmitting the BSE agent, provided they are sourced from cattle subject to ante- and post-mortem inspection, without any additional conditions applied. However, consistent with the conservative approach adopted in respect of BSE, it was considered that some additional conditions for gelatin and collagen prepared from bones are necessary as a safety margin.

2.3.13.1, 1e protein-free tallow (maximum level of insoluble impurities of 0.15% in weight); and Article 2.3.13.15. tallow (other than protein-free tallow as defined in Article 2.3.13.1.)

Supporting evidence for the safety of tallow with regard to BSE transmission:

Tallow refers to a wide range of animal fats and covers edible products and animal by products

a) edible products (such as discrete adipose tissues) produced from animals which have passed ante- and post-mortem inspection are usually melted in dedicated processing facilities. The tissues are gently heat-treated (<95 °C) to maintain the high quality of tallow and it is purified, mainly by separation and filtration, in order to reduce any residual insoluble impurities. Edible tallows have low initial levels of impurity and are usually produced with final total impurity levels of <0.02%

b) products manufactured from animal by products (such as trimmings, bones, certain slaughter offals, etc.) are extracted by rendering a mixture of tissues at < 100°C or are also obtained by pressing after rendering at "133°C/20'/3 bar". The tallow is usually purified to below 0.15% insoluble impurities. The extracted residue is referred to as greaves and can be further refined to produce meat and bone meal.

Experimental data

Tallow contains no detectable infectivity when bioassayed in mice. Insofar as rendering is concerned, the scientific studies with brain-spiked TSE-infected material demonstrate no detectable infectivity in tallow whether filtered or not (Taylor and Woodgate, 2003).

Risk assessment results

With respect to tallow, a quantitative risk assessment (EFSA Journal 2005 221) assuming sourcing of tissues from animals which passed ante and post mortem inspection, calculated different scenarios resulting in different risk levels. The risk assessment indicates that when the tallow is processed either by fat melting or rendering, the risk is virtually negligible. Although the residual BSE risk as expressed in the paper varied by two logs depending upon the retention or removal of SRMs and the BSE risk posed by the country of origin, even the worst case scenario indicated a residual BSE risk considerably below that previously assumed.

However, the input parameters to the supporting risk assessment model sourced animals only from the healthy slaughter sub-population and did not address the scenario where material was sourced from cattle not subject to ante- and post-mortem inspection.

The level of insoluble impurities in the tallow did not significantly affect the risk of exposure, due to the very low risk which tallow inherently presents.

On the other hand, the source of the raw material is significant and may warrant further consideration such as an examination of the worst case scenario in which none of the contributing material was sourced from cattle subject to ante- and post-mortem inspection, but rather from fallen stock animals or animals condemned at AM and PM inspection in general proportions characteristic of the European experience.

In conclusion, there are indications that tallow is not a risk factor in transmitting the BSE agent at least when it is sourced from cattle subject to ante- and post-mortem inspection , without any additional conditions applied.

However, consistent with the conservative approach adopted in respect of BSE, it was considered that some additional conditions are necessary as a safety margin for tallow, which is not protein-free.

2.3.13.16 Tallow derivatives (other than those made from protein-free tallow as defined in Article 2.3.13.1)

Tallow is not used in pharmaceutical and cosmetic products, but tallow derivatives can be used and are considered as safe for any purpose provided these are produced by hydrolysis of tallow by approved methods using high temperature and pressure (*eg* from safe source tissues with a low risk of TSE infection) or by processes which give the same degree of assurance.

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2.3.13.1, 1f Dicalcium phosphate (with no trace of protein or fat); and
Article 2.3.13.15. dicalcium phosphate (other than dicalcium phosphate as defined in Article 2.3.13.1.)

Supporting evidence for the safety of dicalcium phosphate with regard to BSE transmission:

Dicalcium phosphate (DCP) is a co-product of the alkaline or acid gelatin manufacturing process.

Tricalcium phosphate (TCP) can be manufactured as a by-product of the heat and pressure bone gelatine manufacturing processes and the manufacturing processes of hydrolysed collagen.

Both are obtained from degreased bones, almost exclusively from cattle and pigs (SSC, 2003).

The end use of these bovine-derived phosphates is in principle animal nutrition as an additive, mainly in monogastric animals, but they may also be used as fertiliser.

Experimental data

With respect to dicalcium phosphate prepared from bones, studies of mice intracerebrally injected with bone marrow from cattle with naturally occurring clinical BSE have not demonstrated infectivity. These data for BSE are based on transmissions attempted from a very small number of animals but they are, in general, consistent with those in studies of the pathogenesis of BSE in experimentally orally exposed cattle. In a single group of cattle challenged with 100g of BSE infected brain tissue (Wells *et al*, 1996, 1998) and killed sequentially at approximately 4 month intervals from 2-40 months post exposure, infectivity was detected, at a level close to the limit of detectability by mouse bioassay, in the sternal bone marrow from animals killed in the clinical phase of the disease at 38 months p.i. (but not before and not after) (Wells *et al.*, 1999).

The inconsistent result of the absence of detectable infectivity in bone marrow in this study at the later time point of 40 months p.i. has raised, amongst other alternative explanations, the possibility that the finding of infectivity at 38 months p.i. may have been the result of an accidental procedural contamination. Nevertheless, there is limited evidence from previous studies of other TSEs that infection of bone marrow, although not part of the general pathogenesis pattern, could be a rare event occurring late in the incubation period.

Risk assessment results

With respect to dicalcium phosphate, a quantitative risk assessment (EFSA Journal 2006 339) assuming sourcing of tissues from animals which passed ante and post mortem inspection, calculated different scenarios resulting in different risk levels. The risk assessment indicates that when a limit of less than 1 resulting case per year within the exposed population is considered as negligible, no scenario of sourcing bovine bones, derived phosphates from GBR III or GBR IV countries leads to an average residual BSE risk equivalent to less than 1 case of BSE per year in either adult dairy or beef cattle.

This assessment, assuming different levels of contamination of bones with dorsal root ganglia and spinal cord, a very low level of infectivity of bone marrow and an infectivity reduction for the acid process of $10^{4.2}$ - $10^{4.8}$ and for the heat/pressure process of $10^{6.2} - 10^{6.8}$ indicates a very low risk.

However, the input parameters to the supporting risk assessment model sourced animals only from the healthy slaughter sub-population and did not address the scenario where material was sourced from cattle not subject to ante- and post-mortem inspection.

In conclusion, there are indications that DCP and TCP are not a risk factor in transmitting the BSE agent. However, the risk is only estimated for bones exclusively sourced from cattle subject to ante- and post-mortem inspection, which does not reflect the real practice.

Therefore, it was considered that some additional conditions are necessary as a safety margin for dicalcium phosphate with traces of protein or fat.

2.3.13.1, 1g Deboned skeletal muscle meat and
2.3.13.9-11 fresh meat and meat products

Supporting evidence for the safety of deboned skeletal muscle meat with regard to BSE transmission:

Experimental data

From studies of the pathogenesis of experimental BSE in cattle, no infectivity is found in assays of skeletal muscle pools (triceps, masseter, sternocephalicus and longissimus dorsi) completed in inbred mice and in progress in cattle (semitendinosus and longissimus dorsi) from selected kill time points of the oral exposure study (Wells et al, 2005). These studies are being terminated in 2006 and inoculated animals have so far shown no clinical evidence of infection (D Matthews, personal communication).

However, in the transgenic mice over-expressing the bovine PrP gene (Tg bov XV) infectivity was detected in one muscle (semitendinosus), from a single clinical case of BSE in Germany (Buschmann & Groschup 2005).

In light of the discussions above, data from cattle to cattle experiments are regarded as the baseline for inclusion of tissues in the list of SRM. Therefore, pure skeletal muscle meat itself is regarded as safe, if some additional measures involving compliance with recommended stunning practices (The EFSA Journal, 2004 123; TAFS, 2004) and the hygienic removal of SRM (The EFSA Journal, 2005 220) are applied.

The application of the 30 months age cut off for skeletal muscle meat in Article 2.3.13.1 is based on the significantly reduced risk associated with SRM from animals younger than that age (see Article 2.3.13.13 for list of SRM). For controlled risk countries, a lower age cut off could be considered. However, since Article 2.3.13.1 addresses meat from all categories of BSE risk, it was considered that 30 months should be retained as it added an element of safety regarding possible contamination from tissues listed in Article 2.3.13.13 originating from countries of undetermined BSE risk.

In conclusion, experimental evidence indicates that deboned skeletal muscle meat is not a risk factor in transmitting the BSE agent if the recommended mitigative measures are applied to prevent contamination with SRM.

2.3.13.1, 1h blood and blood by-products

Supporting evidence for the safety of blood and blood by products with regard to BSE transmission:

Experimental data

Blood (buffy coat) from experimentally infected BSE cases 6, 18, 26 and 32 months post infection contains no detectable infectivity when inoculated intracerebrally into cattle (Wells et al, 2005).

However, brain damage caused by certain stunning techniques can produce Central Nervous System (CNS) tissue emboli in venous blood draining the head (The EFSA Journal 2004 123).

In conclusion, experimental evidence indicates that blood and blood by-products are not risk factor in transmitting the BSE agent if recommended stunning procedures are applied.

2.3.13.13 List of SRM

The list of SRM was first based on the results of historical experiments on sheep scrapie. These were supplemented by later results from experiments in which tissues from cattle in the pathogenesis study described above were inoculated intracerebrally into mice or cattle. All tissues found positive in these experiments have so far been included in the list.

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Vertebral column and skull are included, although they have not shown to be infected, but because of their close association with the CNS and in the expectation that they will be contaminated as a result of that association and current carcase dressing procedures.

Approximately 90% of infectivity is associated with the brain, spinal cord, dorsal root and trigeminal ganglia. The other 10% is associated with the distal ileum (Comer and Huntly, 2004). Although it cannot be excluded that infectivity may be present in other tissues at a level below the limit of detection in the bovine bioassay, this additional infectivity would constitute less than 1% of the total infectivity associated with the carcase. Some re-adjustment of that calculation may now be necessary following the detection of infectivity in peripheral nerves of clinically affected cattle (Buschmann & Groschup 2005, Iwamaru et al., 2005, Iwata et al., 2006).

The age limit for SRM (except tonsil and distal ileum) from countries that have followed the recommendations to ensure they are in the controlled risk status, is set to 30 months and that for countries of undetermined risk status is 12 months.

The age limits are based on data from the pathogenesis study and the attack rate study (EFSA Journal 2005 220) as well as the observed epidemiology of BSE, especially with respect to age.

Evidence suggests that detectable infectivity appears in the CNS at about ¾ of the incubation time. The average age of BSE cases has generally increased, e.g. from 76 to 95 months in healthy slaughtered animals in the EU from 2001 to 2005, since the implementation of control measures. Therefore, a cut-off at 30 months represents a considerable safety margin for commodities from countries with a controlled risk status. It is however likely that in the few years after the implementation of control measures, such as a fully enforced feed ban, countries may experience small numbers of cases that are younger than those reported from the EU above. This is no surprise as the age range for clinically affected BSE cases can be as little as 20 months in the case of the UK, or extend to the full natural lifespan of cows that are exposed to low dose or infected as adults. The mean age at onset of disease in the UK before the effects of intervention were seen was 60 months, with the majority of affected cattle being four to six years of age. Clinical cases at a young age, or CNS positive cases at an equivalent age, therefore represent only a very small proportion of infected animals, and therefore the likelihood that CNS will test positive and be infectious at less than 30 months is low. They become increasingly rare as the effects of feed bans are seen.

For countries with an undetermined risk the situation remains unclear; imposition of a conservative age limit of 12 months would cover even the youngest animals which might be encountered at the beginning or the peak of an epidemic in this scenario).

2.3.13.2.-2.3.13.5 BSE risk status of the cattle population

History shows that the risk associated with commodities originating within the cattle population of a country, zone or compartment cannot be determined solely on the basis of reported BSE cases, even in the presence of an active, targeted surveillance program.

Therefore, the recommendations concerning BSE classification incorporate assessment of a broader series of considerations. They are primarily based on the outcome of a BSE risk assessment, along with additional considerations listed in Article 2.3.13.2 (factors such as disease awareness programs, a system of notification and investigation of BSE cases as well as available laboratory competence and the aforementioned implementation of a risk-based surveillance system). They are accompanied by assessment of the date of effective implementation of a number of strategic controls within the feed production process.

Only after evaluation of all these factors, countries, zones or compartments can be classified. The former five BSE Status categories: BSE free, BSE provisionally free, minimal risk, moderate risk, and high risk were changed in 2005 into 3 categories: negligible, controlled and undetermined BSE-risk.

For guidelines concerning the factors to consider in conducting the BSE risk assessment recommended in chapter 2.3.13., see Appendix 3.8.5.

MAJOR CATEGORIES OF INFECTIVITY: TABLES Ia, Ib, Ic

The information in these Tables is based exclusively upon observations of tissue infectivity in naturally occurring disease, or primary experimental infection by the oral route (in cattle), and does not include data on models using strains of TSE that have been adapted to experimental animals, because passaged strain phenotypes can differ significantly and unpredictably from those of naturally occurring disease. Assay species include inbred mice, transgenic mice overexpressing the bovine PrP gene or cattle. Because the detection of misfolded host prion protein (PrP^{TSE}) has proven to be a reliable indicator of infectivity, PrP^{TSE} testing results have been presented in parallel with bioassay data. Tissues are grouped into three major infectivity categories, irrespective of the stage of disease:

Ia: High-infectivity tissues: CNS tissues that attain a high titre of infectivity in the later stages of all TSEs, and certain tissues that are anatomically associated with the CNS.

Ib: Lower-infectivity tissues: peripheral tissues that have tested positive for infectivity and/or PrP^{TSE} in at least one form of TSE.

Ic: Tissues with no detectable infectivity: tissues that have been examined for infectivity and/or PrP^{TSE} with negative results.

Data entries are shown as follows:

+	Presence of infectivity or PrP^{TSE}
-	Absence of detectable infectivity or PrP^{TSE}
NT	Not tested
?	Controversial results
( )	Limited or preliminary data

It is possible that the detection of infectivity using transgenic mice that over-express the gene encoding the normal prion protein, or the detection of PrP^{TSE} using various newly developed amplification methods, may be more sensitive than transmission studies in wild-type bioassay animals, and thus may not correlate with disease transmissibility in nature.

It is also important to understand that categories of infectivity are not the same as categories of risk, which require consideration not only of the level of infectivity, but also of the route by which infection is transmitted and the amount of tissue to which a person or animal is exposed.

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Table 1a: High-infectivity tissues

CNS tissues that attain a high titre of infectivity in the later stages of TSE and certain tissues anatomically associated with the CNS		
Tissues	**Cattle BSE**	
	Infectivity[1]	**PrP^{TSE}**
Brain	+	+
Spinal cord	+	+
Retina	+	NT
Optic nerve	+	NT
Dorsal Root ganglia	+	NT
Trigeminal ganglia	+	NT
Pituitary gland[2]	-	NT
Dura mater[2]	NT	NT

Table 1b: Lower-infectivity tissues

Peripheral tissues that have tested positive for infectivity and/or PrP^{TSE} in at least one form of TSE		
Tissues	**Cattle BSE**	
	Infectivity	**PrP^{TSE}**
Peripheral Nervous system		
Peripheral nerves	+	+
Enteric plexuses[3]	NT	+
Lymphoreticular tissues		
Spleen	-	-
Lymph nodes	-	-
Tonsil	+	-
Nictitating membrane	+	-
Thymus	-	NT
Alimentary tract		
Tongue[4]	-	NT
Esophagus	-	NT
Fore-stomach[5]	-	NT
Stomach/ abomasum	-	NT
Duodenum	-	NT
Jejunum	-	NT
Ileum[6]	+	+
Large intestine	-	NT
Reproductive tissues		
Placenta	-	NT
Other tissues		
Lung	-	NT
Liver	-	NT
Kidney	-	-
Adrenal	NT	NT
Pancreas	-	NT
Bone marrow	+	NT
Skeletal muscle[7]	(+)	NT
Blood vessels	-	NT
Nasal mucosa	-	NT
Salivary gland	-	NT
Body fluids		
CSF	-	NT
Blood[8]	-	?

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Table 1c: Tissues with no detected infectivity or PrP^{TSE}

Tissues with no detected infectivity		
Tissues	**Cattle BSE**	
	Infectivity	**PrP^{TSE}**
Reproductive tissues		
Testis	-	NT
Prostate/Epididymis/ Seminal vesicle	-	NT
Semen	-	NT
Ovary	-	NT
Uterus (Non-gravid)	-	NT
Placenta fluids	-	NT
Fetus[9]	-	NT
Embryos[9]	-	NT
Musculo-skeletal tissues		
Bone	-	NT
Heart/pericardium	-	NT
Tendon	-	NT
Other tissues		
Gingival tissue	NT	NT
Dental pulp	NT	NT
Trachea	-	NT
Skin	-	NT
Adipose tissue	-	NT
Thyroid gland	NT	NT
Mammary gland/udder	-	NT
Body fluids, secretions and excr*etions*		
Milk[10]	-	-
Colostrum[10,11]	(-)	-
Cord blood	-	NT
Saliva	NT	NT
Sweat	NT	NT
Tears	NT	NT
Nasal mucus	NT	NT
Bile	NT	NT
Urine	-	NT
Faeces	-	NT

Footnotes

1. Infectivity bioassays of cattle tissues have been conducted in either mice or cattle (or both). Differences in relative levels of infectivity are not indicated.
2. No experimental data about infectivity in bovine pituitary gland or dura mater have been reported, but human cadaveric dura mater patches, and growth hormone derived from cadaveric pituitaries have transmitted CJD to hundreds of people and therefore must be included in the category of high-risk tissues.
3. In cattle, limited to the distal ileum.
4. In cattle, infectivity bioassay was negative, but the presence of PrP^{TSE} in palatine tonsil has raised concern about possible infectivity in lingual tonsillar tissue at the base of the tongue that may not be removed at slaughter [Wells et al., 2005].

5. Ruminant forestomachs (reticulum, rumen, and omasum) are widely consumed, as is the true stomach (abomasum). The abomasum of cattle is also a source of rennet.

6. In cattle only the distal ileum has been bioassayed for infectivity.

7. Muscle homogenates have not transmitted disease to cattle from cattle with BSE. However, intracerebral inoculation of a semi-tendinosis muscle homogenate (including nervous and lymphatic elements) from a single cow with BSE has transmitted disease to over-expressing transgenic mice at a rate indicative of only trace levels of infectivity [Buschmann and Groschup 2005], and recent published and unpublished studies have reported the presence of PrP^{TSE} in skeletal muscle in experimental rodent models of scrapie and vCJD [Beekes et al, 2005], in experimental and natural infections of sheep and goats [Andreoletti et al., 2004]. Bioassays to determine whether PrP^{TSE} is associated with transmissibility in these experimental or natural infections are in progress.

8. A wealth of data from studies of blood infectivity in experimental animal models of TSE has been extended by recent studies documenting infectivity in the blood of sheep with naturally occurring scrapie, and (from epidemiological observations) two blood-associated vCJD transmissions in humans. Blood has not been shown to transmit disease from patients with any form of 'classical' TSE, or from cattle with BSE (including fetal calf blood). However, several laboratories using new, highly sensitive methods to detect PrP^{TSE} claim success in studies of plasma and/or buffy coat in a variety of animal and human TSEs. Because the tests are all in a preliminary stage of development (and do not yet include results on blinded testing of specimens from naturally infected humans or animals), it is too early to evaluate the validity of these tests with sufficient confidence to permit either a negative or positive conclusion.

9. Embryos from BSE-affected cattle have not transmitted disease to mice, but no infectivity measurements have been made on fetal calf tissues other than blood (negative mouse bioassay) [Fraser and Foster 1994]. Calves born of dams that received embryos from BSE-affected cattle have survived for observations periods of up to seven years, and examination of the brains of both the unaffected dams and their offspring revealed no spongiform encephalopathy or PrP^{TSE} [Wrathall et al. 2002].

10. Evidence that infectivity is not present in milk includes temporo-spatial epidemiologic observations failing to detect maternal transmission; clinical observations of over a hundred calves nursed by infected cows that have not developed BSE; and experimental observations that milk from infected cows has not transmitted disease when administered intracerebrally or orally to mice [Middleton and Barlow 1993; Taylor et al. 1995] Also, large volumes of milk and colostrum from experimentally infected cows have been concentrated and tested for the presence of PrP^{TSE}, with negative results [Everest et al 2006].

11. A single bioassay in over-expressing transgenic mice of colostrum from a cow with BSE gave a negative result [Buschmann and Groschup 2005] – See also note 10.

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