REPORT ON THE SAFETY OF SHEEP INTESTINE AND NATURAL CASINGS DERIVED THEREFROM IN REGARD TO RISKS FROM ANIMAL TSE AND BSE IN PARTICULAR

REPORT PREPARED FOR THE TSE/BSE *AD HOC* GROUP OF THE SCIENTIFIC STEERING COMMITTEE

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OBJECTIVES OF THE REPORT

The current report is to provides data and background information useful to determine whether or not the TSE infectious load of a sheep intestine and natural casings derived therefrom can be considered to be negligible, the cleaning efficacy, the age of the source animals, the section of the intestine used for casing production and in which part of the intestine most of the infectivity is located.

INTRODUCTION

The term casing refers to the envelope enclosing an animal product, principally containing meat, offals (like liver) or blood for human consumption, the whole being termed a sausage. This report refers strictly and only to the casing and not to the contents.

Casings may be artificial (non-animal derived) that present no TSE risk, natural (derived from intestines and sometimes stomachs of animals, that might create a TSE risk) and collagen (a manufactured casing derived from animal by-products, mainly cattle, by an industrial process). The TSE risk for casings prepared from bovine collagen depends on the inherent safety of the source material, any cross-contamination from SRM and the process of manufacture. A separate risk analysis is required for this product.

This report refers only to the TSE risks in natural casings derived from small ruminants (sheep and goats). However, it is noted that natural casings are also prepared from cattle, pigs and more rarely, horses and deer. In the EU no casings are made from native-born cattle because cattle intestine is a specified risk material (SRM).

This report concerns only TSE risks from the small intestine (duodenum to ileo-caecal junction inclusive) from small ruminants (sheep and goats) and natural casings derived therefrom. Natural casings from small ruminants are the only commodities harvested and traded internationally from these species. TSE risks in the tripe organs (oesophagus to abomasum inclusive) and the large intestine are excluded. It cannot be excluded that small local enterprises harvest large intestines for local use. Tripe is used in petfood and human food, but for the latter purpose cattle are the predominant source species.

Natural casings must be distinguished from the intestines and chitterlings. Chitterlings are the washed intestines from pigs and other animals including sheep and goats. Chitterlings have minimal post-collection treatment and are anatomically more closely aligned with intestines than casings. If there is TSE infectivity in these materials it will be most concentrated in fresh intestines and chitterlings since the former reflects the infectivity state in the living animal and the latter is cleaned only by removal of manure. Natural casings on the other hand have consistent and severe processes applied to them to remove the mucosal (lumenal) surface, where most TSE infectivity (if present) resides, and the outer serosal and muscular layers (EU CRAFT PROJECT, 1994), see below for a description.

Preparation of natural casings involves licensed abattoirs where intestines are removed from the sheep and sent to the gut room, and a casings preparation plant that is usually in a separate premises. Some pre-processing is done in the abattoir.

This report deals with the generalities of the collection and processing of small intestines of small ruminants and after-procedures up to despatch. It is based largely on the operation in the UK where machines are used for processing. However, following discussion with representatives of the International Casing Industry including the European Natural Sausage Casings Association (ENSCA) and after consultation with members of the Scientific Working Group of the International Casing Associations the following comments are made:

- "The process of desliming intestines (removal of the mucosa and some outer layers) intestines is the most important factor for the quality of natural casings with respect to marketing aspects.
- There are some differences with respect to the species of origin, geographical origin and processing of natural casings:
  - Hog casings – have a worldwide distribution and desliming is achieved by machine-processing
  - Beef-casings – only coming from South-America (GBR Category I countries) – also only machine desliming
  - Sheep-casings – machine processing in EU, Australia, New Zealand, South America and USA.
  - In China, Mongolia and Oriental Countries like Iran, Turkey, Egypt and others the desliming is performed by hand. This gives some better yield with respect to holes and less tears.
- The finished product is not distinguishable whether the intestine is processed by hand or machinery.
- As the market for processing (desliming) machinery is not so big in terms of industrial investment, there are only 3 companies world-wide involved in this business. Some small companies in Non-EU-countries sell "copies" of established machinery. Technically these machines are virtually identical to the prototypes from the established companies".

The report focuses upon the TSE risk in the source material (intestine) and the risk reduction that occurs as a result of the processing.

The report deals principally with intestines and natural casings derived from sheep, but the general principles can be applied to goats as well.

THE HAZARD

In regard to this risk assessment, the hazard under consideration is from the BSE agent. This is because the BSE agent is regarded conclusively as the cause of BSE in cattle and the probable cause of vCJD in man as well as TSE in various other species of BOVIDAE and FELIDAE, none of which provide food for human consumption. In the context of natural casings the hazard could only stem from the intestine because this is the only animal-derived material used in the production of natural casings.

BSE infectivity could result, either from inherent infectivity within the intestine at the time of slaughter or, hypothetically, from cross contamination from other infected tissues within the carcass. Inherent infectivity, if present, does not occur in all parts of the intestine but only in organised lymphoreticular tissue and possibly also in nervous

tissue (nerve plexuses). It follows therefore that, if the lymphoreticular and nervous tissue is completely or partially removed, there will be a complete or partial removal of the inherent infectivity too. This report will discuss this in more detail below.

To date there have been no reports of the occurrence of natural BSE in small ruminants. Thus, in this report, any reference to BSE in these species is related to the experimental production of the disease under laboratory conditions as a result of challenge with brain material from cattle with confirmed BSE. Naturally this would never have happened in the past and could not happen in the future. The source of any potential BSE infection in the past would have been processed mammalian protein in the form of meat-and-bone-meal and derived from infected starting materials, principally SRM. Though there is currently no scientific report available on in-progress studies aimed at determining if natural transmission of experimental BSE from sheep to sheep can occur, such as maternally, this possibility cannot be excluded.

Small ruminants can be naturally affected by scrapie, a TSE naturally confined to these species, caused by various strains of scrapie agent (that is biologically and molecularly different from the BSE agent) and which are not regarded as human pathogens. Much of the risk assessment for BSE in sheep is based on knowledge from natural or experimental scrapie in sheep and goats.

TISSUE DISTRIBUTION OF INFECTIVITY IN SHEEP AND GOATS WITH NATURAL TSE

A summary of the current knowledge is presented by the EC (EC, 2002a).

What the tissue distribution of the BSE agent would be in natural BSE in small ruminants is unknown, because the disease is hypothetical and speculative. However, it is more likely to be similar to the distribution of scrapie agent in natural scrapie in sheep and goats than to natural BSE in cattle, that is, it would have a wide distribution in lymphoreticular tissues and nervous tissues. The intestine, lymph nodes, brain, spinal cord, associated ganglia and possibly other tissues come into consideration. Since the original studies reported by Hadlow *et al* (1979, 1980, 1982) were completed, the *PrP* gene and the PrP gene have been discovered. This has had two effects.

First, some *PrP* genotypes of sheep have such long incubation periods that they are for practical purposes currently regarded as forming a population of scrapie-resistant sheep. Whilst there are some uncertainties still remaining about the completeness of the resistance, such as in relation to the possibility of a carrier status, the information currently available is so robust that several countries are adopting policies to increase the resistance alleles in their national sheep flocks. Also, sheep harbouring at least one ‘resistance’ allele and no allele associated with highly susceptibility appear to have some resistance to TSE and a more restricted or absent distribution of TSE infectivity in peripheral tissues (particularly at young ages) than do completely susceptible sheep.

Second, immunohistochemical detection of PrP, using a range of techniques/antibodies, has been utilised to determine the precise sites of infectivity in infected organs. Some have used these detection methods on gut and lymphoreticular tissue and have equated the finding of PrP with the occurrence of infectivity. Whilst this has been evaluated in brain tissue no validation is yet reported for extraneural

tissues. Since the methods used cannot distinguish PrP^{C} from PrP^{Sc}, reliance has been placed upon the destruction of PrP^{C} by proteinase K. Some studies *e.g.* Keulen *et al,* (1999a, b) have identified PrP not only in the gut-associated lymphoid tissue (GALT) but also in the enteric nerve plexuses (Auerbach's and Meissner's plexus). Comparable infectivity studies have no been done to validate the assumed association between PrP and infectivity. Heggebø *et al,* (2000) remarked upon the importance of the ileal Peyer's patch as an important site for the uptake of scrapie agent. Interestingly they found PrP in the *lamina propria* even of scrapie-free lambs.

For the purpose of this paper it will be assumed:

- That the tissue distribution of infectivity in natural BSE in sheep will be the same as in natural scrapie and be similarly modified by the age and genotype of the host and
- That any infectivity in the small intestine will be in organised lymphatic tissue (GALT) notably Peyer's patches and in the two enteric nerve plexuses.

Thus any TSE-risk reduction resulting from the process of making a natural casing will be related to the completeness of the removal of the GALT and the two nerve plexuses, even though to date there are no reports that infectivity, as distinct from PrP, exists in the nerve plexuses. In this regard it is important that the comparison is made between the fresh, unprocessed intestine collected within minutes of death and as it is in life, and the natural casing. This is because immediately following death (even in a few minutes) the mucosal surfaces starts to auto-digest as a result of exposure to its content of enzymes and those present in the microbiological/cellular content of the lumen.

INFECTIVITY TITRES IN INTESTINAL AND OTHER TISSUES

An important missing component at the time of writing is the absence of data on the amount (titre) of BSE infectivity in any infected tissues. This is because there are no reports of infectivity titrations, including for the intestine. However, in the absence of this information it is assumed that any titres that are present may be closely similar to those published for goats, but only in the clinical phase of scrapie (Hadlow *et* al, 1980 and Suffolk sheep in the pre-clinical and clinical phase of disease (Hadlow *et al,* 1982) and other sheep (Hadlow *et al*, 1979). Unfortunately even these detailed studies did not investigate the titre of infectivity in parts of the small intestine other than the ileum (within 10cm and mostly 4-6cm from the ileocaecal valve, (W.J. Hadlow, Personal Communication) which is rich in lymphatic tissue in the form of Peyer's patches. These authors did, however, report on infectivity in the proximal colon (rich in lymphatic tissue) and distal colon (less rich in lymphatic tissue). Whereas, in Suffolk sheep and in goats intestinal tissue from these sites rich in lymphatic tissue often had detectable infectivity and this was consistent in Suffolk sheep from at least 10 months of age if infectivity was found elsewhere like in the spleen and lymph nodes, it was always less, and sometimes absent, in those parts of the intestine with lower amounts of lymphatic tissue or with no lymphatic tissue. Infectivity was equated with the occurrence of Peyer's patches (GALT = gut-associated lymphatic tissue, (Reynolds and Morris, 1983, 1984, Landsverk, 1984, Lowden and Heath, 1992). In some breeds, individual sheep with natural scrapie confirmed by microscopic examination of the brain had no detectable infectivity at all in the ileum, and in one case in a Montadale sheep, none in any tissue, except the CNS. When

significant levels of infectivity were found in the ileum however they were of the same order of magnitude as in lymph nodes from a wide range of body sites and in spleen and tonsil. The data from these studies showed that in the intestine marginally the highest level of infectivity was in the ileum as compared with infectivity in the colon as defined above[1]. Thus it would seem logical that if a TSE risk was perceived for the intestine, then lymph nodes also would represent a risk. The size of the nodes is irrelevant, what is important is the amount of infectivity they contain. The highest risk part of the intestine is the ileum since it is the part with the highest infectivity and this was related to its high content of GALT.

In regard to natural casings, as distinct from intestine, if the presumed infected lymphatic tissue was removed before sale to the public, the TSE risk in the lymphatic tissue would be removed along with it, disregarding for the moment risks from cross-contamination. Partial removal would result in a risk reduction, though not elimination of infectivity in the GALT. Even if GALT were completely removed any infectivity in Meissner's plexus would remain as this is within the sub-mucosa that forms the casing. It therefore becomes important to determine:

- If infectivity (as distinct from PrP) is present in the sub-mucosal nerve plexus
- How much this contributes to the infectivity of the intestine as a whole.

It is noted that the studies by Hadlow *et al* used random-bred, female Swiss mice for the bioassays, which are likely to underestimate the real infectivity by some unknown factor because of the species barrier between sheep and mice (*cf*. infectivity bioassays in mice and cattle showing that mice underestimate the titre by a factor of 500 times, Wells, 2001). Thus the 'real' titres determined by i/c inoculation of sheep of the same *PrP* genotype may be higher than those reported by Hadlow. Therefore, any estimate of the reduction in risk by processing any material from infected sheep, including natural casings, might be correspondingly larger than currently envisaged (for example, 80% reduction of 6 logs of infectivity is vastly more efficient than 80% reduction of 2 logs of infectivity).

THE AGE OF SOURCE ANIMALS

In Europe most sheep are kept to produce meat and wool with a minority used for milk production that might nevertheless predominate in certain localities. Goats on the other hand are largely kept for milk production. Thus, on average because lamb is the major product from sheep, most will be killed younger than goats. Annually, more sheep are killed as lambs than adults. For example in the UK (disregarding the effect of sheep culling for Foot and Mouth Disease control in 2001), out of a sheep population in excess of 40 millions, 16 million are killed annually for lamb and about 2.5 million as ewes. 'Lamb' is a loose description for an unbred sheep. Lamb production from native-born sheep is seasonal, commencing with lambing in late December in the UK and continuing until June though there are some flocks *e.g.* of Dorset Horn sheep that can lamb out of season or be bred twice in the year. The earliest lambs for a season are produced fat by Easter but the majority is produced fat during the rest of the year to suit the market demand. On average the age of lambs slaughtered in the summer months is lower than in autumn, winter and spring.

[1] Hadlow (1983), shortly after his studies had been published, reported to the USDA that 'virus may not be distributed uniformly in the contents of the distal intestinal tract and may occur there in low concentrations'. He thought more effort should be needed to find virus in faeces.

Hoggets are older lambs that can be over a year old and are predominately killed at the start of the year. Adult sheep (rams and ewes) can be killed at any time but predominantly are killed in the autumn as a result of the need to dispose of defunct sheep that are unsuitable for breeding to produce lamb in the coming season. Different situations may exist in other European countries.

In the UK, and probably in other countries, casings from lambs predominate over casings from ewes. Around 9.5 millions of lambs (60%) are estimated to be six months old or less. The remainder range in age from 6 up to about 14 months. Adults can be of any age with relatively few being over six years old though this will depend on the type of sheep.

Casings are estimated to be collected from about 85% of slaughtered sheep. The age range might be estimated to be as follows in the UK: < 6 months 8.6 millions, 6-12 months 5.5 millions and > 12 months, 1.9 millions.

PART OF THE SMALL INTESTINE USED FOR NATURAL CASINGS

The small intestine starts at the exit from the abomasum (the pylorus) and extends to the junction with the caecum at the ileo-caecal junction. The International Natural Sausage Casings Association (INSCA), North American Natural Casings Association (NANCA), European Natural Sausage Casings Association (ENSCA) and the Natural Sausage Casings Association (NSCA) have been advised for several years to remove the distal ileum or indeed the whole ileum before processing small ruminant intestines for natural sausage casings for human consumption. This is because the highest titres of scrapie infectivity in the intestine occur in the distal ileum (Hadlow *et al*, 1979, 1980, 1982, Hadlow, 1983) that at least in theory might be mirrored if BSE occurred naturally in small ruminants. However there is a deficit of information on the scrapie-infectivity of parts of the sheep intestine proximal to the ileum. Practical methods able to remove the ileum successfully and consistently within the gut room of an abattoir, either manually or by machine have been developed in the UK. In practice the whole ileum and a small part of the jejunum is removed[2].

WHERE THE ILEUM IS REMOVED

Intestines used to produce natural casings are only sourced from animals destined for human consumption, slaughtered in licensed abattoirs following official *ante* and *post mortem* examination and passed fit for human consumption. The whole process of slaughter and subsequent procedures in the abattoir are in principle subject to official control[3].

After the abdominal cavity is opened, stomachs with the intestine attached are sent to the gut room. The intestines (large and small) are cut from the abomasum at the pylorus. The whole ileum and part of the jejunum is removed either using a pulling machine or by hand.

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2 This has recently been shown (T. Hiepe, and P. Comer, Personal Communication) to be simple and provides a virtual total achievement of the objective when carried out by trained individuals.

3 For the EU: in accordance with Council Directives 64/433 and 93/43 and employing hazard analysis and critical control point procedures.

RISKS FROM CROSS CONTAMINATION IN THE ABATTOIR

Embolic spread of infected material *(See EC, 2002b)*

The first risk stems from the possibility of cross-contamination as a result of the stunning procedure applied by law before killing by bleeding out. Currently electrical stunning, which is the most common method used for stunning small ruminants, is regarded as presenting a negligible risk. In some abattoirs (particularly those with a low throughput), may stun sheep by methods that penetrate the skull and damage the brain. A cartridge operated captive bolt pistol can cause brain emboli to enter the venous system in sheep (but not in cattle) and is still permitted in the EU but more research has been advised to confirm the observation, (EC, 2002b).

Captive bolt pistols that inject air under pressure into the cranial cavity, and any brain penetrating method followed by pithing has been shown in some cases to create risks of brain emboli being discharged into the venous system. Such emboli are likely to be trapped in the right side of the heart or the lungs or be present in the blood that exits from the stick wound to the blood trough. Such methods of stunning are now banned in the EU. A recent report on these topics (Scientific Opinion, 2002) recommends that further work be done to investigate the risk of emboli getting to the systemic circulation from risk methods of stunning and thus be distributed to remote tissues that could include the intestine.

In summary, whereas electrical stunning presents no discernible risk, brain-penetrative stunning of any kind may do in small ruminants. This potentially creates the risk of emboli from clinically normal sheep with TSE infection getting at least into the venous blood drainage, the lungs and right side of the heart and potentially to any part of the body. There are alternative methods of non-penetrative stunning that could be used and would eliminate this risk. Alternatively in this situation the application of an approved PrP test on brain material could be used to reject carcases and offals from positive animals from the food chain. This would only be completely effective if the sensitivity of the test was as sensitive as a bioassay. Furthermore, such a test would not detect PrP that could exist in high quantity in peripheral tissues before infectivity had reached detectable levels in the brain.

Risks from accidental cross-contamination from specified risk materials (SRM)

A wide range of tissues could carry BSE infectivity and in theory might be a source of infection for cross-contamination of intestine. However, in practice these theoretical risks can be eliminated by careful application of meat hygiene rules.

The risks from brain have been partly described in the paragraph above. The possible risk from blood (other than within the circulatory system – see above) are negligible because bleeding is done with the carcass in the vertical, hanging position at a point vertically below the abdomen which is not opened until bleeding has ceased. Because the head is removed before the abdomen is opened there is no risk of cross-contaminating intestine by direct or indirect contact if the hygiene rules are followed.

There is no risk either from spinal cord or any part of the vertebral column, since lamb carcases under one year old are not split at all and those over a year old the carcass is not split to expose these structures until after the stomachs and intestine are removed from the abdominal cavity and they enter the gut room. In fact they are not

usually split in the abattoir but in a licensed cutting plant. Rodding (closure and sealing of the oesophagus to prevent leakage of stomach contents) and bagging (sealing of the rectum to prevent faecal leakage) prevent any contamination of the external surface of the intestines from these sources.

On entry to the gut room the intestine is removed from the stomachs by severing the duodenum at the pylorus within about 1 cm of the pyloric sphincter. The next procedure is 'pulling' which separates the small intestine from its suspending mesentery and mesenteric lymph nodes (which are not cut) and from the distal jejunum, ileum and large intestine. The small intestine retained extends from the duodenum to the distal jejunum and no part of the ileum is present and so cannot cross-contaminate the part of the intestine from which the natural casing is made. The risk from cross contamination from the distal ileum or ileum from the same or different sheep can be regarded as negligible. The ileum is an anatomically defined part of the terminal small intestine as follows:

> *The World Association of Veterinary Anatomists authorises the term 'ileum' that is defined in Nomina Anatomica Veterinaria, as "the short terminal part of the small intestine to which the Plica ileocacalis (ileocaecal fold) is attached." The distal ileum is that half of the ileum that is joined to the caecum.*

Pulling can be done by hand or by machine, the latter also acting as a manure stripper. See video programme for details. The following description is quoted from a video tape entitled '*Production of natural sheep casings – Removal of the distal ileum*" and produced on behalf of the Natural Sausage Casings Association UK, to which Association the majority of natural casings manufacturers in the UK belong.

> *'Although the distal ileum comprises only half of the ileum, in practice the whole ileum is removed, with a short part of jejunum as well.*
>
> *Having removed the stomachs from the intestines, the remaining viscera are hung in 'sets' on the carousel of the device, called a pulling machine. Its purpose is to separate the small intestine from the ileum and the large intestine and to remove the majority of the manure that exits from the duodenal and jejunal ends. Several sets of viscera are loaded together. The centre of the small intestine is threaded on to the pulling machine. Several sets of intestine are threaded at the same time.*
>
> *Then, as the pulling machine reaches the last part of the small intestine, the tissue breaks, leaving the residue behind.*
>
> *This method of removing the distal ileum avoids contamination of the intestines used to prepare natural casings.*
>
> *There is a relatively long section of small intestine left behind. This comprises the ileum, including the distal ileum and a small portion of the jejunum, and thus more than satisfies proposed recommendations.*
>
> *Then the 'runners', as they are now called, are placed into barrels of cold water for transport to the casing preparation premises. All waste material is then disposed of.*
>
> *Pulling runners by hand is also practical. The process is similar, but the point at which the bung end of the runner is cut must now be determined by the operator using the end of the ileocaecal fold as a marker for the jejunal end of the ileum. This demands careful training and supervision. Hand-pulling must equate with machine-pulling in all respects.*
>
> *On arrival at the casing preparation plant, the runners are subjected to a rigorous sequence of cleansing and quality inspection; everything in accordance with the*
>
> *Recommendations of the International and the European Natural Sausage Casings Associations.*

The casings are passed through a series of cleaning machines and tanks of hot water.

Both the inner and outer layers of the small intestine are carefully removed.

Finally, the casings are passed through a finishing machine.

Quality control is continual, and additional checks are made, such as measuring the gauge of the casings, and making sure there are no holes. Any faulty casings are discarded.

Then the runners are collected into hanks of 50 and salted.

They are then placed in barrels of salt for a minimum of 30 days, prior to dispatch.'

FACTORS TO BE TAKEN INTO ACCOUNT WHEN MAKING AN ASSESSSMENT OF THE TSE RISK TO THE CONSUMER AND IN REGARD TO NATURAL CASINGS AND BONE-IN MEAT

In this regard when the risk reduction is quantified it is essential to make a comparison between the infectivity present in the intestine as it is in the animal and that which resides in the casing. This is because a natural risk-reduction process occurs immediately the animal is killed and which is attributed to loss of part of the mucosa and lamina propria (in which some of the infectivity resides), as a result of autolysis and the stress of killing. To secure a sound baseline for the infectivity in the intestine as it is in life (as is done by those conducting bioassays for TSE infectivity), it is necessary to take steps to reduce the effects from these processes by simple but important techniques familiar to enteric pathologists.

Furthermore it must be understood that the absolute amount of infectivity remaining in a prepared casing is the important criterion in determining the TSE-risk for the consumer. In this regard it is important to also take account of the dose of infectivity that a consumer might consume at one meal. Casings are only eaten as an envelope of sausages rather than as a commodity on its own. Casings therefore contribute a relatively small amount by weight to a meal of sausages. Thus the dose of infectivity that might be consumed (if residual infectivity was present) will be calculated as a product of the weight of the casing multiplied by the absolute residual infectivity titre per unit mass of the casing. This contrasts with TSE-risk in bone-in meat from the same infected animal. Here there is no risk reduction process applied to the 'meat', *i.e.,* the whole joint comprising meat, bone and its marrow, lymph nodes and nerves in which in an infected animal TSE infectivity might reside. Infectivity in lymph nodes of bone-in meat is of a similar titre to that in intestines (as they exist in the live animal) and if present in the bone marrow or nerves may not be less, particularly if the meat includes vertebral bones.

PARTS OF THE INTESTINE IN WHICH TSE INFECTIVITY MAY RESIDE

The studies conducted by Hadlow *et al* (1979, 1980 and 1982) clearly demonstrate that infectivity appears to be associated mostly, if not entirely to GALT. However, subsequent studies using immunohistochemistry to detect PrP (van Keulen *et al,* 1999, 2000) have shown that in scrapie-affected animals PrP is found not only in the GALT but in the enteric nerve plexuses of Auerbach and Meissner where it existed in neurons and glial cells. This report identified the ileum as being consistently showing PrP in the nerve plexuses but only if the animals were clinically and pathologically confirmed as having scrapie. Such sheep are prohibited from use for human food or for any purpose. Jeffrey *et al,* 2001, showed similarly that the enteric nerve plexuses

showed PrP staining if the animal from which they came had clinical scrapie and/or presence of PrP in the brain but not otherwise.

In a subsequent study, van Keulen *et al*, 2000 reporting on the pathogenesis of natural scrapie in sheep found evidence of PrP in Peyer's patches of all susceptible animals (except those with a *PrP* genotype ARR/VRQ at the age of five months and older but at this age not in the enteric nervous system. By ten months of age it was found in the enteric nervous system.

All parts of the alimentary tract contain nervous tissue in the form of neurons, myelinated and non-myelinated nerve fibres and associated supporting cells. No method has yet been reported to show that the myenteric plexuses of the gut specifically contain infectivity but research in mice (Kimberlin, 1986) and hamsters (Beekes, Baldauf and Diringer, 1996, McBride and Beekes, 1999) has shown that the autonomic nervous system is involved in the transport of infectivity between gut and central nervous system. Thus it is a reasonable assumption to make that in the gut both infectivity and PrP can be present in both Peyer's patches and in the enteric nerve plexuses. The precise timing of the onset of infection in these parts of the intestine may be controlled partly by the age of the animal at the time of exposure, the route of exposure if it is not oral, partly by the *PrP* genotype and partly by the strain of agent.

The contribution to the total infectivity of the intestine that GALT and the enteric nervous system makes, is unknown. However, it might reasonably be suggested that the Peyer's patch contributes more than does the enteric nervous system, based upon the subjective assessment of the PrP staining in the two systems.

Within the Peyer's patches PrP is detected in highest quantity in association with follicular dendritic cells (FDC) where replication is believed to take place (Mabbott and Bruce, 2001). Tingible body macrophages, also harbour PrP. Other cells such as intestinal M cells, transport antigenic macromolecules and some pathogens from the gut lumen to the patch (Trier, 1991). It is therefore possible that M cells transport PrP in this way and so may transiently harbour PrP. A very recent publication (Huang *et al* 2002) reports that, at least in mice, migrating intestinal dendritic cells derived from bone marrow transport PrP^{Sc} from the gut directly into lymphoid tissues *in vivo*. These dendritic cells are quite different from resident follicular dendritic cells located in germinal centres that are believed to replicate/accumulate the agent. The PrP^{Sc} in M cells (if present) and/or in intestinal dendritic cells probably contributes a small amount to the total since lumenal PrP^{Sc} is likely to be transiently present. Tingible body macrophages are believed to scavenge material from dead cells including PrP that accumulates in lysosomes. However, this PrP is truncated with the N-terminal cleaved. At present it is unclear as to whether or not this PrP is infectious. What is clear is that the fixed cells of the patch that contribute most PrP are FDC. Thus complete removal of FDC would presumably have the greatest effect on infectivity reduction from GALT. No work yet reported has informed on this in natural casings. In the intestines of sheep with experimental BSE reported by Foster *et al,* 2001, tingible body macrophages did not stain for PrP as they do in sheep with scrapie (Jeffrey, *et al,* 2000) though this may have been due to the type of antibody used.

Collectively, these studies show that tissue exists in the intestine of sheep that is able to harbour TSE, including BSE infectivity. These tissues are GALT, nerve cells and glia within the two nerve plexuses of the gut. GALT probably contributes more PrP and thus infectivity, than do the nerve plexuses. However it is noted that the two

nerve plexuses are relatively evenly distributed throughout the alimentary tract including the small intestine and from van Keulen *et al* (1999, 2000) studies, if infected would probably contribute a fairly consistent amount of infectivity. By contrast, the Peyer's patches are variable in distribution, the largest being in the ileum but others being present in the jejunum at least. Thus, the anatomical description of the three parts of the small intestine (duodenum, jejunum and ileum) does not inform specifically on the infectivity in any particular small section within these three anatomical parts. The load of infectivity present in such sections contributed by GALT is therefore directly related to the presence or absence of GALT at that site. In general there is more GALT distally than proximally and less GALT exists with increasing age (Reynolds and Morris, 1983). No data exist to quantify the amount of infectivity in different parts of the small intestine or in the parts (GALT or enteric nervous system) that contribute to it. Since no titrations have been done on gut in experimental BSE there is no way of determining the level of risk in the intestine except by cross-reference to the Hadlow studies in scrapie.

In regard to GALT, FDC probably contribute the highest amount of PrP within the Peyer's patch. Intestinal dendritic cells and tingible body macrophages (both of which are mobile cells) and M cells probably contribute less. A critical question to answer therefore, when assessing any TSE risk in a natural sheep casing would be, in regard to GALT, 'How many FDC are still present?' In regard to the two enteric nerve plexuses it is not possible to determine if they contribute equally to the infectivity from these sources or not. For practical purposes it is assumed they contribute equally.

COMPARATIVE STUDIES IN LYMPH NODES, NERVES AND PLACENTA

What is clear is that the titre of infectivity in lymph nodes is of the same order of magnitude (at least in scrapie) as in the ileum or distal ileum (the part of the gut generally assumed to have the highest degree of infectivity). Madec *et al,* 2000 reported some quantitative studies on the amount of PrP in brain and lymphoid organs including the spleen, lymph node and tonsil with natural scrapie. These authors found similar amounts of PrP in the three lymphoid tissues that were generally lower than levels found in the cerebellum but in some cases were greater than levels found in the frontal cortex.

Race, Jenny and Sutton (1998) showed similar levels of infectivity in spleen, lymph node and placenta in sheep with natural scrapie but lower levels than in brain, based on the incubation time in mice. Since pregnant sheep enter abattoirs and might be infected with a TSE there is therefore a possibility that in some circumstances cross contamination events of the carcass meat might occur from the placenta.

Groschup *et al,* (1996) reported on the distribution of infectivity in a range of peripheral nerves that innervate many of the muscles used for human consumption from a single Suffolk sheep with clinical scrapie. The infectivity titres were calculated to be in the range 10^3 - $10^{4.5}$ mouse infectious units per gram whereas the infectivity in the cerebellum was 10^6 mouse infectious units per gram. It is concluded that since these nerves are present in joints of sheep meat (mutton) it is possible that significant levels of infection might be present at the time of slaughter if the sheep was infected (and close to clinical onset). However, the level of infectivity would be expected to be lower in pre-clinical than in clinical animals. Again, these studies used mice to detect infectivity and so the titres quoted are probably underestimates.

THE POTENTIAL PROBLEM OF INFECTED FEED

If BSE-infected MBM was in the diet of small ruminants, whether infected with BSE or not, it is theoretically possible that this MBM could be present in the lumen of the intestine at the time of slaughter. Thus, the mucosal surface of the small intestine could be contaminated with an external source of BSE infection. Since 1994 throughout the EU the inclusion of MBM in the diet of ruminant animals has been prohibited and from 2000 it has not been permitted in the diet of any food animal species. Although there have been some breaches of this legislation in several countries, their frequency in 2002 is regarded as very low. Furthermore, effective removal of SRM and improved standards of rendering make the risk from MBM much lower than previously.

By contrast, the intestine from lambs would be at greater risk than adults from infected feed in the lumen following the feeding of milk substitutes that contained BSE-infected material. Possible sources could be bovine fat not from discrete adipose tissue (though infectivity has not been demonstrated therein) or fat derived from the degreasing of bones (especially vertebral and skull bones) used for the production of bovine bone gelatin. Skulls of cattle are now SRM in the EU and vertebral bones from cattle over a year old are not permitted into any food chain (with certain safe exceptions because comparable measures are in place, *e.g.* in the UK, the Over Thirty Months Scheme). Only fat from discrete adipose tissues is permitted to be used in animal feeds in the EU therefore risks in 2002 from contamination of the lumen of the intestine with BSE-infected feed is remote and hypothetical. The risks can now be considered as negligible.

TSE RISK ELIMINATION FROM THE SMALL INTESTINE

This is a hypothetical objective that it would be impossible to achieve or prove, not least because one of the nerve plexuses traverses the full length of the sub-mucosa that forms the majority of a natural casing.

TSE RISK REDUCTION FROM THE SMALL INTESTINE

This is an achievable objective but it is not possible to accurately quantify what the level of reduction would be, not least because, no natural cases of BSE exist in small ruminants and because there is no knowledge of the titre of infectivity in any part of the intestine. Any estimates of the risk reduction are therefore speculative and subjective. Some guidance can be given from the starting titres in scrapie but there is no knowledge of the infectivity left in natural casings from scrapie-infected sheep. The best judgements can come from studies showing the intestinal tissues that are removed when intestines are processed into casings. These are as follows:

- Complete removal of the whole ileum and part of the jejunum.

  Comments: *Possible with close to 100% efficiency, auditable macroscopically in the abattoir and effective in removing 100% of the infectivity in the parts removed.*

- Complete removal of the serosa, outer longitudinal muscle, Auerbach's nerve plexus and inner circular muscle.

Comments: *Normally achievable with close to 100% efficiency. Not easily auditable except by using histological methods. Effective in removing close to 100% of infectivity in Auerbach's plexus thus accounting for the removal of approximately 50% of the infectivity in the enteric nervous system of the intestine.*

- Removal of manure.

  Comments: *Achievable to the naked eye with close to 100% efficiency. Auditable also by microbiological methods.*

- Removal of the mucous membrane (epithelium and lamina propria and its contents).

  Comments: *Normally achievable with 100% efficiency in parts and estimated to be > 80% efficiency overall, provided the machinery is working efficiently. Not easily auditable except by using histological methods. Where the removal is 100% any GALT within the lamina propria will be removed with 100% efficiency. However, where a Peyer's patch penetrates through the muscularis mucosae remnants of the patch may remain on the casing. It is not uncommon that where this situation pertains part of the mucous membrane may co-exist also. Inexplicably, small pieces of mucous membrane may still be detectable at irregular intervals.*

The final composition of a sheep casing is of the sub-mucosal layer bounded on one side by the *muscularis mucosae* and on the other by the junction with the inner circular muscle and Meissner's nerve plexus. From place to place there may be small remnants of mucous membrane including epithelium and lamina propria and any GALT present at that site. In other places there may be parts of Peyer's patches retained, particularly where these structures penetrate through the *muscularis mucosae*.

Overall, some 50% of the nerve plexus tissue is removed (because that 50% lies between the two muscle layers which are removed).

In regard to the percentage of GALT removed overall, this is estimated to be greater than 80% and may be 100% in places. However, this estimate may vary between authors (*e.g.,* EU CRAFT Project 1994, 100% removal and Parisi, Julini and Pinzone, 1979 - 100% removal; other studies *e.g.* Gjevre and Toubro, 1997*,* indicate some retention of mucosa with traces of lymphoid tissue and lymphoid cells detected by immunohistochemistry using leucocyte common antigen). No studies report detection of residual FDC in natural casings but almost certainly appropriate methods were not available or not used.

In an unpublished study by R Bradley (Personal Communication) ten sets of bovine casings (which retain the serosal and muscular coats unlike in sheep) examined at five sites along their length showed that out of 47 adequate sections, 13 retained some mucosa, 19 showed lymphocyte infiltration (probably not relevant to containing TSE infectivity), up to 9 showed the presence of lymphoid nodules and 9 showed remnants of Peyer's patches. Overall it was determined that Peyers's patches were substantially reduced in bovine casings compared with the fresh intestine and the loss of organised lymphatic tissue was estimated to be 50%. This was repeated for ten bovine casings at five sites prepared using different machinery. The number of respective sections retaining structures were as follows: Mucosa 6, lymphocyte infiltrations 6, lymphoid

nodules 6 and Peyer's patches 16. Overall it was determined that as in the first study Peyer's patches were substantially reduced in bovine casings compared with the fresh intestine and the loss of organised lymphatic tissue was estimated to be 50%. A very small number of sheep intestines were examined from animals of an unknown age and whilst confirming the main residual tissue to be sub-mucosa, the removal of Peyer's patches was improved over that for bovine intestine but still incomplete. It is clear that it is not possible to completely remove potentially infected tissue from intestines but it is equally clear that substantial risk reductions are possible and that these are likely to be greater for small ruminant than for cattle casings, taking account of the more efficient removal of the mucosa and Peyer's patches and the total removal of half of the nerve tissue.

OTHER FACTORS

The removal of the ileum removes the largest Peyer's patch and the only tissue in which there is consistent and definite knowledge of infectivity. Furthermore the natural casing production process clearly will reduce the level of infectivity in its final product as compared to the raw intestine. It is clear, however, also that if BSE actually was found in sheep none of the processes used could guarantee that all infectivity was removed.

Two other factors need to be considered. The first is the dose of infectivity likely to be consumed at a meal. That consumed by one person from a casing enveloping the number of sausages making a meal would be a very small proportion of the total mass of sausage consumed. The natural sheep casing is on average 0.11 mm thick (EC CRAFT Project, 1994) and length 18-34 metres. The weight of a casing is 11% to 23% of the weight of the manure-stripped intestine from which it is derived. This range of variation is attributed to the age of the sheep, the time of year (that also influences the mean age of lambs for slaughter) and the diet. The mean initial weight of ten sets of intestines is 5.9kg and of ten sets of casings derived from them is 1.36kg (R Harder, personal communication). This means that each metre of a casing weighs 4.0g – 7.5g and that the weight of casing consumed in five sausages would be between 2.0g and 3.75g (assuming that about 0.5 metres of casing was required to envelop five sausages). Very little of the casing would be infected tissue though it is not possible currently to estimate what it is. By contrast, if the popliteal lymph node was consumed from a leg of lamb about 30-600 human oral ID_{50} could be consumed from a node weighing some 1.9g (AFSSA 2002).

The second factor is cooking. Sausages are usually fried or grilled in which the external temperature is around 175ºC which is applied to the outside of the sausage and thus to the casing, for some minutes. The comparable figure for roasting a joint of meat is somewhat higher, around 200ºC and achieved for an hour or more. However only the surface of the meat reaches this temperature and bone marrow, deep lymph nodes and nerves may reach a substantially lower temperature. There is likely to be some reduction in infectivity for at least surface meat but it should no be relied upon to destroy TSE agents. It is however likely that overall a well-cooked sausage casing would achieve a higher and more lethal temperature than the deep parts of a roast joint of meat.

SHORT ANSWERS TO THE QUESTIONS

Disregarding the effect of age and *PrP* genotype that may limit the level of infectivity in the intestine, if the host animal is infected then the intestine is unlikely to have a negligible infectious load. However, a quantitative measurement of this infectious load for the BSE agent is not possible because titration data are not available.

In regard to the infectivity of a natural casing derived from the duodenum and jejunum only, the infectious load will be significantly reduced but it may not be negligible. This is because a quantitative measurement of this infectious load for the BSE agent is not possible because titration data are not available.

The cleaning efficiency removes 100% of the infectivity in the ileum. Any infectivity present in the small part of the terminal jejunum that is discarded is also removed completely. (Since ALL the ileum and a small part of the jejunum is completely removed without cross contamination, any infectivity present in these structures is expected to be completely removed too)

In regard to the remainder of the intestine (duodenum and the majority of the jejunum) the cleaning efficiency of any infectivity in Auerbach's plexus is close to 100%. The cleaning efficiency in regard to removal of Meissner's plexus is inefficient and none may be removed. The cleaning efficiency for the mucous membrane including any lymphoreticular material it may contain in the lamina propria is 100% in places but 0% in others. Overall the efficiency of removal is considered to be >80% but this is a subjective assessment that needs more formal investigation. The cleaning efficiency for GALT in the form of Peyer's patches can be 100% in places where the GALT does not penetrate the muscularis mucosae but in other parts it may be incompletely removed or perhaps rarely not reduced at all. It is uncertain as to whether the GALT remaining contains FDC in which it is presumed the majority of infectivity exists.

GALT is present in higher quantities in young animals than in older ones and is most conspicuous in the terminal part of the small intestine, notably the ileum but exists elsewhere, though not in a continuous patch.

Most infectivity is anticipated to be in the distal ileum and in the proximal colon and to a lesser extent in the distal colon and rectum because these sites contain GALT. None of these tissues are collected by the natural casings industry in the UK and they are not traded. All parts of the alimentary tract contain enteric nerve plexuses and if infectivity is confirmed in them the best that can be expected is that half is removed from casings. It is noted that similar nerve plexuses exist in other organs like the heart. It cannot be excluded that infectivity exists in GALT proximal to the ileum if the host was infected. Indeed it is likely that it does. When judging the level of infectivity by morphological methods it should be measured mainly on the amount of GALT that remains in the casing rather than making a judgement based on the anatomical section of small intestine under consideration (excluding the ileum).

CONCLUSIONS

Natural casings prepared by the European and international natural casings industry are products that have had a TSE risk-reduction process applied to them.

It is impossible to completely remove TSE infectivity from an intestine during the processing to make a natural casing.

It is possible to reduce the intestinal infectivity considerably by removal of the ileum and by the normal methods of natural casing manufacture. Approximately 50% of any infectivity in the nervous tissue is likely to be removed by the latter process and an unquantifiable amount of the intestine in other cell types (e.g., of lymphatic tissues) in the rest of the casing. Nevertheless this amount is likely to be substantial, perhaps estimated overall to be in the region of > 80%. Some parts of the length of the casing may be completely decontaminated and others not.

Cooking may reduce infectivity still further also by an unquantifiable amount.

By contrast to natural casings, meat sold on the bone has no risk reduction process applied to it and heat applied to it during cooking would only secure the same temperature as achieved in the sausage casing in the superficial layers. TSE infectivity would exist in the carcass lymph nodes at levels comparable to those in intestines from the same age and genotype of animal. Infectivity might also exist in peripheral nerves and in bone marrow.

Collectively this report indicates that if there is a TSE risk in intestines the risk would be lower from natural casings than from the intestine. There are a number of serious deficits in knowledge such as titration data for different tissues in sheep experimentally challenged with the BSE agent. There is insufficient knowledge about the contribution made by GALT on the one hand and enteric nervous tissue on the other to the total infectivity. No studies have been reported about the deposition of PrP in neurones of the autonomic nervous system in other body organs such as the heart for example, which are still allowed for human consumption. There is also insufficient knowledge about the contribution that M cells, intestinal dendritic cells and tingible body macrophages (as distinct from FDC) make to the total infectivity in the intestine.

In the EU, in 2002 if all the rules are enforced there should be a virtual absence of any infectivity in the gut contributed by feed. Risks from this source could be regarded as negligible.

There are reasons to be concerned that the continuing use of penetrative stunning of sheep might contaminate other tissues sold legally to the consumer in certain circumstances.

It is accepted that there are likely to be other ways to protect the consumer from exposure to the BSE agent in sheep, which is not part of this report. For example, by permitting only tissues including bone-in meat and natural casings from certain ages of animal or from certain *PrP* genotypes into the human food chain.

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