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OPINION ON

ADIPOSE TISSUE ASSOCIATED WITH THE DIGESTIVE TRACT OF CATTLE, SHEEP AND GOATS: AN APPRECIATION OF POSSIBLE TSE RISKS

ADOPTED BY THE SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF 28-29 JUNE 2001

ADIPOSE TISSUE ASSOCIATED WITH THE DIGESTIVE TRACT OF CATTLE, SHEEP AND GOATS: AN APPRECIATION OF POSSIBLE TSE RISKS

1. MANDATE

At its meeting of 29-30 March, the Scientific Steering Committee (SSC) was invited to address the following question:

In its opinion of 7-8 December 2000, the SSC concludes that the entire bovine intestine from duodenum to rectum is a risk issue. Can the discrete adipose tissue associated with the digestive tract of cattle, sheep and goats be used for the preparation of foodstuffs, for example after treatment in a fatmelter? Or should it be considered as a risk material and handled as such, because it contains also lymph-nodes and because the fat melting in any case is at temperatures below 100°C?

The SSC asked the TSE/BSE *ad hoc* Group to prepare a scientific report on the issue to serve as the basis for an opinion. At is meeting of 26 April 2001. The *ad hoc* Group adopted the report given in Section 3.

2. OPINION

The possible risk linked to discrete adipose tissue associated with intestine is depending upon sourcing and production process.

a. Discrete adipose tissues directly associated with the digestive tract can contain all cell types and tissues that are implicated in TSE pathogenesis after peripheral exposure: parts of autonomic nervous system and lymphoid cells from secondary lymphoid organs. If contamination with intestine occurs, neural cells and Peyer patches derived cells could also be part of the starting material.

b. For cattle, due to the infectivity titre that could be theoretically reached in nervous tissues and in some parts of intestine, and due to the risk of contamination with intestine tissue, adipose tissue associated with the digestive tract could pose a risk in countries with a geographical BSE risk higher then I (GBR > I) unless the production process leading to the desired final product has been validated to sufficiently clear TSE agents. As under experimental conditions infectivity has been found in the distal ileum in cattle a short period of time after experimental oral exposure the possible risk in adipose tissues associated with the digestive tract exists very soon after oral infection in young animals.

   However, when considering the classification of digestive-tract-associated discrete adipose tissues as a specified risk material, it needs to be kept in mind that not the whole mass of tissue constitutes a possible risk. In fact, the tissues possibly carrying infectivity are the mesenteric nerves which are situated near the arteria mesenterica. If slaughter practices permit the removal of the mesenteric lymph nodes and the mesenteric tissue including the area around the arteria mesenterica, the rest of the discrete adipose tissue (including the omentum) should not be considered to be an SRM if there is no contamination with intestine tissues. If this separate removal is not feasible, the whole mesenterium should be considered to be an SRM. The SSC did not address the issue whether such separate removal is feasible or not under practical conditions.

c. Small ruminants infected with BSE agent. On the basis of results obtained with small ruminants experimentally infected with BSE, which show similarities

between the pathogenesis of scrapie and BSE in sheep and the involvement of the lymphatic system, the risk associated with adipose tissues of small ruminants may be higher in BSE infected small ruminants than in cattle. If BSE in small ruminants would become evident or probable under natural conditions, all adipose tissue associated with the digestive tract, including the omentum, should be considered to carry a risk and should be included in the list of specified risk materials[1].

3. REPORT FROM THE TSE/BSE AD HOC GROUP

According to the question posed this risk assessment deals with adipose tissue associated with the digestive tract of cattle, sheep and goats and includes tissues such as the omentum[2]

a. In theory, adipose tissue associated with the digestive tract from cattle or ovine and caprine may contain some constituents that are target for TSE agent replication, i.e. lymph nodes, peripheral nerves, and, if infected at all, blood. The possible risk could be increased by any rough dissection of this tissue which occurs routinely during slaughtering processes and which would contaminate it with intestine tissue itself.

b. TSEs in animal models (general). There are several experimental data which support the role of the lympho-reticular system in TSE agent replication after peripheral routes of infection in animal models. Moreover, the peripheral nerve system (PNS) has been demonstrated to permit neuroinvasion after peripheral routes of exposure. TSE agent route of neuroinvasion is depending upon the genetic background of the host, the species, the route of inoculation and the TSE agent strain. For example, in mouse exposed by intraperitoneal route, scrapie agent is first detectable in liver during the first hours of infection, and as soon as day 5, infection of the spleen occurs, rapidly followed by replication in mesenteric lymph nodes. The same TSE agent strain, when administered by oral route, first replicates in Peyer patches, then in mesenteric[3] lymph nodes and significantly later on in spleen and lymphoid organs that are not associated with the digestive tract. How the TSE agent is transported from the first site of replication to the secondary lymphoid organs is not known, but it is believed that there might be a role for B cells and dendritic cells. In the secondary lymphoid organs, infection is restricted to follicular dendritic cells (FDC), and recently, two publications (Mabbott *et a*l, 2000; Montrasio *et al*, 2000) have reported independently that blockade of the maturation of FDC results in a delay of neuroinvasion and an increase of incubation time. Neurovinvasion may occur through small nerve endings that could be evidenced in lymphoid organs. The mechanisms that permit TSE agent transport inside the peripheral nerve system is not still elucidated (retroaxonal, other?).

   There is also evidence of the possibility of a direct entry of TSE agent after peripheral inoculation, through a deposition of the inoculum into a direct contact to a nerve ending in the peritoneum after *i/p* infection; this could also occur after oral exposure through infection of cells from Meissner or Auerbach plexuses, which are composed of cells from neural origin in the intestinal

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1 See also the SSC OPINION of 8-9 February 2001 providing a Pre-emptive risk assessment should BSE in small ruminants be found under domestic conditions.

2 **Omentum:** A peritoneal fold that passes from the stomach to other viscera. It does not contain suspended intestine.

3 **Mesentery:** A fold of peritoneum, which connects the greater part of the small intestine to the parietal peritoneum at its junction with the dorsal wall of the abdomen. Vessels and nerves, embedded in fat pass in a fan like fashion to and from the dorsum to and from the intestine. Large mesenteric lymph nodes embedded in fat are located in a row at the ventral part of the mesentery and close to the intestine.

mucosae. For example, SCID mice, which are devoid of any functional immune cells, can be infected by *i/p* route in some experimental conditions.

Infectivity has been evidenced in large nerves in both experimentally infected mouse and sheep.

c. BSE in cattle. Infectivity has never been detected outside of the CNS and the retina in naturally BSE affected cattle. However, while some 50 tissues have been examined from such animals, the numbers of animals examined have been very few and the sensitivity of the detection methods used is limited.

As under experimental conditions infectivity has been found in the distal ileum in cattle 6 months[4] after experimental oral exposure (at 4 months of age) with 100 g infectious brain material the possible risk in adipose tissues associated with the digestive tract exists very soon after oral infection in young animals. So far no BSE infectivity has been found in the distal ileum, or in any other extra neural tissue in natural cases of the disease in this organ.

The single available bioassay of mesentery and mesenteric fat (midrum fat) from a naturally occurring case of BSE showed no detectable infectivity. At its ventral border the mesentery contains numerous lymph nodes and the small intestine**.** Mesenteric lymph nodes from clinical cases of BSE have shown no detectable infectivity following bioassay in both mice and in cattle. Furthermore, mesenteric lymph nodes from cattle experimentally infected with BSE showed no detectable infectivity at any stage of incubation as determined by bioassay in mice. (It is noted that bioassays, of mesenteric lymph nodes from cattle experimentally infected orally with BSE, in cattle [within species], whilst currently negative are not yet complete.) The midrum fat itself is thus likely to have a low risk of harbouring BSE infectivity. Nevertheless, with its contiguous intestinal component, or if it is cross-contaminated, there could be an associated BSE risk, especially if the distal ileum was still attached to the fat. Moreover, one has to consider that the detection of infectivity in most of the studies has been using mouse bioassay; this implies a species barrier which from comparison of titrations of inoculum in cattle and mice is of the order of a difference of 500 fold. From all available data on TSE's after oral exposure one could hypothesise that infection of Peyer patches and/or autonomic nervous system occurs and that the agent is transported to the spinal cord via autonomic nervous system pathways[5].

No infectivity studies have been done on the omentum, but anatomically this is not associated with intestine nor any other tissues in which BSE infectivity has been demonstrated in cattle. Also, the omental nerve fibres are not considered to be a logical route for the spread of BSE infectivity to the central nervous system after oral exposure to the agent.

d. scrapie in sheep. In sheep, in both natural and experimental disease, infectivity and PrP-res can be detected in intestine and in lymph nodes. In experimental disease and in appropriately genetically susceptible animals, this occurs very soon (within the first two months) after oral exposure. In experimental models, infectivity in lymphoid organs can reach 10^5 - 10^7 ID50/g, and 10^3 - 10^4 in peripheral nerves.

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[4] The range was 6-18 months and 36-40 months (p.i.) [no data available for 22 and 26 months p.i.] relative to an I incubation period range with a minimum of 35 months. .

[5] There is no evidence that the dorsal route ganglia (DRG) become infected before spinal cord. Also, they represent only a sensory neural pathway to the CNS, which seems less likely to be involved. DRG are probably infected from spinal cord.

Because of the possible recycling of the scrapie agent, which is not considered to be a human pathogen, the use of fats of small ruminants as a raw product in feed could promote the spread of infectivity to these animal species.

e. Experimental BSE in sheep. Recent data (Jeffrey *et a*l, 2001a; Jeffrey *et al*, 2001b) indicate that PrP-res is detectable in periphery before the onset of clinical signs in animals experimentally exposed to the BSE agent by the oral route. In this model (Romney ARQ/ARQ sheep exposed to 5 g of BSE affected brain homogenate), PrP-res could be detected in a minority of animals at 4 and 10 months post-infection and 50 % have positive immunodetection of PrP-res at 16 months following infection; with clinical disease occuring between 20 to 26 months p.i.. It should be noted that one of the three positive controls had PrP-res detectable only in the CNS at the time of clinical disease. These data suggests that PrP-res could be found in almost all of the secondary lymphoid organs except the thymus, although individual variation in the distribution of PrP-res deposition was evident. Abnormal prion protein could also be detected in enteric associated nervous system mainly in small and large intestines: mucosal and submucosal plexuses are positive in animals in which Peyer Patches have a detectable amount of PrP-res. One should keep in mind that lymph nodes draining nasopharynx can harbour PrP-res. There are no completed infectivity data available for those experimentally infected animals. Nevertheless, one should remember that the BSE strain does not induce large amounts of PrP-res accumulation in experimentally infected rodents (Lasmézas *et al*, 1996) when compared to several strains of scrapie; this means that only few molecules of PrP are associated with 1 IU of BSE agent (the number of 10^5 molecules of PrP corresponding to 1 IU has been obtained in hamster scrapie model; this constitutes a very optimistic estimation of the situation for BSE agent). This means that all things being identical, the detection of a given amount of PrP-res in a BSE-infected sheep might correspond to higher infectivity than the one observed in sheep scrapie.

4. REFERENCES:

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5. ACKNOWLEDGEMENT

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