



EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Scientific Steering Committee

OPINION ON

**BSE RISK OF THE
BOVINE AUTONOMIC NERVOUS SYSTEM**

**ADOPTED BY THE SCIENTIFIC STEERING COMMITTEE
AT ITS MEETING OF 6-7 MARCH 2003**

OPINION

Mandate

Following a request from the German Federal Authorities, Commission Services invited the Scientific Steering Committee (SSC) to prepare an opinion on the question whether the evaluation of the safety of the bovine autonomic nervous system carried out by scientists of the Federal Institute for Consumer Health Protection and Veterinary Medicine (BgVV), contained elements and scientific data justifying a revision of the previous SSC opinions relating to bovine specified risk materials (SRM). 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. The *ad hoc* Group adopted the attached report.

Opinion

Infectivity has been shown to be present in the vagus nerve and the sympathetic mesenteric ganglia of laboratory animals and sheep infectivity with scrapie agents. As far as cattle are concerned, the only evidence to date of infectivity possibly being present in the autonomic nervous system of BSE-affected animals is that of sporadic immuno-chemistry detection of PrP accumulation in the ganglion cells of the myenteric plexus of naturally and experimentally clinically affected cattle. Therefore, while it cannot be excluded that parts of the autonomic nervous system may contain BSE-infectivity at some point in the course of BSE in cattle, that infectivity levels can be expected to be low or undetectable. Furthermore, it is unclear as to whether the hypothetical involvement of the autonomic nervous system proposed in experimental models of rodent scrapie or in natural scrapie, is applicable to the pathogenesis of BSE. However, it cannot be excluded that other structures of the autonomic nervous system also carry infectivity in BSE-infected cattle. Therefore, it is recommended that such tissues collected from BSE field cases and cattle in pathogenesis studies should be the subjects of further research. Inoculation studies should be performed with these tissues in order to estimate the infectivity associated with structures of the autonomic nervous tissue, in particular the mesenteric ganglia and the vagus nerve. The attached report of the TSE/BSE *ad hoc* Group elaborates further on the protocol for such studies.

Depending upon the outcome of these studies, it may then become necessary to investigate the feasibility of removal of additional components of the autonomic nervous system during slaughter and to weight its advantage (elimination of an hypothetical prion-hazard) against the risk of possible spread of infectivity, if present, over the carcass meat especially if the same knife¹ is used for making incisions in the carcass. However, substantial parts of the autonomic nervous system are currently removed under current regulations and there is no evidence of the involvement of peripheral nerves to muscles. There is therefore currently no justification for further action in respect to removal of such nerves.

¹ The Scientific Committee on Veterinary Measures relating to Public health adopted on 20-21 June 2001 an opinion on the Cleaning and Disinfection of Knives in the Meat and Poultry Industry (adopted on)

REPORT ON

BSE RISK OF THE BOVINE AUTONOMIC NERVOUS SYSTEM

PREPARED BY THE TSE/BSE AD HOC GROUP

Rapporteurs: G.Wells and H.Kretzschmar

1. MANDATE

Following a request from the German Federal Authorities, Commission Services invited the Scientific Steering Committee (SSC) to prepare an opinion on the question whether the evaluation of the safety of the bovine autonomic nervous system carried out by scientists of the Federal Institute for Consumer Health Protection and Veterinary Medicine (BgVV), contained elements and scientific data justifying a revision of the previous SSC opinions relating to bovine specified risk materials (SRM). The translation into English of this document is attached. 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. The *ad hoc* Group adopted the attached report.

2. PREAMBLE

2.1. The draft opinion of the BgVV which evaluates possible BSE infectivity of the autonomic nervous system, particularly the *nervus splanchnicus*, *nervus vagus* and mesenteric ganglia of cattle, summarises its assessment and recommendations as follows:

- Given the current state of scientific knowledge, it must basically be assumed that in infected cattle BSE agents could be present in the peripheral nervous system, which connects the intestine to the central nervous system, and in particular could multiply in the ganglia enclosed therein. However, at present, given the scarcity of test results available, it is not possible to assess adequately the potential infectivity of these nerve tissue structures in BSE-infected cattle.
- The BgVV therefore recommends that the ganglia of the peripheral nervous system accessible from the body cavities be removed systematically from a sufficient number of clinically BSE-infected cattle and cattle which tested positive for BSE after slaughter, and be examined for the presence of pathogenic prion proteins and infectivity.
- Until these test results are available, it is recommended, for reasons of preventive consumer protection (in line with the import rules for British XEL beef), at all stages of meat production and processing to remove visible nerve and lymph tissue from the muscles and organs and (as far as possible, together with the SRM) to dispose of it safely. Intensive training of personnel and the drawing up of detailed working instructions could in

this context make an important contribution to reducing any risk to consumers from the autonomic nervous system.

2.2 Previous relevant SSC opinions

The following SSC Opinions are cited in relation to issues arising in the Report: (EC, 1998, 2000, 2001, 2002a and 2002b)

3. REPORT

3.1. Background

Research in sheep and laboratory animal models has shown that the infectious agents of transmissible spongiform encephalopathies (TSE) may be present in the peripheral nervous system, in particular in the autonomic nervous system of the digestive tract. Investigations of the pathogenesis of TSE, primarily experimental scrapie in mice and hamsters (Kimberlin and Walker, 1980; Kimberlin et al., 1983; Kimberlin and Walker 1989; Beekes et al., 1996; Baldauf et al., 1997; Beekes et al., 1998; McBride and Beekes, 1999; Glatzel and Aguzzi, 2000; Beekes and McBride, 2000; McBride et al., 2001), but also natural scrapie of sheep (van Keulen et al., 2000), indicate that after oral intake TSE agents replicate (as demonstrated by infectivity studies and/or accumulations of disease specific PrP) in both the lymphatic and/or neural tissue of the intestine and access the brain and spinal cord via the tracts of the autonomic nervous system. Current knowledge also suggests that after reaching the CNS the infection spreads from the point of entry in both rostral and caudal directions within the CNS and from there may spread further in the peripheral nervous system (PNS). The PNS pathways and structures implicated in the spread of TSE agent from the gastro-intestinal tract to the CNS are: the autonomic (efferent –sympathetic and parasympathetic) ganglia of the enteric nervous system and the general visceral (afferent) sensory fibres, all within the wall of the intestines; the splanchnic nerves (from the intestines to the spinal cord) containing autonomic (efferent –sympathetic) fibres, which reach the spinal cord via the coeliac/mesenteric ganglia and the (afferent) sensory fibres which reach the spinal cord via the dorsal root ganglia, the vagus nerve (from the intestines to the brain stem passing adjacent to abdominal and thoracic viscera through the neck), containing parasympathetic (efferent) fibres and (afferent) sensory fibres, the last reaching the brain via the nodose ganglion (distal ganglion of the vagus –adjacent to the base of the skull). The involvement of the sympathetic chain of ganglia and the sympathetic trunk (efferent), which extends bilaterally from the cranial cervical ganglion (adjacent to the distal ganglion of the vagus nerve), caudally through the cervico-thoracic (stellate) ganglion and into the thoracic and lumbar parts of the chain of ganglia situated ventrolateral to the vertebrae (McKibben 1975, Sieferle and Böhme, 1992), would it seems, according to the hypothesis, become involved secondarily only as collateral spread in the sympathetic chain i.e the hypothesis suggests preferential routing of infectivity along certain parts of the autonomic (efferent) nervous system.

3.2 The pathogenesis of BSE in cattle

3.2.1 Infectivity assays and immunohistochemical detection of PrP

Bioassays of infectivity of PNS from naturally occurring clinical cases of BSE are confined to the splanchnic nerve of one cow and the sciatic nerve of one bull (Fraser and Foster, 1994). Tissues were assayed by intracerebral and intraperitoneal inoculation of RIII mice with negative results (limit of detection of assay approximately $10^{1.4} \log_{10}$ mouse (i.c./i.p.) ID_{50}/g ; estimated effect of the species barrier = $10^{2.7}$, see EC2002b). The examination of distal ileum of 29 naturally occurring cases of BSE by PrP immunohistochemistry detected minimal immunostaining in neurons of the myenteric plexus of 9 of the cattle and no immunostaining in the submucosal plexus of any (Terry et al., 2003, in press), suggesting probable low levels of infectivity in the enteric nervous system in the clinical phase of BSE. Interestingly also, no immunostaining for PrP^{Sc} was detected in any of the follicles of Peyer's patches in these cases.

Experimental study of the spread of the BSE agent in cattle is limited to a single experiment and this was not designed specifically to address the infectivity of the PNS in relation to pathogenesis. However, the results of this study to date indicate that, in contrast to the evidence from the studies of scrapie in experimental animal models or of natural scrapie in sheep, infectivity outwith the central nervous system is found localized to the distal ileum through out much of the disease course and infectivity in the peripheral nervous system has been detected in certain ganglia (closely associated with the CNS), only in the late pre-clinical and clinical phase.

The experimental study of the pathogenesis of BSE after oral exposure of cattle conducted in the UK (Wells *et al* 1994, Wells *et al.* 1996, Wells et al., 1998, EC 2002, Terry et al., 2003) has examined certain PNS tissues. Thirty Friesian/Holstein calves, were each dosed orally at four months of age with 100g of pooled brain stems from cases of BSE. Groups of exposed animals and controls were killed sequentially at mainly 4 month intervals, with the final kill at 40 months p.i. Tissues were sampled aseptically for infectivity assays in conventional/wild type mice. After each sequential kill, inocula were prepared from 44 tissues, representing principally the lymphoreticular system (LRS), the peripheral nervous system (PNS) and the central nervous system (CNS), alimentary tract, striated muscles and major viscera (see Table 3.1, Wells and others, 1996). Inoculum pools of each tissue were made from all of the exposed cattle at each time point. Infectivity was detected in the distal ileum from 6 to 18 and from 36 to 40 months after exposure. The first animal to show clinical signs was at 35 months after exposure. Of the tissues of the peripheral nervous system assayed for infectivity: dorsal root ganglia (cervical and thoracic levels) (DRG), trigeminal ganglion, the nodose ganglion (distal ganglion of the vagus), the stellate ganglion (cervicothoracic ganglion), the phrenic, sciatic and facial nerves, - infectivity was detected only in the

trigeminal ganglion and the DRG, 32 to 40 months after exposure (Wells *et al.*, 1998; EC 2002b).

Although for completeness here discussion has included data on all aspects of the PNS, only the PNS providing the innervation to the alimentary tract, particularly the efferent motor pathways of the autonomic nervous system have been implicated pathogenetically in experimental and natural scrapie. It is, given the alimentary route of infection, implausible that certain somatic afferent or efferent nerve pathways, such as those of the phrenic and sciatic nerves, could be involved in transporting the pathogens from the alimentary tract into the CNS, because of their anatomical position and the structures they innervate. Spread of agent to such structures would probably occur only after a considerable delay as a result of retrograde/centrifugal progression of the infection after reaching the CNS. In further studies of tissue infectivity in the experimental oral exposure of cattle to the BSE agent, pooled sciatic and radial nerve trunks from the 6, 18, 26 and 32 months sequential kill points of the previously mentioned pathogenesis study have been assayed by intracerebral inoculation of cattle. These assays remain in progress (see section II.5 and Table 5, Update of the Report on Tissue Infectivity Distribution in Ruminant Tissues, EC 2002b) but survival data to date (January 2003), already indicate that if infectivity were present at any time in the incubation period it would be at a concentration of < 10 cattle (i.c.) ID₅₀/g. It seems likely therefore that in BSE, as in studies of scrapie (see Table 1 Update of the Opinion on Tissue Infectivity Distribution in Ruminant Tissues, EC, 2002), involvement of somatic segmental spinal peripheral nerves is an event which occurs, if at all, only in the clinical phase of disease and subsequent to infection of DRG. Therefore the structures theoretically relevant for spread of the pathogens from the alimentary tract to the CNS, and examined by mouse bioassay in the experimental study of the pathogenesis of BSE, include the pooled thoracic and cervical dorsal root ganglia [*includes general visceral afferent supply from intestines, i.e. not autonomic/motor*] nodose ganglion (distal ganglion of the vagus) [*general visceral afferent supply from abdominal viscera, not autonomic NS, synapsing on to neurons of the ganglion*], the stellate ganglion (cervico-thoracic ganglion [stellatum]) [*a large paravertebral ganglion, part of the sympathetic chain of ganglia, providing communicating general visceral efferent, or autonomic, fibres supplying the enteric nervous system via the splanchnic nerve and the coeliac/mesenteric ganglia*] and possibly also certain cranial nerve elements, the trigeminal ganglion and facial nerve trunk. The trigeminal ganglion contains neurons which give rise to sensory fibres innervating nasal, buccal cavity and palatine regions and the facial nerve contains fibres distributed to alveoli of the teeth; all structures which are possible sites of primary exposure to the agent resulting from ingestion of infection (further discussed in 3.2.2 below).

Studies to examine the distribution of disease specific PrP by immunohistochemistry in the distal ileum of cattle from the same pathogenesis

study (Terry et al., in press), confirmed PrP accumulation in some Peyer's patches, at kill times consistent with mouse infectivity results for distal ileum, but showed sparse immunostaining confined to the myenteric plexus in neurons of the enteric nervous system (ENS), in only one of three animals (at 38 months after exposure) and one of two animals (at 40 months after exposure), when CNS involvement was apparent. Because of the species barrier the negative results of mouse infectivity assays can be regarded only as a general indication of very low infectivity of the peripheral nervous system of BSE-infected cattle compared with the CNS and the general agreement obtained thus far between the results of tissue infectivity by mouse bioassay and by intracerebral inoculation of cattle, gives a measure of confidence in the former results over the range of tissues examined. However, the only evidence to date of infectivity in the autonomic nervous system of cattle with BSE is that of inconsistently detectable PrP accumulation in the ganglion cells of the myenteric plexus (but not the submucosal plexus) of naturally and experimentally clinically affected cattle (Terry et al., 2003). Therefore, while it cannot be excluded that parts of the autonomic nervous system may contain infectivity at some point in the course of BSE in cattle, it seems likely that infectivity levels can be expected to be low or undetectable. Further examinations of elements of the autonomic nervous system for infectivity over the course of the disease are required either by the intracerebral inoculation of cattle, or by assays conducted in transgenic mice expressing bovine PrP (Safar et al., 2002) to address this issue.

3.2.2 *Neuropathological evidence of possible PNS involvement in BSE*

Based on experience of field cases of BSE and studies of the distribution and relative severity of vacuolar changes, it has been speculated that the solitary tract nucleus and the spinal tract nucleus of the trigeminal nerve are possible primary sites of neural pathogenesis in BSE (Wells 1993). Subsequent study of early disease specific PrP distribution in the brains of cattle infected with BSE agent by the oral route (Ryder, Bellworthy and Wells 1999) indicated PrP accumulation initially in these same medullary nuclei, suggesting, possibly neuroinvasion via sensory pathways (SOLITARY TRACT NUCLEUS: general visceral afferent [GVA] and special visceral afferent [SVA] of VII, IX, X & SPINAL TRACT NUCLEUS OF V: general somatic afferent [GSA] of V, VII, IX, X), in contrast to natural scrapie (Ryder et al. 2001) and experimental BSE in sheep (Ryder, Bellworthy and Wells 1999), in which preclinical accumulation of PrP was found solely in the dorsal nucleus of the vagus nerve (autonomic/parasympathetic, general visceral efferent [GVE]). This might suggest some differences in pathogenetic events between BSE in cattle and scrapie and BSE in sheep with respect to entry of the agent into the CNS. The late occurrence and paucity of PrP immunostaining in neurons of the ENS in BSE could be consistent with the apparent less pivotal role of the vagus, suggested from studies in other TSE hosts. Evidence from the British pathogenesis study of BSE (Wells et al., 1998, Update of the Opinion on Tissue Infectivity Distribution in Ruminant Tissues, EC 2002b) also suggests

that there is no clear disparity in the temporal involvement (as indicated by mouse infectivity and PrP immunohistochemistry) in the spinal cord (and DRG) and the brain stem, an observation which can best be explained, as in the rodent models and in natural scrapie, by dual routes of neuroinvasion, but in BSE with the neuroinvasion of brain resulting from sensory pathways. The recent finding of infectivity in tonsil 10 months after oral exposure in the pathogenesis study (see, Update of the Opinion on Tissue Infectivity Distribution in Ruminant Tissues, EC 2002b) , raises the possibility that such a pathway for infection could arise in the innervation of the soft palate/tonsil. It is also of interest that in an experimental model of transmissible mink encephalopathy (TME) in the hamster (Bartz et al 2003) rapid neuroinvasion of the CNS resulted from inoculation of prions into the tongue, implicating a cranial nerve route of spread which was more efficient than other non neural routes, including another intramuscular route of inoculation.

3.3 Bovine autonomic nervous system and cranial PNS in relation to SRM

3.3.1 Current recommendations and/or regulations

Here the parts of the autonomic nervous system described in 3.1 above are each considered in relation to current recommendations.

In its opinion of 7-8 December 2000 (EC 2000), the SSC concluded that the entire bovine intestine is a risk issue and Commission Regulation (EC) No. 270/2002 (14th February 2002) ANNEX II designates “the entire intestines from the duodenum to the rectum and the mesentery of bovine animals of all ages;” as SRM. Also, in the SSC opinion of 28-29 JUNE 2001, Adipose tissue associated with the digestive tract of cattle, sheep and goats: an appreciation of possible TSE risks (EC 2001) the view was expressed that 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 than I (GBR > I) (...). 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.*”

In its Opinion and Report: Assessment of the human BSE risk posed by bovine vertebral column including dorsal root ganglia. Adopted by the SSC at its meeting of 16 May 2002 (EC2002c) it was concluded that the risk assessments were

scientifically sound but applicable only to the UK and to Ireland. The risk estimates produced cannot be generalised for other countries, because of consumption patterns (quantities consumed by individuals, parts of the carcass used for the production of meat-on-the-bone, frequency of consumption of meat-on-the-bone and other carcass parts to which dorsal root ganglia may be attached, age distribution of the animals slaughtered) and because BSE incidences are different.

They also concluded that the assumption made by the SSC on 12 January 2001, i.e., that in general, as a reasonable worst case assumption, the dorsal root ganglia and the spinal cord are considered to pose a higher risk as from the second half of the incubation period, remains valid. Commission Regulation (EC) No. 270/2002 (14th February 2002) ANNEX II designates “the vertebral column (...) including the DRG and spinal cord of bovine animals over 12 months (...)” as SRM. While it cannot be assumed that infectivity will be confined to these ganglia (DRG and trigeminal ganglion), the evidence available suggests that these ganglia are involved only late in the incubation and in association with established infectivity within the CNS.

Trigeminal ganglia (included in skull) are also disposed of as SRM. And the cranial cervical ganglion (sympathetic) and the adjacent distal ganglion (nodose) of the vagus nerve are removed together with the head if the latter is removed as SRM. They are so closely associated with the base of the skull that they too would be disposed of also when the skull is SRM. Any new evidence of the primary involvement of PNS pathways in the head, in the migration of infectivity from the buccal cavity and/or pharynx to the CNS in cattle, would require reconsideration of the whole head as SRM (see EC 2002b - Update of the Opinion on TSE Infectivity distribution in ruminant tissues (Initially adopted by the Scientific Steering Committee at its meeting of 10-11 January 2002 and amended at its meeting of 7-8 November 2002) following the submission of (1) a risk assessment by the German Federal Ministry of Consumer Protection, food and Agriculture and (2) new scientific evidence regarding BSE infectivity distribution in tonsils.

Thus after removal of organs as SRM the components of the autonomic nervous system which remain in the carcass are the abdominal, thoracic and cervical sympathetic trunk and ganglia and the entire vagus nerve. The largest ganglion of the sympathetic chain is the stellate ganglion (*ggl. Cervicothoracicum[stellatum]*), a fusion of the most caudal cervical and the most cranial thoracic paravertebral ganglia. It communicates with the *ggl. cervicale medium and craniale* and has average dimensions in cattle of 21mm craniocaudally, 7mm dorsoventrally and 4mm mediolaterally. It is situated on

the ventrolateral aspect of the *m. longus colli* ventral to the costovertebral junction of the first rib and the first intercostal space.

During slaughter the vagus nerve is in part removed from the carcass along with the viscera of the thorax and stomachs. It does not seem possible, however, to remove all parts of the vagus nerve from the carcass.

3.2.3. *Potential infective load*

It is suggested (McBride, et al., 2001) from evidence of low infectivity in the vagus nerve in relation to CNS in the clinical stage of the 263K hamster model of scrapie that the nerve trunk is a conduit for transport and not a site of amplification of the agent. There are insufficient data on the potential infectivity of the autonomic nervous system of cattle infected with BSE and on the relative weights of ganglia and nerve trunks implicated to make any quantitative estimate of the possible infective load if the hypothesis were applied to cattle. Considering, nevertheless the stellate ganglion of adult cattle (the largest sympathetic ganglion, but not directly implicated in the theoretical routing of infectivity from the intestines in the model) as one that has been tested in the pathogenesis study by mouse bioassay. The average weight of this ganglion in adult cattle (S. A. C. Hawkins, unpublished) is 2.3g; it is a bilateral ganglion, so the total weight of the ganglion is 4,6g. The limit of detection of the mouse assay conducted on these ganglia in the pathogenesis study was approximately $10^{1.4} \log_{10}$ mouse (i.c./i.p.) ID₅₀/g. If the estimated effect of the species barrier is a factor of $10^{2.7}$ (see EC, 2002b) then it can be calculated that in an individual bovine animal infected with BSE there would remain the potential for an infective load in this ganglion of $<10^{2.1} \log_{10}$ mouse (i.c./i.p.) ID₅₀'s or $<10^{4.8}$ cattle (i.c.) ID₅₀'s. This however, would be equivalent to only approximately <0.1 cattle (oral) ID₅₀.

This estimation can only serve as an example of the potential infectivity present in one ganglion of the autonomic nervous system, further assessment would require not only more information on the results of more sensitive assays of tissue, but the application of an additional hypothesis to that proposed for scrapie to explore the possible extent of the involvement of the autonomic nervous system in cattle.

3.4. **Conclusions and Recommendations**

3.4.1. *Conclusions*

- Review of the BgVV Report provides no justification for the revision of previous SSC Opinions in relation to bovine SRM and in this respect concurs with the BgVV opinion that “ the test results available to date do not constitute sufficient grounds for a compulsory classification (*of the tracts of the autonomic nervous system, particularly the nervus vagus, the nervus splanchnicus and mesenteric ganglia*) as SRM.“
- Provision has been made for advice on the removal of substantial parts of the autonomic nervous system structures in cattle that are potentially

involved in BSE from knowledge acquired from studies of scrapie agents in experimental models of TSE pathogenesis (see 3.3.1). This includes provision for removal of the mesenteric ganglia (*ggl. coeliacum*, *ggl. mesentericum craniale* and *ggl. mesentericum caudale*) which lie in the fat at the *radix mesenterii*. The BgVV report indicates that during slaughter they usually remain connected to the root of the mesentery and are removed with the intestine. On evisceration the *radix mesenterii*, including the ganglia in the adhering fat, can therefore be largely loosened and removed as SRM together with the intestine and mesentery.

- It seems likely also that trimmed out blood vessels, aorta, vena cava etc. and body cavity fat potentially containing nerve tissue, would not, as is customary nowadays in many cases, be used for making meat products, or be rendered, but at all stages of marketing be disposed of safely. The further need to remove the ganglia of the *truncus sympathicus*, which lie in the fatty tissue at the ventral edge of the vertebral column and can remain there when the fillet muscle is removed should be investigated in the event of additional data on infectivity of autonomic nervous system in cattle infected with BSE. It is suggested in the BgVV Report that careful removal of stellate ganglion (*ggl. Cervicothoracicum[stellatum]*) could be achieved in conjunction with extraction of sawing residues and fatty tissue, but, the present need for this is not apparent. Also, it seems likely that it would involve a specific dissection and not a trimming simple exercise.
- Similarly, partial removal of the fibres of the *n.vagus* from the organs intended for human consumption or pet food is, as suggested by the BgVV Report, requires further data on the potential for involvement of the autonomic nervous system in BSE in cattle before specific implementation.

3.4.2. Recommendations

- Tissue samples appropriate to improving an understanding of the role of the PNS and particularly the autonomic nervous system should be collected from BSE field cases and cattle in pathogenesis studies¹. Inoculation and disease specific PrP studies should be performed with these tissues, using the most sensitive methods available, in order to

¹ Archived PNS tissues (fresh and fixed) available for examination from VLA, UK study (SE1736) in which 100 calves, dosed orally with 100g BSE brain homogenate, 100 calves received 1g and 100 undosed controls, were killed sequentially throughout the incubation period and disease course: cranial cervical ganglion [nodose], cervicothoracic ganglion [stellate], stomachs, small and large intestine (for examination of ENS), trigeminal ganglion and somatic nerve trunks of facial, phrenic, radial and sciatic nerves. In addition the following tissues have been archived for the EU FAIR CT 98-3651 Project from field cases of BSE: cranial cervical ganglion [nodose], cervicothoracic ganglion [stellate], coeliaco-mesenteric ganglia, vagus nerve, splanchnic nerve, stomachs, small and large intestine (for examination of ENS), trigeminal ganglion and somatic nerve trunks of facial, phrenic, radial, femoral and sciatic nerves

estimate the possible infectivity/PrP associated with structures of the PNS in particular the mesenteric ganglia and vagus nerve.

- Should evidence of significant infectivity in autonomic nervous pathways be shown in cattle incubating or affected with BSE then the feasibility of the specific removal of those nerve trunk components and ganglia of the system may require further investigation. Such an investigation would require including not only the practicality of dissection of such structures from the carcass at slaughter but also issues of ease and likelihood of compliance with the proposed measures.

Such dissection could also be counterproductive in leading to possible spread of infectivity, if present, over the carcass meat, especially if the same knife is used for making other incisions in the carcass. The advantage (elimination of an hypothetical prion-hazard) should be weighed against this risk.

IV. SUMMARY

Infectivity has been shown to be present in the vagus nerve and the sympathetic mesenteric ganglia of experimental animals and sheep infectivity with scrapie agents. Although experimental data from cattle are insufficient at present, infectivity in these tissues of cattle has not been shown other than by the inconsistent presence of disease related PrP in the myenteric plexus of cattle during the clinical phase of disease. Furthermore, it is unclear as to whether the hypothetical involvement of the autonomic nervous system proposed in experimental models of rodent scrapie or in natural scrapie, is applicable to the pathogenesis of BSE. However, it cannot be excluded that other structures of the autonomic nervous system also carry infectivity in BSE-infected cattle. Therefore, it is recommended that such tissues collected from BSE field cases and cattle in pathogenesis studies should be the subjects of further research. Inoculation studies should be performed with these tissues in order to estimate the infectivity associated with structures of the autonomic nervous tissue. It may then become necessary to investigate the feasibility of removal of additional components of the autonomic nervous system during slaughter. However, substantial parts of the autonomic nervous system are currently removed under current regulations and there is no evidence of the involvement of peripheral nerves to muscles. There is therefore currently no justification for further action in respect to removal of such nerves.

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ATTACHMENT: TRANSLATION INTO ENGLISH OF THE ASSESSMENT OF THE BSE RISK OF THE AUTONOMIC NERVOUS SYSTEM AND ITS GANGLIA, CARRIED OUT FOR THE GERMAN FEDERAL INSTITUTE FOR CONSUMER HEALTH PROTECTION AND VETERINARY MEDICINE (BGVV)

Letter dated: Berlin, 17 January 2002

From: Federal Institute for Consumer Health Protection and Veterinary Medicine (BgVV)

To: Federal Ministry of Consumer Protection, Food and Agriculture

Subject: **BSE risk of the autonomic nervous system and its ganglia**

Ref.: 5222-04-167948

Rapporteurs: Dr. Schütt-Abraham and Dr. Voigt (FG 503)

Order 322-7010-501/3 AK of 6.12.2001 instructed the BgVV to test and evaluate possible BSE infectivity of the autonomic nervous system, particularly the *nervus splanchnicus*, *nervus vagus* and mesenteric ganglia of cattle.

The draft opinion of the BgVV set out below was forwarded by letter dated 4.1.2002 to both the BAFF (*Bundesanstalt für Fleischforschung* - Federal Institute for Meat Research) in Kulmbach and the BFAV (*Bundesforschungsanstalt für Viruskrankheiten* - Federal Research Institute for Viral Diseases in Animals) - Institute for Immunology - with a request for their opinion. The opinion of the BAFF Kulmbach is enclosed; we have not yet received a reply from the BFAV.

Summary assessment

Given the current state of scientific knowledge, it must basically be assumed that in infected cattle BSE agents could be present in the peripheral nervous system, which connects the intestine to the central nervous system, and in particular could multiply in the ganglia enclosed therein. However, at present, given the scarcity of test results available, it is not possible to assess adequately the potential infectivity of these nerve tissue structures in BSE-infected cattle.

The BgVV therefore recommends that the ganglia of the peripheral nervous system accessible from the body cavities be removed systematically from a sufficient number of clinically BSE-infected cattle and cattle which tested positive for BSE after slaughter, and be examined for the presence of pathogenic prion proteins and infectivity.

Until these test results are available, it is recommended, for reasons of preventive consumer protection (in line with the import rules for British XEL beef), at all stages of meat production and processing to remove visible nerve and lymph tissue from the muscles and organs and (as far as possible, together with the SRM) to dispose of it safely. Intensive training of personnel and the drawing up of detailed working instructions could in this context make an important contribution to reducing any risk to consumers from the autonomic nervous system.

Our replies to the individual questions are as follows:

1. **In the light of current scientific knowledge, are there any grounds for assuming infectivity of the *nervus vagus* in particular but also of the *nervus splanchnicus* and mesenteric ganglia which could pose a threat to consumers' health?**

BSE in cattle

The information currently available to the BgVV does not permit a reliable assessment of the BSE infectivity of peripheral neuronal structures of infected cattle. However, all the test results available on TSE agents, primarily scrapie, indicate that after oral intake TSE agents replicate in both the lymphatic and/or neural tissue of the small intestine and rise to the brain and spinal cord via the tracts of the autonomic nervous system (parasympathicus and sympathicus) (Glatzel and Aguzzi, 2000). The precondition for this spread is the presence of endogenous prion proteins, as found in all mammals tested so far. Regardless of the actual infection pathway, after reaching the CNS the infection spreads from the point of entry in both a cranial and caudal direction within the CNS and can return from there to the body via nerve paths leaving the CNS.

If this infection pathway also applies to BSE agents after oral intake by cattle, the pre-, para- and vertebral ganglia that lie on the route outlined must be passed by the pathogens. At present, however, only the vertebral ganglia (spinal ganglia) are to be disposed of safely as SRM and - in countries in risk group 5 - additionally the trigeminal ganglia. Both spinal and trigeminal ganglia have been shown to be infective in some cases in the British pathogenicity studies. However, we know of no reasons that would justify the assumption that multiplication of the BSE agent is confined to these ganglia.

There are only a few studies concerned explicitly with the spread of the BSE agent in cattle and the infectivity of its neuronal structures located outside the CNS. The most extensive study to determine by means of titration tests on mice the infectivity of the different body tissues of cattle infected with BSE naturally and experimentally was carried out in the United Kingdom in the 1990s. However, its results have so far been described only in a few publications, in most cases with insufficient details of how the tests were carried out.

At a meeting of the Scientific Veterinary Committee about BSE held on 14 and 15 September 1993, the results of infectivity tests on tissues from nine BSE cattle slaughtered in 1989 were explained (Fraser and Foster, 1994). However, as far as peripheral nerve tissue is concerned, only the splanchnic nerve (*n. splanchnicus*) of one cow and the sciatic nerve (*n. ischiadicus*) of one bull were examined, which on intracerebral injection did not trigger a BSE disease or histopathological changes in the brain in any of the R III mice used or at any of the dilution stages. These results cannot, however, be regarded as representative of the peripheral nervous system of BSE-infected cattle.

Pathogenicity studies on a total of 30 BSE-infected cattle slaughtered at regular intervals after oral administration of substantial quantities of pathogens showed infectivity in the distal ileum from 6 to 18 and from 38 to 40 months after infection. The earliest appearance of clinical signs was observed 35 months after infection in one animal. Infectivity was also detected in the cervical and thoracic spinal ganglia 32-40 months after inoculation (Fraser and Foster, 1994; Wells *et al.*, 1998).

Of the tissues of the peripheral nervous system tested - according to data (unfortunately, undated) from Wells published on the Internet, these were the trigeminal ganglion, the *ggl. nodosum* (distal ganglion of the *n. vagus*), the *ggl. stellatum* (nowadays called the cervicothoracic ganglion), the *n. ischiadicus* and the *n. facialis* - infectivity was detected only in the trigeminal ganglion 36 and 38 months after inoculation (Wells *et al.*, 1998). Tissues of the same type from in each case three cattle slaughtered at different times after infection were pooled. As the *n. ischiadicus*, the *n. facialis* and the trigeminal ganglion can be ruled out for transporting the pathogens from the intestine into the CNS on account of their position and anatomical sequence and would probably be reached only with a considerable delay as a result of progression of the infection after it had reached the CNS, of the structures theoretically relevant for spreading the pathogens only the *ggl. nodosum* (distal ganglion of the *n. vagus*) and the *ggl. stellatum* were therefore tested.

The negative results of these infection tests can therefore be regarded only as an indication of an appreciably lower infectivity of the peripheral nervous system of BSE-infected cattle compared with the CNS. In view of the small number of tests carried out they do not, however, justify the conclusion that peripheral nerve tissue of BSE-infected cattle does not in principle entail any risk of BSE.

Studies of aggression of the peripheral nervous system in other species of animals infected with the BSE agent experimentally or naturally (via feed) are available for sheep (BSE) and, anecdotally, for cats (FSE). They will be described briefly below, even though no direct conclusions as regards the behaviour of the pathogens in cattle can be drawn from them.

BSE in sheep

In sheep that were in the final stage of an experimentally induced BSE infection, pathogenic prion proteins (PrP^{Sc}) were found in significant and in some cases high concentrations in almost all parts of the lymphatic system examined and in low to significant concentrations in parts of the peripheral nervous system too. These included the *n. vagus* (positive in three out of six sheep tested) and the *ggl. coeliacum* (positive in two out of two sheep tested). On the other hand, no PrP(Sc) discoloration showed up in the *n. radialis* and *n. ischiadicus* (seven sheep tested in each case) or in the *ggl. stellatum*, which was, however, tested in only two sheep, and the *ggl. nodosum* (distal ganglion of the *n. vagus*), which was tested in one sheep only (Foster *et al.*, 2001). Jeffrey *et al.* (2001) too found predominantly a spread of pathogens in the lymphoreticular system of Romney sheep of differing genotype infected orally with the BSE agent.

The spread of the BSE agent is thus largely comparable to that of the scrapie agent in the body of sheep. A similar observation was made by Maignien *et al.* (1999) when comparing the spread of mouse-adapted scrapie and BSE agents in the lymphoreticular tissue of mice. It is therefore probably less pathogen-specific but predominantly host-specific.

Feline spongiform encephalopathy (FSE)

Findings so far indicate that cats displaying symptoms of a FSE were infected by feed containing BSE agents. Disease-specific prion protein was found in the Peyer's patches of the ileum of one infected cat. An immunohistochemical discoloration

indicative of pathological prion protein concentrations also appeared in the neurones of the *plexus myentericus* in four cats tested for this, but not in the *n. ischiadicus* of one cat tested. The restrictive development of disease-specific prion proteins in cats differs clearly from that in sheep and is similar to that determined so far in BSE infections of cattle. The massive discoloration of the *plexus myentericus* points to the possibility of the pathogens being spread via the nervous system without the lymphatic system being involved (Ryder *et al.*, 2001).

2. Should these structures be included in the list of risk materials?

Including the above-mentioned nerve tissue structures in the list of specified risk materials (SRM) would have far-reaching consequences, since their complete removal, staining and disposal up to incineration would have to be comprehensively documented and officially supervised in the same way as for all other SRM. Given the financial and human resources available, this is scarcely feasible. Furthermore, in our opinion the test results available to date do not constitute sufficient grounds for a compulsory classification as SRM.

In the light of the various theories about the pathogenesis of TSE diseases (see Annex 1), which are based predominantly on animal experiments, there is, however, ample suspicion that the BSE agent too could be carried up into the brain along the tracts of the autonomic nervous system. That is why it is recommended that as far as possible, for reasons of preventive consumer protection, this nerve tissue be removed from the food chain until such time as test results are available that permit a reliable assessment of the risk that it poses. This could be done by declaring visible nerve tissue from the body cavities of cattle in general to be unfit for human consumption. Although this would not prevent (currently permissible) further processing into meat products, it would at least considerably reduce it. The removal and safe disposal of these parts could - like the arrangements to be applied to impurities in the meat and abscesses not discovered until later processing stages - take place at all stages of meat production and processing. In addition, by modifying the cutting rate - as proposed by the Veterinary Medicine Working Group at the Free University of Berlin - the production of ganglion-free muscle meat could be promoted already at the slaughtering and cutting stages.

On the other hand, in our opinion the classification of the whole of the intestine from duodenum to rectum, including the mesentery and the *radix mesenterii* together with the ganglia contained in them, as SRM would not entail any significant additional expenditure. They should therefore be removed wholesale and as intact as possible and disposed of as SRM without further processing. A recommendation of the BgVV to this effect was already made in opinion 5222-04-152419 of 30 July 2001.

3. Is removal of the *n. vagus* from the carcass technically feasible in view of its complex sequence?

A working group at the Free University of Berlin has analysed the nerve paths leading from the intestine to the central nervous system that might be responsible for the spread of the BSE agent (including the ganglia contained in them) and identified them on the carcass. A workshop was held on this subject on 4.12.2001 in Berlin (see Annex 2). The aim of this preliminary work was to create the conditions for a modified cutting rate when quartering carcasses, so that the peripheral autonomic nerve tissue can be largely removed from the food chain.

The *n. vagus*, which runs from the splanchnicus to the oesophagus and together with the latter crosses the thoracocervical area, is during slaughter largely removed from the carcass along with the viscera of the neck, thorax and stomach. However, this does not apply to the *ggl. stellatum* or *cervicothoracicum*, which communicates with the *ggl. cervicale medium* of the *n.vagus*, is up to 2 cm long in cattle and constitutes the prevertebral ganglion located furthest in the cranium and in the thoracocervical area remains in the fatty tissue beneath the *m. longus colli* adjacent to the vertebral column. Careful removal of this ganglion too in conjunction with extraction of sawing residues and fatty tissue seems possible, however, and should be tested for feasibility.

Partial removal of the fibres of the *n.vagus* from the organs intended for human consumption or pet food is possible by removing (trimming) the visible externally adherent nervous and fatty tissue.

In addition, the ganglia of the *truncus sympathicus*, which lie in the fatty tissue at the ventral edge of the vertebral column and can remain there when the fillet muscle is removed, should also be taken into consideration and removed as potentially pathogen-containing material.

Body cavity fat potentially containing nerve tissue and removed by trimming should not, however, as is customary nowadays in many cases, be used for making meat products or rendered but at all stages of marketing be disposed of safely as impounded material.

4. Are the mesenteric ganglia also removed when the mesentery is removed from the carcass?

The mesenteric ganglia (*ggl. coeliacum*, *ggl. mesentericum craniale* and *ggl. mesentericum caudale*) lie in the fat at the *radix mesenterii*. During slaughter they usually remain connected to the root and are removed with the intestine. On evisceration the *radix mesenterii*, including the ganglia in the adhering fat, can therefore be largely loosened and removed as SRM together with the intestine and mesentery.

It is recommended that working instructions be drawn up in order to ensure that the whole of the intestine, including the mesentery and *radix mesenterii*, is removed during evisceration.

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http://www.fda.gov/dockets/ac/00/slides/3635s1_06.pdf

(signed)

Dr D. Arnold

Director and Professor

ANNEX : REVIEW OF THE LITERATURE ON THE CONTRIBUTION OF THE PERIPHERAL NERVOUS SYSTEM TO THE PATHOGENESIS OF TSE INFECTIONS AND ON THE INFECTIVITY OF PERIPHERAL NERVE TISSUE

While the peripheral nerve tissue in BSE-infected cattle has so far been investigated only sporadically, in the case of **scrapie** systematic studies have in the meantime yielded evidence of extensive infectivity not only of the lymph tissue but also of the peripheral nerve tissue.

On the basis of these findings it can be assumed that scrapie agents ingested with the feed are first of all replicated in the lymphoreticular tissue. Such tissue is present in all sections of the intestine but forms larger nodules in the Peyer's patches of the ileum. Transport of the pathogens from there to the central nervous system apparently needs an intermediary PrP-expressing tissue (Glatzel and Aguzzi, 2000a). The tracts of the autonomic nervous system are suitable for this purpose. Replication of the pathogen is also conceivable in the nerve tissue plexus of the intestinal wall (*plexus myentericus*), regardless of the presence of lymph tissue (Glatzel and Aguzzi, 2000b).

Infection tests on **mice** yielded early indications of a spread of scrapie infection along the tracts of the peripheral nervous system:

- **CW mice** (Sinc p7) infected with scrapie strain 139A showed consistently similar results despite inoculation into four different places on the body. Thus, multiplication in the CNS began in the thoracic vertebrae after around 35% of the incubation period had elapsed (25-42%). Only then did replication begin simultaneously in the brain and the lumbar vertebrae. It was difficult to reconcile the findings with a haematogenic spread of the pathogen, but they tallied with the assumption of a spread via the peripheral nervous system, especially the sympathetic (Kimberlin and Walker, 1980).
- In **CW mice** infected intraperitoneally with scrapie strain 139A, infectivity in the peripheral nervous system reached a plateau long before the end of the incubation period, although the highest titers found here were much lower than in the CNS. It was concluded from this that replication in the peripheral nervous system was limited. The beginning of replication in the spinal cord, spinal ganglia and spinal nerves suggested, however, that there was a spread from the central to the peripheral nervous system (Kimberlin *et al.*, 1983).
- As a result of direct inoculation of the scrapie agent into the sciatic nerve in *mice*, the pathogen spread directly as far as the brain at a speed of 1.0-2.0 mm a day. In the clinical stage of the disease, the titers in the peripheral nervous system were, however, appreciably lower than in the CNS (Kimberlin *et al.*, 1983b).
- Inoculation of **CW mice** with scrapie strain 139A via the stomach led almost immediately to replication of the pathogens in the Peyer's patches, even before it began in the spleen. A splenectomy had no effect on the incubation period. In the CNS, replication was ascertained first of all in the thoracic vertebrae and later in the brain. This suggested a neuronal spread of the pathogen via the autonomic nervous system (Kimberlin and Walker, 1989).
- By inoculating **mice** with a tenfold dilution of tissue homogenates from a scrapie-infected sheep, infectivity was found in all the peripheral nerves tested, with the exception of the *n. saphenus*. The following nerves were examined: *n. axillaris*, *n. ulnaris*, *n. medianus*, *n. ischiadicus*, *n. tibialis*, *n. fibularis* and *n. saphenus*

(Groschup *et al.*, 1996). This points to an extensive spread of the pathogens in the late stage of the infection. The splanchnic nerves connecting the intestine with the CNS, on the other hand, were not tested.

- Tissue containing PrP is needed for spreading the pathogen from the intestine to the brain, since PrP-less *mice* in whose brain nerve tissue containing PrP was implanted were infected only intercerebrally but not peripherally (Blaettler *et al.*, 1997). However, the changes caused by prions were restricted to the implant. PrP was found on the cell surface of neurons and peripheral nerve tissue in all the vertebrates tested to date. The propagation of the pathogenic form of the prion protein apparently occurs as a result of an autocatalytic process through direct contact with physiological prion protein along the nerve paths containing PrP (Laplanche, 1997).
- **Transgenic mice** which overexpressed PrP showed, after inoculation of scrapie strain RML into the *n. ischiadicus* or the sole of the foot, the pathological form of the prion protein in certain sections of the peripheral sensory nerve paths. The propagation rate of infectivity in peripheral nerve tissue was calculated at 0.7 mm a day. The infectivity of the *n. ischiadicus* was appreciably higher in the transgenic tga 20 mice than in the wild mice. The authors concluded from this that the intraneural transport capacity is influenced by the excessive supply of physiological PrP (Glatzel and Aguzzi, 2000a).
- Oral and intraperitoneal infection of **transgenic mice** with scrapie strain 263K led with expression of hamster PrP in the peripheral nerve tissue to infection of the brain. At high doses of the pathogen, prior replication in the lymphoreticular tissue did not seem to be required. On the other hand, expression of various PrPc molecules protected the majority of the mice against the outbreak of a clinical disease (Race *et al.*, 2000).

Also in **hamsters** infected **orally** with the scrapie strain 263K, the results available indicate that the autonomic nervous system plays an important part in the spread of the pathogens:

- 70 days after inoculation, precipitates of pathological PrP were found within or in the vicinity of neurons and other cells in the nerve plexus of the intestinal wall and in its lymphatic tissue, especially the Peyer's patches (Beekes and McBride, 2000).
- After a third of the incubation period had elapsed, low concentrations of pathological PrP precipitates were found in the nerve plexus of the intestinal wall and the autonomic ganglia of the *n. splanchnicus* or *vagus*. These precipitates appeared before similar ones in the sensory ganglia. The authors concluded from this that the pathogen reaches its targets in the brain and spinal cord along the autonomic ganglia connected by synapses and along the efferent tracts of the *n. vagus* and *splanchnicus* (McBride *et al.*, 2001).
- In another study, the pathological process became apparent in the CNS first of all in the region of the fourth to ninth thoracic vertebral segment, from where it spread anteriorly and posteriorly at a speed of 0.8-1 mm a day. There were indications of a possible alternative route from the periphery to the brain, bypassing the spinal cord (Beekes *et al.*, 1996).
- TSE-specific amyloid protein was found first of all in the dorsal motor nucleus of the *n. vagus*, quickly followed by the nuclear area of the *tractus solitarius*, where

the sensitive and sensory terminal nuclei of the *n.vagus* also lie. The cervical vertebrae were not affected until after this. These findings prove that the pathogen had reached the brain via the *n. vagus* and not via the spinal cord (Beekes *et al.*, 1998).

- Pathological PrP precipitates were ascertained in the *ggl. nodosum* (distal ganglion of the *n. vagus*), the spinal ganglia and the mesenteric ganglia. In one case, a low-density precipitate was also detected along a few axons of the *n.vagus* (McBride and Beekes, 1999).
- A high infectivity was found in both trigeminal ganglia. On inoculation of the pathogen via a dental pulp, infectivity and pathological PrP(Sc) were found only in the equilateral trigeminal ganglion. Both these findings indicate a neuronal spread of the pathogen (Ingrosso *et al.*, 1999).

Following **intraperitoneal** infection of hamsters with scrapie strain 263K, the infection was found first of all in the thoracic vertebral segments T 7-9 and secondarily in the lower thoracic vertebrae and at the junction of the dorsal and lumbar vertebral columns. There were also strong indications of the existence of direct access to the *medulla oblongata*, bypassing the spinal cord (Baldauf *et al.*, 1997).

After intraperitoneal inoculation of the scrapie agent, **sheep** and **hamsters** showed prominent granular PrP(Sc) precipitates in the ganglion cells and a few satellite cells of the trigeminal ganglia, spinal ganglia, *ggl. coeliacum*, *ggl. thoracica* and *ggl. nodosum* (distal ganglion of the *n. vagus*). In addition, scattered adaxonal precipitates were also found along the nerve fibres close to the ganglia of the peripheral nervous system (Groschup *et al.*, 1999).

Scrapie-specific PrP was also detected in the peripheral ganglia of scrapie-infected sheep (Jeffrey *et al.*, 2001).

On the other hand, scrapie-associated fibrils (SAF) were not found in the *n. ischiadicus* of any out of ten sheep which were in the final stage of a scrapie infection (Stack *et al.*, 1998).

Chronic Wasting Disease (CWD)

The spread of the TSE agents via the *n. vagus* as far as its nuclear area in the obex was also investigated by means of immunohistochemical staining in six mule deer infected naturally with **Chronic Wasting Disease**. PrPres was found in the *truncus vagosympathicus* of all the animals. One each of the animals had PrPres in the sciatic nerve, the *truncus sympathicus* or the *plexus brachialis*. Brain and spinal cord were positive in all six animals, the intramural nerve plexus of the intestine in five of them. The *ggl. nodosum* (distal ganglion of the *n.vagus*) was tested in only two animals and was positive in both cases. The trigeminal ganglion (tested in five animals), the spinal ganglia (tested in four animals) and the *ggl. cervicale craniale* (tested in one animal) were negative in all samples taken (Sigurdson *et al.*, 2001).

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