Autonomous Nervous System with Respect to Dressing of Cattle Carcasses and Its Probable Role in Transfer of PrP\text{res} Molecules

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MS 02-289: Received 28 August 2002/Accepted 3 December 2002

ABSTRACT

Pathogen prions are widely recognized as the causative agent in bovine spongiform encephalopathy (BSE) and other transmissible spongiform encephalopathies. However, more research on the possible transmission routes of this agent once it has reached the host is needed. There is evidence based on the anatomy and physiology of the autonomous nervous system (ANS), as well as observations for different animal species, that the ANS might be involved in the axonal drainage of pathogenic prions toward the central nervous system. In this context, more attention should be paid to the cranial cervical ganglion, the stellate ganglion, the chain of paravertebral ganglia next to the first six thoracic vertebrae, the chain of the paravertebral ganglia next to the first six thoracic vertebrae, the vagus nerve in the neck region and in the mediastine, and the esophagus (because of its close connection to the vagus nerve). For a more detailed risk analysis with respect to these tissues, the ANS of animals having shown clinical signs of BSE might be examined to corroborate the evidence presented here. In the meantime, as a precautionary measure, the tissues addressed should be taken out of the human food chain, taken out of animal feed, and handled as if they were specified risk material. It is technically possible to remove these parts during cutting and dressing.

THE ANS

First, the ANS must be distinguished from the somatic nervous system. The ANS consists of sympathetic, parasympathetic, and enteric (intramural) subunits. Its efferent and afferent functions, as well as the numbers of synapses associated with it, are given in Table 2. Moreover, one might separate the ANS into sections within the CNS (the brain and the spinal cord) and on the periphery (outside the skull and the vertebral column). Territories of nerve cell bodies are called nuclei if they are located within the CNS, and they are called ganglia if they are located outside the CNS. With regard to BSE, four regions of ganglia can be identified in the ANS (tissues from some of these locations have already been declared SRM):

1. The dorsal root ganglia (spinal ganglia), located near the respective intervertebral foramen or lateral vertebral fo-
TABLE 1. Specified risk material (SRM) according to regulation (EC) 270/2002 (3)

<table>
<thead>
<tr>
<th>Type of animal</th>
<th>SRM in member states in general</th>
<th>SRM in the UK, Northern Ireland, and Portugal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All ages</td>
<td>Intestines from duodenum to rectum, including the mesenterium</td>
<td>Intestines from duodenum to rectum, including the mesenterium</td>
</tr>
<tr>
<td>&gt;6 mo old</td>
<td></td>
<td>Entire head, excluding the tongue, including the brain, eyes, and trigeminal ganglia; tonsils; vertebral column, including spinal cord and dorsal root ganglia; thymus; spleen</td>
</tr>
<tr>
<td>&gt;12 mo old</td>
<td>Skull,(^a) including the brain and eyes; tonsils; vertebral column, including the spinal cord and dorsal root ganglia, excluding the tail bones</td>
<td></td>
</tr>
<tr>
<td>Ovine/caprine</td>
<td>Spleen</td>
<td>Spleen</td>
</tr>
<tr>
<td>All ages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;12 mo old</td>
<td>Skull,(^a) including the brain and eyes; tonsils; spinal cord</td>
<td>Skull,(^a) including the brain and eyes; tonsils; spinal cord</td>
</tr>
</tbody>
</table>

\(^a\) Under certain circumstances, particular parts of the head may be used for deboning (3).

anatomical sites and evidence for the deposition of PrPres molecules

Ganglia located in the head. Located underneath the skull base, the cranial cervical ganglion (no. 1\(^{11}\) in Figs. 1 and 3) is a part of the sympathetic nervous system. It is easily accessible and quite large, especially in cattle (2 to 3 cm in diameter) (6), whereas the other ganglia of the head are small or not accessible. Sigurdson et al. (19) did not recover a deposition from this ganglion in the mule deer but did recover a deposition from the nodose ganglion of the vagus nerve, which is located near the cranial cervical ganglion and is hardly visible (no. 1\(^{19}\) in Figs. 1 and 3). However, Foster et al. (9) did not recover PrPres depositions from the nodose ganglion in sheep.

The trigeminal ganglion (no. 1\(^{999}\) in Figs. 1 and 3) is part of the somatic nervous system. It is located within the skull (intracranial). Evidence for the deposition of PrPres in the trigeminal ganglion has been obtained from studies involving hamsters experimentally infected with scrapie agent 263K (13) and bovines (22). In its “tentative summary,” the Scientific Steering Committee (18) attributes low infectivity titers to the trigeminal ganglion (on the basis of experimental data obtained for bovines). The trigeminal ganglion has been designated SRM in the United Kingdom, Northern Ireland, and Portugal (3).

The vagus nerve of the parasympathetic nervous system. The vagus nerve contains parasympathetic efferent

TABLE 2. The autonomous nervous system\(^a\)

<table>
<thead>
<tr>
<th>Physiological systemic subunit</th>
<th>Impulse direction within fiber(^b)</th>
<th>No. of neurons</th>
<th>No. of synapses</th>
<th>Location of synapses</th>
<th>Ganglia present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sympathetic</td>
<td>Efferent</td>
<td>(\geq 2)</td>
<td>(\geq 1)</td>
<td>Far from target organ</td>
<td>Paravertebral ganglia and prevertebral ganglia</td>
</tr>
<tr>
<td>Parasympathetic</td>
<td>Efferent</td>
<td>(\geq 2)</td>
<td>(\geq 1)</td>
<td>Near target organ</td>
<td>Parasympathetic ganglia of the head</td>
</tr>
<tr>
<td>Sympathetic and parasympathetic</td>
<td>Afferent</td>
<td>1</td>
<td>0</td>
<td>—</td>
<td>Dorsal root ganglia and sensory ganglia of the head</td>
</tr>
<tr>
<td>Enteric nervous system</td>
<td>Efferent and afferent</td>
<td>Plexus</td>
<td>Several</td>
<td>Target organ</td>
<td>Auerbach and Meissner plexuses</td>
</tr>
</tbody>
</table>

\(^a\) Some tissues are SRM according to regulation (EC) 270/2002 (3).

\(^b\) Efferent, motor and secretory; afferent, sensory.
FIGURE 1. The ANS as related to the skull and the vertebral column. 1, ganglia of the head (1′, ganglion cervicale craniale; 1″, ganglion nodosum; 1‴, ganglion trigeminale); 2, dorsal root ganglia; 3, paravertebral ganglia (3′, ganglion stellatum; 3″, nervi splanchnici); 4, prevertebral ganglia (4′, ganglion coeliacum; 4″, ganglion mesentericum craniale; 4‴, ganglion mesentericum caudale); 5, truncus vagosympathicus (5′, vagus nerve; 5″, dorsal motor nucleus of the vagus nerve); 6, ENS.

and afferent nerve fibers. Large parts of the organs in the thoracic cavity and the greater part of the organs in the abdominal cavity are modulated by this nerve (no. 5″ in Fig. 1).

The dorsal motor nucleus of the vagus nerve (no. 5″ in Fig. 1) is located in the obex region of the medulla oblongata of the brain stem; it is the root of efferent parasympathetic neurons of the vagus nerve. Nerve cell bodies of its fibers are located in the nodose ganglion (no. 1″ in Fig. 1) and project to the nucleus of the solitary tract, from which axons project to the hindbrain and perhaps farther (14). The dorsal root ganglia project to the nucleus of the solitary tract as well.

Between the head and the thoracic aperture, the nerve follows the arteria carotis communis together with the sympathetic system ("vagosympathetic trunk," no. 5 in Figs. 1 and 3). Between the heart and the diaphragm, both the vagal branches and the esophagus are closely connected. In the abdominal cavity, the nerve projects to the intestinal tract via the coeliac and the cranial mesenteric ganglion (no. 4′, 4″, 4‴ in Fig. 1).

Foster et al. (9) recovered PrPres in the dorsal root ganglia (no. 2 in Figs. 1, 2, and 4; cervical, thoracic, and lumbar) in some animals but not in every animal. In a study involving experimentally infected bovines, Wells et al. (22) reported positive findings for the cervical (3rd to 6th vertebra) and thoracic (5th to 8th vertebra) dorsal root ganglia. The Scientific Steering Committee (18) estimated the infectivity titers of the dorsal root ganglia to be low. Spinal ganglia have already been classified as SRM.

Sympathetic nervous system (thoracolumbal system): paravertebral ganglia (sympathetic trunk) including the stellate ganglion (or ganglion cervicothoracicum). The stellate ganglion (no. 3″ in Fig. 1) is located near the apertura thoracis. It is of major importance because of its weight (ca. 1 g) and size (6). It serves as a relay to the nerve structures of cranial parts of the body. However, in studies involving sheep, depositions could not be recovered in this ganglion (9).

The subsequent paravertebral ganglia (no. 3 in Figs 1, 2, and 4) are located near the column from the thoracic vertebral column to the tail (6) as follows:

1. Thoracic cavity: located near the musculus longus colli from the first to the sixth vertebra, and subsequently (from the 6th to the 13th vertebra) located very close to the vertebral column.
2. Lumbar (lumbar) vertebra: located near the musculus psoas major and the m. psoas minor from the first to the sixth vertebra.
3. Farther down the pelvis; closely connected to the sacrum, in cattle relatively large compared with ganglia of the adjacent regions.
4. From the first tail vertebra on: very small.

Sigurdson et al. (19) found tissue in the sympathetic trunk to stain. The vertebral column is already classified as SRM and must legally be destroyed.

Sympathetic nervous system (thoracolumbal system): prevertebral ganglia. Prevertebral ganglia are located in the mesentery, surrounding the origin of the large visceral arteries (no. 4 in Fig. 2). Foster et al. (9)
ROLE OF ANS IN TRANSFER OF PrPres

FIGURE 2. The sympathetic nervous system as related to the spinal cord and the vent cavity. 2, dorsal root ganglia; 3, paravertebral ganglia (3°, nervi splanchnici); 4, prevertebral ganglia; 6, ENS.

FIGURE 3. Cranial nerves and some ganglia of the head. 1, ganglion cervicale craniale; 1′, ganglion nodosum; 1″, ganglion trigeminale; 5, truncus vagosympathicus.

FIGURE 4. The lumbar vertebral column. 2, dorsal root ganglia; 3, paravertebral ganglia. (a) Spinal cord. (b) T-bone steak with m. psoas major. (c) T-bone steak with m. psoas minor.

found the coeliac-mesenteric ganglion of sheep to stain, and Groschup et al. (10) found the coeliac ganglion of sheep to stain as well, whereas for mule deer, Sigurdson et al. (19) did not detect any deposition of PrPres in the coeliac ganglion. In a study involving sheep, van Keulen et al. (20) addressed the transfer of PrPres via prevertebral ganglia to the thoracic spinal cord as a possible route for the trafficking of the agent.

The nervi splanchnici system (no. 3° in Figs. 1 and 2) interconnects between paravertebral and prevertebral ganglia (ganglion coeliacum, ganglion mesentericum craniale) (7, 14). Recent evidence suggests that neuroinvasion occurs via sympathetic fibers of the splanchnic nerves (21). With the whole mesenterium being classified as SRM, ganglia and nervous structures of the alimentary tract are already out of the human consumption chain.

The ENS (no. 6 in Figs. 1 and 2). With respect to the alimentary tract, studies involving naturally infected sheep have revealed that PrPres accumulation appears first in lymphoid tissues of the gut (21) and next in the cranial mesenteric and coeliac ganglia, indicating the possible onset of the trafficking of PrPres from the gut. With the exception of the forestomach and the stomach system, these tissues have already been classified as SRM.

DISCUSSION

The first step in a risk analysis approach is the identification of a risk. On the basis of given anatomical information and experimental data obtained for different species, this paper identifies potential risk with respect to particular sites of the ANS staining positive for depositions of PrPres, indicating that the ANS might be involved in the transfer of the agent (Table 3).

Systematic surveys involving hamsters have demon-
TABLE 3. Parts of the autonomous nervous system in which PrP<sub>res</sub> has been found (9, 10, 13, 19, 21, 22)

<table>
<thead>
<tr>
<th>Site</th>
<th>Stain result</th>
<th>SRM?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteric nervous system</td>
<td>+</td>
<td>Yes</td>
</tr>
<tr>
<td>Parasympathetic nervous system</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vagus nerve</td>
<td>+</td>
<td>No</td>
</tr>
<tr>
<td>Vagusosympathetic trunk</td>
<td>+</td>
<td>No</td>
</tr>
<tr>
<td>Nodose ganglion of the vagus nerve</td>
<td>+</td>
<td>No</td>
</tr>
<tr>
<td>Dorsal motor nucleus of the vagus nerve</td>
<td>+</td>
<td>Yes, intracranial</td>
</tr>
<tr>
<td>Sympathetic nervous system</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevertebral ganglia</td>
<td>+</td>
<td>Yes</td>
</tr>
<tr>
<td>Paravertebral ganglia</td>
<td>+</td>
<td>No</td>
</tr>
<tr>
<td>Stellate ganglion</td>
<td>−</td>
<td>No</td>
</tr>
<tr>
<td>Cranial cervical ganglion</td>
<td>−</td>
<td>No</td>
</tr>
<tr>
<td>Dorsal root ganglia</td>
<td>+</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Stratified the dissemination of the agent via the ANS and the possible trafficking of PrP<sub>res</sub> via axonal transport (4, 15, 16). In these studies, positive staining results were obtained for the enteric nervous system (including gut-associated lymphoid tissue), the sympathetic (prevertebral) nervous system (coeliac and mesenteric ganglia, splanchnic nerves), afferent neurons (spinal ganglia, the nodose ganglion, the nucleus of the solitary tract), and the parasympathetic nervous system (the vagus nerve and the dorsal motor nucleus of the vagus nerve). The deposition of PrP<sub>res</sub> in the myenteric plexus of the ENS, in the vagosympathetic trunk, and subsequently in the nodose ganglion of the mule deer (19) supports this approach.

Still, the role of the ANS in PrP<sub>res</sub> infection of bovines is undetermined because of a lack of data for cattle. If the hypothesis that the intestine is the site of invasion should turn out to be true, particular sites in cattle carcasses must be observed in more detail with respect to dressing, cutting, and deboning. However, other factors that must also be considered in this context are possible differences in modes of infection for different species, possible differences between results for naturally and experimentally infected animals, possible differences in results for different PrP<sub>res</sub> strains used in experimental studies, and detection limits of presently available test systems.

In view of the limited information available at present, this approach is preliminary, but it is nonetheless necessary: according to article 7 of regulation (EC) 178/2002 (2), to prevent possible risks to humans, any tentative preventive measure should be taken until more scientific evidence has been gathered. In conclusion, the following tissues should be observed more closely:

1. **Ganglia located in the head**. During deboning, some ganglia of the head are accessible (Fig. 3). Ganglia that are not accessible are removed with the skull as SRM. This is true especially for the somatic trigeminal ganglion, which is located inside the skull and is not likely to be cut out during deboning (8). However, during cut-

ting, the cranial cervical ganglion might be collected with other edible tissue. Its weight and its location (close to the suspect nodose ganglion) should be reason enough to take it out of the human food chain. Again, no positive staining has been reported for the cranial cervical ganglion.

2. **The vagus nerve**. With regard to the vagosympathetic trunk and the stellate ganglion (nos. 3’ and 5 in Fig. 1), adipose and connective tissue of the neck and the thoracic aperture should be considered for removal during dressing, which can be accomplished easily. Also, more attention should be paid to the esophagus because of its close connection to the vagus nerve (11, 12), which has been corroborated by findings with regard to nervous tissue along the caudal part of the esophagus (20).

3. **The sympathetic nervous system**. The stellate ganglion (no. 3’ in Fig. 1) and the other parts of the paravertebral ganglia (sympathetic trunk (no. 3 in Figs. 1, 2, and 4) in the course of the vertebral column are accessible during dressing and cutting. The stellate ganglion and subsequent paravertebral ganglia along the m. longus colli are accessible, and ganglia close to the filet (m. iliopsoas with m. psoas major and m. iliacus) and close to the m. psoas minor are accessible, too. If the respective connective and adipose tissues were deliberately removed, they would be prevented from entering the human food chain. Ganglia in the second part are located close to the column, and thus, remaining in their natural site, they would be removed with the vertebral column as SRM anyway.

REFERENCES
11. Hildebrandt, G., K. Rauscher, S. Buda, K.-D. Budras, T. Eggers, and


