Evaluation of Methods for Removing Central Nervous System Tissue Contamination from the Surface of Beef Carcasses after Splitting

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ABSTRACT. Since high levels of prions, the causative agent of bovine spongiform encephalopathy (BSE), accumulate in the brain and spinal cord, contamination of beef carcasses with central nervous system tissue (CNST) may occur at post-slaughter process. In this study, we investigated CNST contamination on the surface of beef carcasses using glial fibrillary acidic protein as a marker after splitting and evaluated the effects of washing procedures on contamination removal. High levels of CNST contamination was detected immediately after splitting, especially in the area close to the spinal column. This suggests that spinal cord fragments are attached to carcasses at the time of splitting even though the spinal cords have been removed by vacuum before splitting. Steam cleaning or manually washing with normal-pressure water, either steam cleaning or manually washing with normal-pressure water could reduce CNST contamination to almost negligible levels. These results are useful for preparation of appropriate sanitation standard operating procedures to reduce the risk of CNST contamination of carcasses for prevention of exposure to BSE prion via the food chain.

KEY WORDS: BSE, carcass splitting, central nervous system tissues, glial fibrillary acidic protein, post-slaughter.

Since the epidemiological linkage of bovine spongiform encephalopathy (BSE) to the occurrence of variant Creutzfeldt-Jakob disease in human was reported, BSE has been recognized as a foodborne disease [13]. Prion infectivity exists abundantly in the brain and spinal cord of BSE-affected cattle. BSE prion has also been detected in the trigeminal ganglion, dorsal root ganglia and, though at low levels, peripheral tissues such as the retina, bone marrow, tonsil and distal ileum [2, 11, 12]. The brain, spinal column including the spinal cord and dorsal root ganglia and distal ileum are removed as specified risk materials (SRMs) and thus kept out of the chain of consumption to reduce the risk of foodborne BSE infection. Recently, the existence of prion in tissues other than SRMs became evident; an abnormal isoform of prion protein, PrPSc, which is thought to be a major component of prion, was detected in the peripheral nerves [5] and adrenal glands of BSE-affected cattle [7].

There are reports of carcasses and meat products contaminated with CNST in spite of washing [6, 8, 14]. These reports determined the levels of CNST using glial fibrillary acidic protein (GFAP) as a marker and indicated that carcasses were contaminated with CNST under the following conditions: (1) brain tissue, destroyed by penetrating captive bolt stunning or pithing, is carried into muscle tissue via blood flow [1, 3]; (2) spinal cord fragments are attached to the surface of carcasses during splitting [4]; and (3) carcass washing after splitting is insufficient to remove spinal cord fragments from the carcass surface [8]. In addition, cross-contamination from SRM-contaminated carcasses is also a potential source of contamination [8].

In this collaborative study of 8 slaughterhouses in Japan, we investigated CNST contamination on the surface of the carcasses using GFAP as a marker for CNST. First, we investigated the contamination of carcasses with CNST immediately after splitting to understand the actual situation of contamination in Japan. Contamination was detected at all slaughterhouses examined, although the positive rate and degree of contamination varied among the slaughterhouses. Carcasses must be rid of CNST contamination in slaughterhouses before they are shipped as food products. Herein, we report the level of CNST contamination and effectiveness of washing procedures for removing CNST contamination on the beef carcass.

MATERIALS AND METHODS

Slaughtering process and analysis objectives: This study was conducted at eight domestic slaughterhouses (A-H) between August and November 2006. The general slaughtering process is outlined in Fig. 1A. Following stunning, exsanguination, and head removal, the carcasses were hung up by the hind legs. A tube was inserted into the spinal canal to suck out the spinal cord, and then the carcass was split. The carcasses were subsequently washed automatically or manually with high-pressure water. Prior to washing with high-pressure water, either steam cleaning or manual washing with normal-pressure water was carried out...
as preliminary washing to ensure the efficacy of high-pressure washing. At the time of planning of this research, most of slaughterhouses manually washed carcasses with high-pressure water without a preliminary washing step. Therefore, this study was designed to first investigate the actual situation of CNST contamination on the surface of carcasses immediately after splitting (at slaughterhouses A to H). Next, we attempted to evaluate the effects of steam cleaning (at slaughterhouses F and H) and manual washing with normal-pressure water (at slaughterhouses B and C) on CNST removal as a preliminary washing step and the effect of manual washing with high-pressure water on the CNST removal as the main washing step.

GFAP detection by enzyme immunoassay: One hundred square centimeters of sectioned muscle or fat on the split surface of wet carcasses were wiped off with dry sterile cotton applicators around the third cervical vertebra or last thoracic vertebra (Fig. 1B). The swabs of the applicators carrying the wiped-off materials were put into 2 ml sterile tubes for immediate laboratory testing.

Ridascreen Risk Material 10/5 (R-Biopharm GmbH, Germany), an ELISA-based test, was used to detect GFAP in the wiped-off samples. Following the manufacturer’s instructions, the levels of CNST in the samples were determined from standard curves and were expressed as GFAP amounts per 100 cm². Samples with a GFAP concentration above 3 ng/100 cm², the detection limit of the kit used, were judged to be positive. Data analysis was performed assuming that GFAP concentrations below the detection limit were equal to 0 ng/100 cm². If the GFAP concentration exceeded the quantitation range, the sample was diluted for remeasurement and subsequent calculation of the actual GFAP concentration in consideration of the dilution ratio.

RESULTS

Residual CNST on the carcass surface immediately after splitting: Beef carcasses were investigated immediately after splitting in eight slaughterhouses to determine the levels of residual CNST on their surfaces (Table 1). Among these slaughterhouses, slaughterhouse D, which did not withdraw the spinal cord by suction, showed the highest GFAP positive rate (100%) around both the third cervical vertebra and last thoracic vertebra. The other slaughterhouses, at which the spinal cord was withdrawn by suction before splitting, showed GFAP positive rates ranging from 21.7 to 85.0%. In contrast, the residual levels of CNST exceeded 1 µg/100 cm² around the third cervical vertebra in slaughterhouse C and around the last thoracic vertebra in slaughterhouses B and H. This suggests that the level of contamination may not depend on removal of spinal cord, but instead scattering of saw residue during splitting may cause contamination in a limited area. Thus, removal of the scattered residue by washing is essential to reduce contami-
nation of carcasses with CNST.

Effects of steam cleaning on CNST removal: The effects of steam cleaning on CNST removal were examined in slaughterhouses F and H. Steam cleaning was performed by spraying the surfaces of carcasses with approximately 80°C steam and simultaneously sucking substances attached to the surface of the carcasses through a suction nozzle moving across the carcass surface. Table 2 shows the mean levels of CNST residue before and after steam cleaning and the corresponding rate of decrease. In addition, the maximum and minimum levels of CNST residue are also indicated. In each slaughterhouse, the maximum levels of CNST residue decreased after washing. All cases showed a 42.9 to 70.9% decrease in the mean CNST levels after steam cleaning, demonstrating that this treatment is effective for removing CNST residue attached to carcasses.

Effects of manual washing with normal pressure water on CNST removal: The effects of manual washing with normal pressure water on CNST removal were examined in slaughterhouses B and C. The entire split surfaces of carcasses were washed evenly for 15 seconds. Table 3 shows the mean levels of residual CNST before and after washing and the corresponding rate of decrease. In each slaughterhouse, the maximum levels of CNST residue decreased after washing. All cases showed a 42.9 to 70.9% decrease in the mean CNST levels after steam cleaning, demonstrating that this treatment is effective for removing CNST residue attached to carcasses.

Table 1. Residual levels of CNST immediately after splitting

<table>
<thead>
<tr>
<th>Slaughterhouse</th>
<th>Wiped section</th>
<th>n</th>
<th>Positive rate (%)</th>
<th>GFAP (ng/100 cm²)</th>
<th>Rate of decrease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean Standard deviation Maximum Minimum</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>C.V. 60</td>
<td>60</td>
<td>21.7</td>
<td>2.4</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>T.V. 60</td>
<td>60</td>
<td>73.3</td>
<td>22.3</td>
<td>31.4</td>
</tr>
<tr>
<td>B</td>
<td>C.V. 20</td>
<td>20</td>
<td>85</td>
<td>89</td>
<td>150.3</td>
</tr>
<tr>
<td></td>
<td>T.V. 20</td>
<td>20</td>
<td>70</td>
<td>156.3</td>
<td>283.1</td>
</tr>
<tr>
<td>C</td>
<td>C.V. 70</td>
<td>70</td>
<td>32.9</td>
<td>27.6</td>
<td>138.5</td>
</tr>
<tr>
<td></td>
<td>T.V. 70</td>
<td>70</td>
<td>42.9</td>
<td>19.4</td>
<td>80.2</td>
</tr>
<tr>
<td>D</td>
<td>C.V. 111</td>
<td>111</td>
<td>100</td>
<td>49.6</td>
<td>49.1</td>
</tr>
<tr>
<td></td>
<td>T.V. 111</td>
<td>111</td>
<td>100</td>
<td>149.6</td>
<td>132.6</td>
</tr>
<tr>
<td>E</td>
<td>C.V. 40</td>
<td>40</td>
<td>30</td>
<td>5.7</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>T.V. 40</td>
<td>40</td>
<td>22.5</td>
<td>12</td>
<td>28.2</td>
</tr>
<tr>
<td>F</td>
<td>C.V. 55</td>
<td>55</td>
<td>43.6</td>
<td>8.7</td>
<td>26.5</td>
</tr>
<tr>
<td>G</td>
<td>C.V. 40</td>
<td>40</td>
<td>45</td>
<td>9.8</td>
<td>27.9</td>
</tr>
<tr>
<td></td>
<td>T.V. 40</td>
<td>40</td>
<td>52.5</td>
<td>16.9</td>
<td>32</td>
</tr>
<tr>
<td>H</td>
<td>C.V. 100</td>
<td>100</td>
<td>44</td>
<td>36.4</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>T.V. 100</td>
<td>100</td>
<td>24</td>
<td>32.2</td>
<td>152.2</td>
</tr>
</tbody>
</table>

a) C.V. and T.V. indicate the third cervical and last thoracic vertebra areas, respectively.

Table 2. Effects of steam cleaning

<table>
<thead>
<tr>
<th>Slaughterhouse</th>
<th>Wiped section</th>
<th>n</th>
<th>GFAP concentration (ng/100 cm²)</th>
<th>Rate of decrease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before steam cleaning</td>
<td>After steam cleaning</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean Maximum Minimum Mean Maximum Minimum</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>C.V. 20</td>
<td>20</td>
<td>11.7 190.6 &lt;3</td>
<td>3.4 51.2 &lt;3</td>
</tr>
<tr>
<td>H</td>
<td>C.V. 100</td>
<td>100</td>
<td>36.4 648.8 &lt;3</td>
<td>3.9 13.6 348.2 3.4</td>
</tr>
<tr>
<td></td>
<td>C.V. 100</td>
<td>100</td>
<td>32.2 1,328.4 3.4</td>
<td>18.4 493.0 3.5</td>
</tr>
</tbody>
</table>

a) Steam Vacuum System Model CV-1 (Jarvis, USA) was used.

Table 3. Effects of manual washing with normal pressure water

<table>
<thead>
<tr>
<th>Slaughterhouse</th>
<th>Wiped section</th>
<th>n</th>
<th>GFAP concentration (ng/100 cm²)</th>
<th>Rate of decrease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before washing</td>
<td>After washing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean Maximum Minimum Mean Maximum Minimum</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>C.V. 20</td>
<td>20</td>
<td>115.8 651.8 &lt;3</td>
<td>21.7 73.9 &lt;3</td>
</tr>
<tr>
<td></td>
<td>T.V. 20</td>
<td>20</td>
<td>155.4 1,076.4 &lt;3</td>
<td>17.2 116.8 &lt;3</td>
</tr>
<tr>
<td>C</td>
<td>C.V. 10</td>
<td>10</td>
<td>115.1 1,112.3 &lt;3</td>
<td>0.8 4.6 &lt;3</td>
</tr>
<tr>
<td></td>
<td>T.V. 10</td>
<td>10</td>
<td>63.1 578.0 &lt;3</td>
<td>15.6 106.6 &lt;3</td>
</tr>
</tbody>
</table>

a) The entire split surfaces of carcasses were washed evenly for 15 seconds.
the corresponding rate of decrease after washing. The max-
imum levels of CNST residue decreased after washing, and
both slaughterhouses showed a decrease in the mean CNST
levels of more than 75% after manual washing with normal
pressure water, demonstrating that this treatment is also
effective for CNST removal from carcasses.

**Effects of manual washing with high-pressure water on CNST removal**: In Japan, slaughterhouses generally conduct manual washing of carcasses with high-pressure water using a gun-type nozzle if automatic high-pressure washers are not available. At present, more than 60% of slaughter-
houses have introduced manual washing with high-pressure
water (unpublished observation). Therefore, the effects of
manual washing with high-pressure water on CNST
removal were examined in slaughterhouses D, E and G.
Ebara Pressure washers (Ebara Corp., Japan), Plunger Pump
units (Arimitsu Industry Co., Ltd., Japan) and Rocky wash-
ers (Arimitsu Industry Co., Ltd., Japan) were used in slaugh-
terhouses D, E and G, respectively.

Figure 2 shows the changes in the average levels of
CNST residue during manual washing with high-pressure
water. In slaughterhouse D, the average residue level of
CNST around the last thoracic vertebra was as high as 159.7
ng/100 cm² before washing, but decreased to 24.7 ng/100
cm² after washing for 15 seconds and then to 3.4 ng/100 cm²
after washing for 60 sec. Similarly, the average CNST resi-
due level around the third cervical vertebra was as high as
59.3 ng/100 cm² before washing, but decreased to 19.7 ng/
100 cm² after washing for 15 sec and then to 4.8 ng/100 cm²
after washing for 60 sec (Fig. 2A). In slaughterhouse E, the
average CNST residue levels were relatively low before
washing (12.1 ng/100 cm² around the last thoracic vertebra
and 5.7 ng/100 cm² around the third cervical vertebra). The
levels fell below the detection limit around the last thoracic
vertebra after washing for 45 sec and around the third cervi-
cal vertebra after washing for 30 sec.

We also examined the efficacy of manual washing with
high-pressure water in terms of washing order (Fig. 2B). In
slaughterhouse G, the upper part of the split surfaces of car-
casses in particular were washed for 40 sec and then the
lower parts of the split surfaces of some of the carcasses
were washed intensively for 30 or 45 sec. The average
CNST levels around the third cervical vertebra before wash-
ing, 8.1 ng/100 cm², increased to 14.4 ng/100 cm² when
only the upper part of the carcass was washed, but decreased
below the detection limit when the lower part of the carcass
was also washed for at least 45 sec. The average CNST resi-
due level around the last thoracic vertebra, which was 7.1
ng/100 cm² before washing, decreased significantly even
when only the upper carcass was washed and decreased
below the detection limit when the lower section of the car-
cass was also washed.

**DISCUSSION**

There are several reports available concerning CNST
contamination on the surface of carcasses after high-pres-
sure washing in slaughterhouses. Prendergast et al. [8]
reported high contamination near the splitting line on the
internal surfaces of carcasses, and Lim et al. [6] reported
that the internal surfaces of carcasses were more contamin-
ated than the external surfaces. According to their find-
ings, they believe that contamination on the surface of
carcasses depends not only on the slaughtering method but also on the splitting or washing method. According to Pren-dergast et al. [9], the longer splitting takes, the larger the amount of sawing residue accumulation on the blade and thus the greater the CNST contamination of the carcass.

In the present study, we showed that the degree of CNST contamination was higher in a slaughterhouse where the spinal cord was not removed before splitting than in those where the spinal cord was removed before splitting. Thus, removal of the spinal cord before splitting is believed to be effective in reducing the occurrence of CNST contamination during splitting. Similarly, Helps et al. [4] reported that CNST contamination on the carcass surface is reduced by removal of the spinal column with an experimental oval saw that can remove it without exposing the spinal cord. These reports indicate that spinal cord suction plays a role in reducing the risk of CNST contamination of carcasses. However, it is difficult to completely remove the spinal cord by suction, and substantial amount of residual spinal cord and dura matter still remain in the spinal column after suction. In fact, we showed that the residual levels of CNST varied greatly from over 1 µg/100 cm² to below the detection limit, even though the spinal cord was removed by vacuum before splitting. Such contamination on the surfaces of carcasses is possibly due to scattering of spinal cord fragments remaining in the spinal columns as sawing residue, as pointed out by Ramantani [7]. Thus, adequate washing of carcasses after splitting is indispensable for reducing the risk of CNST contamination.

Steam cleaning and manual washing with normal pressure water are 2 possible methods of ensuring the efficacy of washing with high-pressure water in terms of reducing CNST contamination. In this study, we showed that both methods are useful in reducing the residual levels of CNST but that they cannot completely remove them. Steam cleaning, unlike washing with high-pressure water, does not scatter pieces of tissue, and therefore, it appears to be useful especially in removing spinal cord and dura mater fragments attached around the spinal column without scattering them. Helps et al. [4] reported that steam cleaning would contribute to dissemination of CNST fragments onto the external surface of carcasses by movement of the nozzle. During steam cleaning, the nozzle should not be moved from the internal surface to the external surface of the carcass and must be washed after cleaning of each carcass to prevent spread of CNST via the nozzle itself. As a preliminary washing procedure, washing with normal pressure water can be easily introduced without any special changes in the slaughtering line or installation of expensive equipment. Also, washing with normal pressure water is advantageous for preventing the spread of contamination because it scattering less water than manual washing with high-pressure water.

As shown in Fig. 2, manual washing with high-pressure water for 60 seconds can reduce even large amounts of residual CNST to nearly below the detection limit. However, the levels of CNST residue around the third cervical vertebra increased if only the upper part of the carcass was washed (Fig. 2B). This is probably due to CNST dripping down to the third cervical vertebra area when the carcasses were hung up by the hind legs. Therefore, in order to reduce CNST contamination below the detection level, it is important to perform washing in the correct order and for the appropriate duration. In addition, it is necessary to separate the washing area from the surroundings to prevent spread of CNST via the water if washing with high-pressure water is adopted.

It is important to understand the effect of the washing methods on CNST removal in order to choose a method that works properly. In the present study, we reported the actual situation of contamination of carcasses by CNST and showed the effects of different methods of carcass cleaning on CNST removal. Our results suggest an efficient method for removal of CNST contamination from the surface of carcasses. To minimize CNST contamination on the surface of carcasses, vacuum removal of the spinal cord is a prerequisite. High levels of CNST contamination, possibly caused by splitting, should be reduced by a preliminary washing step such as steam cleaning or washing with normal pressure water, and washing with high-pressure water should then be performed to reduce the CNST contamination to a negligible level. We believe that these results will provide useful information for creating sanitation standard operating procedures and thus contribute to reducing the risk of spread of BSE prion via carcasses and meat products contaminated with CNST.

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