



Effect of Spinal Cord Removal before or after Splitting and Washing on CNST Decontamination of Beef Carcasses

D. G. Lim, D. H. Kim¹ and M. Lee*

Department of Agricultural Biotechnology, College of Agriculture and Life Science, Seoul National University
San 56-1, Sillim-Dong, Gwanak-Ku, Seoul, 151-921, Korea

ABSTRACT : Beef carcasses were examined to explore the effects of spinal cord removal and washing on central nervous system tissue (CNST) decontamination of the surface during the slaughtering process. A total of 15 carcasses were split by sawing centrally down the vertebral column and left sides of split carcasses were used for analysis. Samples were collected by swabbing the surface from 4 defined parts on the interior and 4 on the exterior of carcasses from the abattoir and analyzed using an ELISA-based test. The results showed that automatic and manual spray washing decreased CNST contamination, especially on the interior ventral parts of carcass surfaces ($p < 0.01$), but did not decrease CNST on the interior dorsal parts. Increasing washing time to 60 s did not affect the reduction of CNST contamination. Samples following spinal cord removal prior to splitting showed lower calculated levels of "risk material" than the stated limit of detection (0.1%) of the ELISA kit on interior and exterior carcass parts ($p < 0.01$). Therefore, spinal cord removal prior to splitting could be a very effective way to minimize CNST contamination of beef carcasses. (**Key Words :** Beef Carcass, CNST Dissemination, Spinal Cord Removal, Carcass Washing)

INTRODUCTION

BSE is transmitted from cattle to humans when infected beef carcasses are cut up, ground, or processed and consumed by people. Brain and spinal cord were identified as high risk materials, which contributed to spreading of the abnormal prion material to the other carcass areas and organs. The consumption of the specified risk materials (SRM) was banned by the European commission in 1997. The goal of the ban was to avoid health risks related to BSE, which linked to new variant Creutzfeldt-Jakob Disease in humans (Smith, 2005; Choi, 2007). According to Prendergast et al. (2003), to limit the transmission of BSE prions by preventing the transfer of SRM, it is essential to be able to monitor and prevent the dissemination of brain and spinal cord material onto the carcass. Prendergast et al. (2003) also suggested that current slaughtering conditions and procedures may result in widespread dissemination of SRM within abattoirs, contaminating equipment, carcasses surfaces, operators and carcasses destined for human

consumption. The critical step in the dissemination of the CNST onto the carcass surfaces and meat is the carcass splitting process (Schmidt et al., 2001; Helps et al., 2002; Helps et al., 2004). However, it has generally not been practical to remove the spinal cord before the splitting process because the spinal cord is located within the vertebrae column.

The majority of abattoirs use warm water (30-60°C) for washing, which has no decontaminating effect and is used to remove bone dust, blood colts and blood splashes (Sheridan, 1998; Doherty et al., 1999). Bone dust arises from splitting the vertebrae and contains CNST from cutting the spinal cord (Schmidt et al., 2001). However, there is no available information on the effect of washing methods on reducing the CNST dissemination on the carcass surface.

It has also been demonstrated that stunning of cattle with captive bolt guns (CBG) causes neural emboli to enter the bloodstream and potentially contaminate other tissues (Garland et al., 1996; Schmidt et al., 1999; Anil et al., 2002). Regulations require slaughterhouses to implement measures to prevent any CNST (especially those considered SRM) from cross-contaminating other tissues at slaughter. Such measures include cleaning tissue debris near the stun wound and physical scraping of the spinal column to remove the

* Corresponding Author: M. Lee. Tel: +82-2-880-4804, Fax: +82-2-873-4804, E-mail: moohalee@snu.ac.kr

¹ National Institute of Animal Science, R. D. A., 564 Omoekchun-Dong, Suwon 441-350, Korea.

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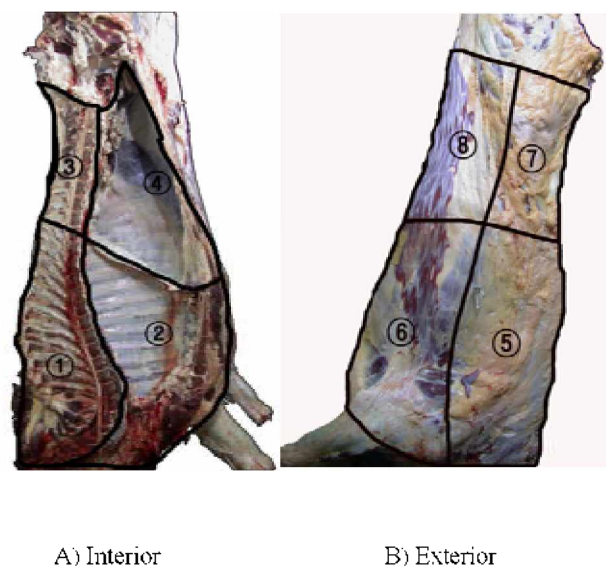


Figure 1. Schematic diagram of the eight parts swabbed on the split beef carcasses. A) Interior and B) Exterior area.

spinal cord (Lopes et al., 2006). Helps et al. (2002) have demonstrated that splitting during the dressing process could result in the dissemination of spinal cord material over the carcass, operator and environment. To avoid and decrease the dissemination of the risk material, Troeger (2004) introduced several new slaughtering and splitting techniques that are conceivable. The techniques were suction of the spinal cord prior to carcass splitting, removal of the complete vertebral column or paramedian sawing and de-boning without carcasses splitting (Troeger, 2004). The efficiency of the suction method is not satisfactory yet, and a complete spinal cord removal is made more difficult because of the occurrence of occasional breaks in vertebral column or dislocation of vertebrae (Troeger, 2004).

Therefore, in order to make the prevention of CNST contamination more practical, a method should be developed to avoid the CNST dissemination during splitting. However, there is little information on the effect of spinal cord removal before or after splitting on the CNST dissemination on the carcass surface. Therefore, this study was conducted to examine the effect of spinal cord removal before or after splitting and of washing procedures on CNST contamination.

MATERIALS AND METHODS

Sample collection and preparation for analysis

Beef carcasses were from the commercial abattoir (Young Nam Industry) in Chang-Nyeong, Korea, with the capacity to slaughter about 30 animals per day. This slaughterhouse used the penetrating captive bolt gun (PCB) for stunning. A total of 15 carcasses were split along the center of the spinal column vertically with a band saw

(Jarvis Buster IX, Jarvis Products Corporation, Middletown, Conn., USA). The splitting saw has a built-in washing function, other than that the saw was not washed additionally between the splitting. Left sides of split carcasses were used for analysis.

Two groups of beef carcasses were used. The first group was conducted to examine the effect of different washing times and methods after spinal cord removal on CNST decontamination. An automatic washing system (Hyundai slaughter machinery Co., Korea) built in the slaughterhouse was compared with manual washing equipment (PA[®] T47 INOX, Italy) with respect to CNST decontamination. Water pressure of automatic and manual washing was 100 bar (1.450 psi). The experimental vacuum suction cleaner (KARCHER NT 65/2 Eco 1750 W, Germany) was used to suck out the spinal cord. Interior carcass surfaces (left hand side) of the split carcasses were used (Figure 1). The sampling positions for the experiment were as follows: (i) the surface of the left side of the carcass without washing after splitting (control) (ii) the surface of the left side of the carcass, immediately after it had been automatically spray-washed with 5°C cold water (20 sec), (iii) the surface of the left side of the carcass, immediately after it had been manually spray-washed with 5°C cold water (20, 40 and 60 sec), (iv) the surface of the left side of the carcass, immediately after automatic (20 sec) and manual spray-washing (20 sec) with 5°C cold water following the spinal cord removal. Samples for detecting CNST were collected by swabbing the surface from four defined areas on the interior surface (left hand side) of the split carcasses (Figure 1).

The second group was conducted to compare the effect of spinal cord removal before or after splitting on interior carcasses for CNST decontamination. Interior and exterior carcass surfaces (left hand side) of the split carcasses were used (Figure 1). Before carcasses splitting, the vacuum suction device equipped with air pump system (KOOK Bo Tech Co. Ltd., Korea) built in the slaughterhouse was used to remove the spinal cord and cerebrospinal fluid. This device for sucking the spinal cord was done by manual pulling the tip of the hose into the vertebral channel upwards. After carcasses splitting, spinal cord was removed with the device (KOOK Bo Tech Co. Ltd., Korea). Each carcass was spray washed with 5°C water at a pressure of 3 bar. The samples were collected from eight defined parts of the split carcass surface after carcasses washing (Figure 1).

Along the vertebrae, interior and exterior side of the split carcass were divided into 4 parts each (interior parts were from part 1 to part 4 and exterior parts from part 5 to part 8): part 1 was sagittal section of dorsal spines and centra of thoracic vertebrae, part 2 was ventral section of costae and internal of thoracic cavity, part 3 was centra of lumbar vertebrae, part 4 was the internal surface of the

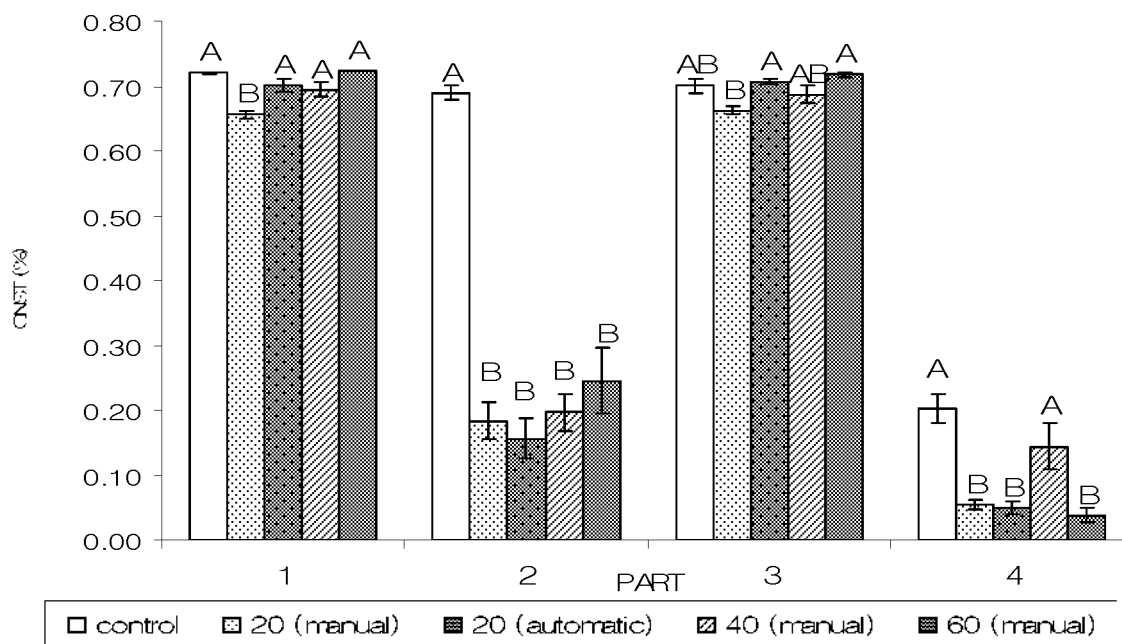


Figure 2. The effect of automatic and manual washing time on interior carcass for CNST decontamination (n = 15). Standard errors appear on each bar. The control is without washing after splitting. Part 1-4 (as defined in Figure 1) were swabbed and analyzed for GFAP ELISA test. ^{A,B} Means with different letters marked on the figure are significantly different from each other (p<0.01). Kit detection limit is 0.1% and values falling below detection limit are considered negative and those above it are positive (Hajmeer et al., 2006).

abdominal cavity, parts 5, 6, 7 and 8 were the external side of parts of 1, 2, 3 and 4, respectively (Figure 1). Samples were prepared by dipping a Dacron[®] fiber-tipped sterile swab (Fisher Scientific, Houston, TX, USA) five times into the sample while rotating the swab. The swab was removed and squeezed into a 2 ml test tube containing 1 ml of 0.5% SDS sample dilution buffer (Ridascreen, R-Biopharm, Darmstadt, Germany). The aliquoted samples were stored at 4°C and measured at one day after collection.

Glial fibrillary acidic protein (GFAP) enzyme linked immunosorbent assay (ELISA)

Detection of CNST was performed on samples using a commercial ELISA-based test - the Ridascreen[™] risk material 10/5 kit (R-biopharm, AG Darmstadt, Germany), which detects glial fibrillary acidic protein as a marker. All procedures for the assay kits were provided by the manufacturer (Art. No. R6703, 10/5, Ridascreen[™] risk material, R-biopharm, AG Darmstadt, Germany). A 50 µl aliquot of sample was transferred from each test tube to an assay well. A sufficient number of antibody-coated wells were inserted into the micro-well holder to accommodate the number of samples and all standards tested (four standards, containing 0%, 0.1%, 0.2% and 0.4% CNST composed of brain and spinal cord, were provided with the Ridascreen[®] assay). Enzyme conjugate (50 µl) was added to each well containing four standard and samples, and the plate was incubated for 10 minutes at room temperature

(20-25°C). After incubation, the liquid was poured out to empty the wells, and the micro-well holder was tapped upside down thoroughly against absorbent paper, so that the liquid added earlier was completely removed from the wells. The wells were washed again with 250 µl of washing buffer and emptied as described above. A 100 µl volume of substrate/chromogen mixture was added to each well and mixed thoroughly, after which time the plate was incubated for 5 minutes at room temperature in the dark. The reaction was stopped by adding 100 µl of stop solution to each well. Color intensity or optical density (absorbance) was determined by photometric evaluation using a microtiter spectrophotometer (Microplate Reader 550, BioRad, Hercules, CA, USA) with a filter corresponding to 450 nm. Results were interpreted as indicated in the Ridascreen[®] risk material 10/5 kit information booklet. Based on the Ridascreen[®] assay kit booklet, the detection limit of the kit was 0.1% for CNST contamination in meat. Values falling below detection limits were considered negative and those above it were positive (Hajmeer et al., 2006).

Statistical analysis

The values are expressed as mean±SE. Statistical analyses were performed by one way analysis of variance (ANOVA) using linear models. The models for this analysis included as single factor (washing time, washing method and spinal cord removal before or after splitting). Significant differences were detected by Duncan's multiple

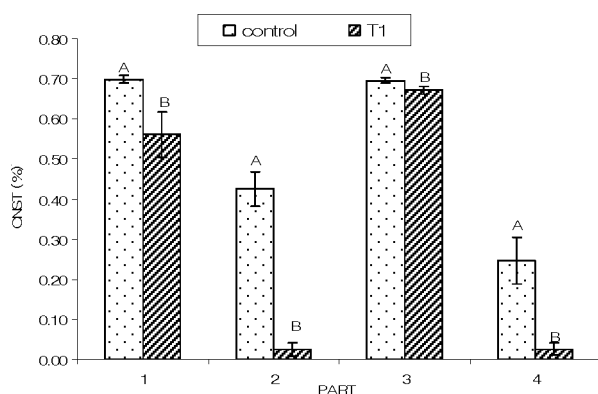


Figure 3. The effect of combination washing methods after spinal cord removal on interior carcass for CNST decontamination ($n = 15$). The control is without washing after splitting. T1 is with automatic (20 s) and manual (20 s) washing after spinal cord removal on splitting carcass. Parts 1-4 (as defined in Figure 1) were swabbed and analyzed for GFAP ELISA test. ^{A, B} Means with different letters marked on the figure are significantly different from each other ($p < 0.01$). Kit detection limit is 0.1% and values falling below detection limit are considered negative and those above it are positive (Hajmeer et al., 2006).

range test using the Statistical Analysis System software (SAS, 1997). An alpha level $p < 0.01$ was used to determine significance.

RESULTS AND DISCUSSION

Effects of different washing time and methods after spinal cord removal on CNST decontamination

Based on the assay instructions, positive CNST contamination existed at more than 0.1% level of CNST (Hajmeer et al., 2006). The effect of automatic and manual washing time on CNST decontamination is shown in Figure 2. The four defined interior carcass parts (Figure 1) were evaluated and the results showed that the automatic and manual spray washing decreased CNST contamination, especially in parts 2 and 4 ($p < 0.01$). Overall, the results showed that the level of CNST contamination was significantly lower after the washing than before washing ($p < 0.01$). Part 2 showed the highest effect of the washing ($p < 0.01$). Increasing washing time to 60 sec did not affect the level of reduction of CNST contamination ($p < 0.01$). From this result, automatic and manual washing were effective in reducing CNST contamination for parts 2 and 4 ($p < 0.01$), but not parts 1 and 3. During splitting of carcasses, CNST was transferred to the internal carcass surfaces 1 and 3 especially (Helps et al., 2002). Prendergast et al. (2004) evaluated the CNST contamination on carcass surfaces from 3 different abattoirs. They reported that washing was completely effective in removing CNST from part 4 of the carcasses, in abattoir B, and from parts 2 and 4 of carcasses in abattoir C ($p < 0.01$), but did not significantly reduce the

CNST concentration of any of the four parts of the carcasses in abattoir A, or parts 1, 2 and 3 in abattoir B.

The effects of washing methods after spinal cord removal using vacuum suction for CNST decontamination is shown in Figure 3. Initial contamination of CNST was 0.70, 0.43, 0.70 and 0.25% for parts 1, 2, 3 and 4, respectively. The spinal cord removal with combination washing could reduce the CNST level to 0.56, 0.02, 0.67 and 0.03% for parts 1, 2, 3 and 4, respectively. Overall, the results showed that the level of CNST contamination was significantly lower after the treatment relative to the control ($p < 0.01$). This result showed that the spinal cord removal with combination washing was very effective in reducing CNST contamination for parts 2 and 4 of carcasses ($p < 0.01$), however, it was not able to remove all CNST contamination for parts 1 and 3. The process of splitting the carcass was the main one responsible for dissemination of CNST on the carcass surface (Helps et al., 2002). Their study also showed that carcass washing and steam or vacuum cleaning did not reduce CNST levels. Environment samples from the slaughterhouse indicated considerable CNST on the splitting saw and in water from the saw. The automatic hot water spray, which is used between each carcass to clean the saw, did not remove the CNST in some parts of the saw and associated equipment. This is consistent with previous research indicating that washing did not significantly affect the contamination of CNST on the parts 1 and 3 of the carcass surface. One negative effect of spray washing was a redistribution or spreading of a localized microbial population over a much larger area (Cabedo et al., 1996). Spray washing resulted in a similar redistribution or spreading of CNST to adjacent beef carcass surfaces. Presently, the majority of abattoirs in Korea use water for beef carcass washing. The purpose of this is used to remove bone dust, blood clots and blood splashes (Prendergast et al., 2004). Bone dust arises from cutting the vertebrae and contains CNST from cutting the spinal cord (Schmidt et al., 2001). The current results showed that spinal cord removal combined with washing was very effective in reducing CNST contamination from parts 2 and 4 of the internal surface of the carcass. However, it was not able to reduce all of CNST contamination on parts 1 and 3.

Effects of spinal cord removal before and after splitting on CNST decontamination

The effect of spinal cord removal before and after splitting on carcasses for CNST decontamination is shown in Figure 4 and 5. Eight defined carcass parts on the split beef carcass were evaluated (Figure 2). Figure 4 shows that the level of CNST contamination following spinal cord removal after splitting was still more than 0.1% levels in parts 1, 2 and 3 of the interior of the carcass. However, the level of CNST contamination following spinal cord removal

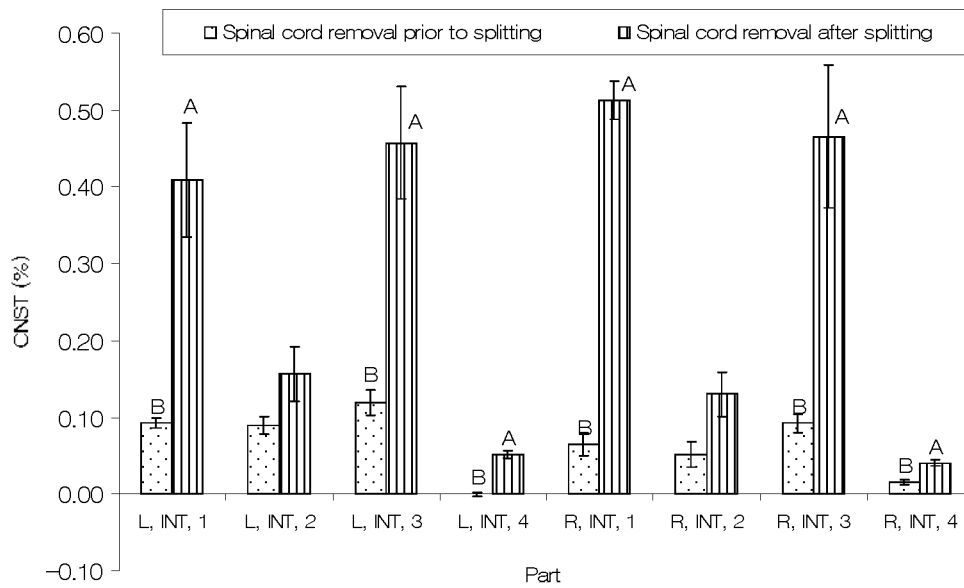


Figure 4. The effect of spinal cord removal before and after splitting on interior carcasses for CNST decontamination (n = 15). Each carcass was spray washed after splitting with cold water. Part 1-4 (as defined in Figure 1) were swabbed and analyzed for GFAP ELISA test. ^{A, B} Means with different letters marked on the figure are significantly different from each other (p<0.01). L = left half, R = right half, INT = medial surface. Kit detection limit is 0.1% and values falling below detection limit are considered negative and those above it are positive (Hajmeer et al., 2006).

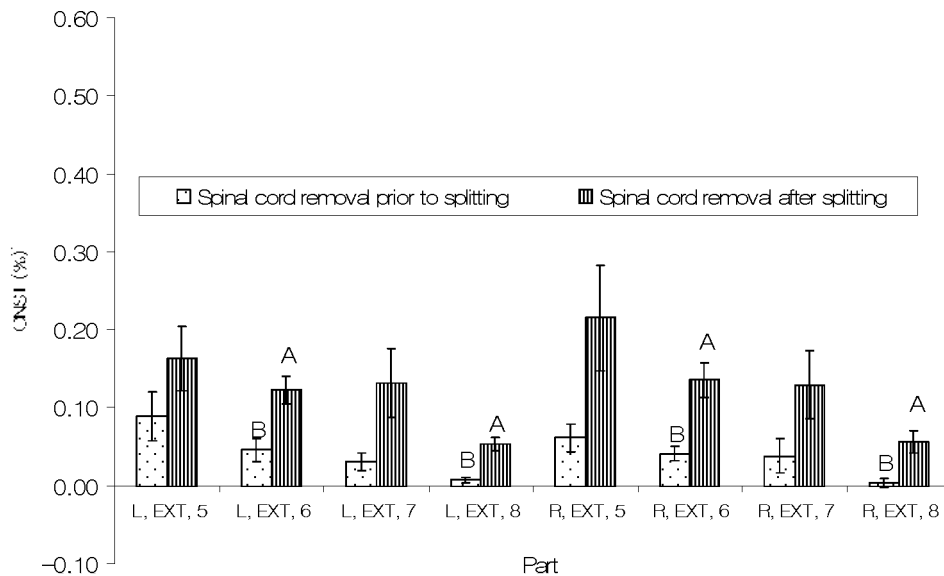


Figure 5. The effect of spinal cord removal before and after splitting on exterior carcasses for CNST decontamination (n = 15). Each carcass was spray washed after splitting with cold water. Part 5-8 (as defined in Figure 1) were swabbed and analyzed for GFAP ELISA test. ^{A, B} Means with different letters marked on the figure are significantly different from each other (p<0.01). L = left half, R = right half, EXT - lateral surface. Kit detection limit is 0.1% and values falling below detection limit are considered negative and those above it are positive (Hajmeer et al., 2006).

prior to splitting was significantly lower than for removal after splitting at less than 0.1% level in all parts of the interior surface of the carcass (p<0.01).

Similar results are shown in Figure 5 for the exterior

surfaces of carcasses with the level of CNST contamination following spinal cord removal after splitting being more than 0.1% levels in parts 5, 6 and 7, but the level following spinal cord removal prior to splitting was significantly

lower at less than 0.1% levels in all parts ($p < 0.01$). From these results, it can be seen that the spinal cord and cerebrospinal fluid removal using the vacuum suction device before splitting resulted in significantly less CNST contamination in all interior and exterior carcass parts ($p < 0.01$). These results indicate that spinal cord removal prior to splitting could be very effective to minimize CNST contamination of beef carcass.

Most of the CNST was detected in parts 1 and 3, along the vertebral column, as has been observed by Helps et al. (2002), Schwagele et al. (2002) and Lim et al. (2007). In this experiment, the level of CNST contamination following spinal cord removal after splitting was also observed at more than 0.1% levels in parts 5, 6 and 7 of exterior carcass. It was unexpected but could have been due to transfer of CNST from the internal surface by the cleaning process (Help et al., 2002). One method for removing the spinal cord includes a vacuum hose, which could reduce the risk of contamination, however it was not completely successful because the spinal cord frequently breaks (Lucker et al., 2002; Schwagele et al., 2002). Troeger (2004) suggested that it would be unnecessary to saw through the spinal cord if the entire carcass was boned hot or cold before it was separated into primal cuts. Rotterud et al. (2006) introduced the alternative method to reduce the CNST contamination by hot boning and cutting the carcass horizontally. According to their results there was no contact between the contaminated knife and the meat and, as a result, CNST contamination was not detected. Frederick et al. (2004) and Smith (2005) described key elements of carcasses splitting and spinal cord removal as follows: (i) the spinal canal should be fully exposed on both sides of the carcass; (ii) the splitting saw should be sterilized between carcasses to remove protein build-up thereby decreasing potential contamination from carcass to carcass; (iii) missplit carcasses should be handled on the harvest floor to ensure complete removal of the spinal cord and meninges (sheath); (iv) one operator on an elevator or two operators (one high, one low) may be needed to remove all of the spinal cord in the sacral-vertebral area; (v) an auditing system should be implemented on the harvest floor to ensure that no SRM remains attached to the carcass if a deviation occurs. Appropriate corrective actions and preventive measures should be taken and documented. Hajmeer et al. (2006) reported that beef carcass samples from advanced meat recovery (AMR) and hand-boning methods showed lower calculated levels of "risk material" than the stated limit of detection (0.1%) of the ELISA kit. Troeger (2004) suggested that to avoid dissemination of the CNST to carcass and meat at slaughtering, (i) electrical stunning (ii) cutting without opening the vertebral channel (iii) head remaining on the carcass may be considered practically to exclude a contamination risk. The review also indicated that

if conventional slaughtering processes are retained (captive bolt stunning, longitudinal sawing with opening of the vertebral channel), (i) suction of the spinal cord from the whole carcass (by means of PVC hose and vacuum), changing hose for every batch (ii) machine skinning of head; closing of captive bolt aperture and foramen occipitale magnum can be used to minimize the risk of spreading CNST on carcasses.

Our experiments were carried out under small-scale circumstance so the detailed results should be approached with some caution. Further studies should be considered and tried such as electrical stunning, removal of the whole vertebral column and deboning without splitting (Troeger, 2004). Nonetheless, as would be expected, spinal cord removal prior to splitting does appear to be effective in minimizing CNST contamination on beef carcasses.

IMPLICATIONS

In light of current consumer concern about BSE, a disease believed to be transmitted by consumption of CNST, there is concern about dressing procedures such as the severing of the spinal cord along the vertebral column during carcass splitting, because it may cause cross-contamination of the carcass with SRM. Therefore, regulations may require slaughterhouses to implement measures to prevent any CNST contamination (especially those considered SRM). In this study, samples from the carcass surface following spinal cord removal prior to splitting showed lower calculated levels of "risk material" than the stated limit of detection (0.1%) of an ELISA kit in all surfaces of the interior and exterior of carcasses ($p < 0.01$) relative to samples when the spinal chord was removed after splitting. Therefore, it was concluded that the spinal cord removal prior to splitting was the more effective way to minimize the risk of spreading CNST on beef carcasses.

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