Journal of Food Protection, Vol. 69, No. 6, 2006, Pages 1388–1392

Alternative Cutting Methods To Minimize Transfer of Nervous System Tissue during Steak Preparation from Bone-in Short Loins

M. LOPES, J. D. STOPFORTH, K. SUCRE, R. R. MIKSCH, E. GIDDENS, M. C. S. REDDY, R. S. YEMM, AND M. SAMADPOUR*

Institute for Environmental Health, Inc., 15300 Bothell Way N.E., Seattle, Washington 98155, USA

MS 05-452: Received 31 August 2005/Accepted 21 January 2006

ABSTRACT

Fresh beef products, such as steaks, may become contaminated with potential specified risk materials (SRMs), such as central nervous system tissue, during the fabrication of bone-in loin subprimals. The objective of this study was to evaluate current and alternative cutting methods that could be used to minimize the transfer of nervous system tissue (NST) tissue during preparation of steaks from bone-in short loins. Bone-in short loins were cut according to three methods. (i) Cutting method I—The vertebral column bones were removed prior to cutting the loin into steaks from the medial (vertebral column) to lateral (flank) side. (ii) Cutting method II—The loin was cut into steaks from the vertebral column side to the flank side prior to removal of the vertebral column bones. (iii) Cutting method III—The loin was cut into steaks from the flank side to the vertebral column side prior to removal of the vertebral column bones. Results indicated that surface areas along the vertebral column cutting line had detectable (0.10 and 0.22% NST/100 cm^2) and, thus, higher potential SRM contamination than resulting steak surfaces or the cutting blade. Overall, there were no detectable ($<$0.10% NST/100 cm^2) differences in NST contamination of steaks produced by the three cutting methods. Immunohistochemical evaluation of areas on excised and ground steak surfaces indicated that regardless of cutting method, there was generally ''no'' to ''moderate'' staining, suggesting that detectable (0.137 to 0.201% NST) contamination from these samples was most likely due to peripheral nerve detection. These results imply that steaks may be cut from bone-in short loins prior to removal of the vertebral column bones without affecting the transfer of NST to resulting steaks at concentrations $<$0.10% NST/100 cm^2.

Bovine spongiform encephalopathy (BSE) is a neurodegenerative disorder of cattle caused by the accumulation of an abnormal form of prion protein in central nervous system (CNS) tissue. The etiologic agent of a similar disease affecting humans, namely variant Creutzfeldt-Jakob disease, has been shown to be equivalent to the agent found in BSE *(7)*. Thus, consumption of beef tissue infected with BSE may cause variant Creutzfeldt-Jakob disease. In light of recent occurrences regarding detection of BSE in the U.S. cattle supply, the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) issued regulations (9 CFR 310.22) regarding control and removal of specified risk materials (SRMs) from cattle during preparation of beef products. SRMs are parts of the cattle that may contain BSE infectivity. These are considered (9 CFR 310.22) the brain, skull, eyes, trigeminal ganglia, spinal cord, vertebral column (excluding the vertebrae of the tail, the transverse processes of the thoracic and lumbar vertebrae, and the wings of the sacrum), and dorsal root ganglia of cattle 30 months of age and older, as well as the tonsils and lateral ileum of all cattle. Despite these regulations, routes still exist whereby carcasses may inadvertently become contaminated with SRMs because of transfer by equipment used in cattle slaughter and meat processing. It has been demonstrated that stunning of cattle with captive bolt guns causes neural emboli to enter the bloodstream and potentially contaminate other tissues *(1, 2, 5)*. Severing the spinal cord along the vertebral column during carcass splitting may also cause cross-contamination of the carcass with SRMs *(3, 4, 6)*. Regulations require slaughterhouses to implement measures to prevent any CNS tissues (especially those considered SRMs) 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 spinal cord.

According to the USDA-FSIS (USDA-FSIS Notice 9-04, 23 January 2004), removal of the vertebral column in cattle ≥30 months of age is not required during slaughter, provided that procedures are in place to ensure that the vertebral column is adequately tracked and properly disposed of during later stages of processing. Steak preparation often involves the use of a band saw to cut steaks from subprimals, such as bone-in short loins. The potential of spreading associated CNS tissue (residual spinal cord not completely removed at slaughter or associated nerve tissue) during mandatory removal of the vertebral column bone is the primary concern with producing steaks from bone-in subprimals.

The objective of this study was to investigate the effect of three cutting methods on nervous system tissue (NST)

* Author for correspondence. Tel: 206-522-5432; Fax: 206-306-8883; E-mail: mansour@u.washington.edu.

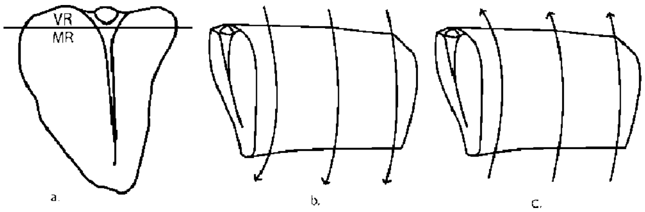

FIGURE 1. *Representation of the methods of steak cutting examined in the study. (a) Diagram of the vertebral surface region (VR) and the meat surface region (MR); (b) transverse cut from the vertebral column side to the flank side of the loin used in cutting methods I and II (prior to removal of the vertebral column bones); and (c) cut from the flank side to the vertebral column side of loin prior to removal of the vertebral column bones used in cutting method III.*

contamination of steaks prepared from bone-in short loins containing the vertebral column.

MATERIALS AND METHODS

Steak preparation. Bone-in short loins (ca. 7 kg each) were obtained from a slaughterhouse in the midwestern United States where the majority of animals processed are 30 months of age and older. Short loins containing the vertebral column (spinal cords removed during slaughter) were each cut into six steaks of similar thickness (ca. 3.8 cm) according to three methods (Fig. 1). Method I—the vertebral column (an SRM) was removed via a longitudinal cut down the column prior to transversely cutting the loin into steaks. The first cut in method I was performed with a band saw as close as possible to the vertebral column, thereby generating two pieces of the loin: the meat portion with the meat surface region and the vertebral column portion with the vertebral surface region. Steak cutting in method I was performed transversely from the medial (vertebral column) to the lateral (flank) side of the loin. Method II—the loin was cut into steaks from the vertebral column to the flank side prior to removal of the vertebral column. Method III—the loin was cut into steaks from the flank side to the vertebral column side prior to removal of the vertebral column. All steaks were cut by a licensed butcher from a local supermarket with a Delta Shopmaster BS220LS band saw (Delta Machinery, Jackson, Tenn.). The cutting blade and the housing surrounding the blade were washed with hot (ca. 55°C) water and detergent (7X Cleaning Solution, ICN Biomedicals, Inc., Aurora, Ohio) between every cut.

Meat product sampling: vertebral column cut surfaces and nerve tissue. After the initial cut was performed according to method I, the meat surface and vertebral surface regions of the meat and vertebral column portions, respectively, were sampled. Powder-free latex gloves (Microflex Corp., Reno, Nev.) were worn for all sampling procedures. Individually wrapped sterile cotton-tipped swabs (Curtin Matheson Scientific, Houston, Tex.), premoistened in sample dilution buffer containing 0.05% sodium dodecyl sulfate (SDS; R-BioPharm, Darmstadt, Germany), were used to swab areas measuring 10 by 10 cm (100 cm^2). Three swab samples were taken on each of the meat surface and vertebral surface regions at the cranial end (I), the medial portion (II), and the caudal end (III). A stainless steel Bone Dust Scraper (Hubert, Harrison, Ohio) was subsequently used to remove debris from both surfaces, and the debris samples were placed in sterile 50-ml centrifuge tubes (Fisher Scientific, Pittsburgh, Pa.) for testing. The same areas (10 by 10 cm) on both surfaces were also swabbed after scraping. Dorsal root ganglion tissue was removed from the vertebral column by a veterinary pathologist with a sterile scalpel and forceps and placed in a separate sterile 50-ml centrifuge tube (Fisher) for testing.

Meat product sampling: steak surfaces. The top and bottom steaks from each short loin were discarded. Immediately after cutting, each of the six remaining steaks were swabbed in areas measuring 10 by 10 cm (100 cm^2) on both sides (A and B) with sterile, cotton-tipped swabs (Curtin Matheson) premoistened in sample dilution buffer (R-BioPharm).

Cutting blade sampling. The Delta Shopmaster BS220LS band saw blade (30.5-cm cutting area, 0.7-cm-wide blade) was swabbed on both sides (43-cm^2 area) with sterile, premoistened cotton-tipped swabs (Curtin Matheson) at several points throughout the procedure. During cutting method I, the blade was swabbed before and after the initial cut was made to remove the vertebral column bone. The blade was then cleaned with hot (ca. 55°C) water and detergent (ICN Biomedicals) and swabbed before and after each of the six steaks were cut. During cutting methods II and III, the blade was cleaned between each use, and swab samples were taken before and after each cut was performed.

Preparation of standards and samples for GFAP-ELISA. Four glial fibrillary acidic protein (GFAP) risk material standards equivalent to concentrations of 0, 0.1, 0.2, and 0.4% risk material in samples, provided in a ready-to-use state as part of the RidaScreen Risk Material 10/5 test kit (R-BioPharm), were utilized in the enzyme-linked immunosorbent assay (ELISA). The four risk material standards were run with each ELISA and were placed in the first four wells of a microtiter plate coated with anti-GFAP antibodies supplied with the kit. Swabbed samples were squeezed thoroughly against the wall of sterile 1.5-ml microfuge tubes (USA Scientific, Ocala, Fla.) containing 1 ml of sample dilution buffer with the 0.05% SDS provided with the RidaScreen kit. All diluted samples were vortexed before testing, and a volume of 50 μl of swab extract was directly utilized in the ELISA.

GFAP-ELISA. Each of the four standards and swab samples (50 μl) were added to wells of the antibody-coated microtiter plate. All reagents used were provided in the RidaScreen Risk Material 10/5 test kit (R-BioPharm). Peroxidase-conjugated antibody against GFAP (50 μl) was dispensed into each well of the plate and mixed by gentle hand rocking. Following a 10-min incubation at room temperature (20 to 25°C), the plate was washed three times with 250 μl of washing buffer (10 mM phosphate-buffered saline [pH 7.4] with 0.05% Tween 20) per well and blotted after each wash. Red Chromogen Pro substrate-chromogen containing tetramethylbenzidine was added (100 μl) to each well. The plate was gently rocked and then incubated in complete darkness for 5 min at room temperature (20 to 25°C). Stop-reagent containing 1 N sulfuric acid was added to each well (100 μl), and the absorbance was read within 15 min at 450 nm against four air-blanks. A Stat Fax 303 Plus spectrophotometer (Embee Diagnostics, Delhi, India) was used for all absorbance measurements. The detection limit of the RidaScreen test kit is 0.10% risk material per 100 cm^2 for CNS in meat products, as specified by the manufacturer.

Ground steak sampling. After all steak surfaces had been swabbed, three areas (A, B, and C) on each steak were identified and removed by the licensed butcher (Fig. 2). Each portion removed was comparable in size and weighed approximately 30 to 50 g. For steaks cut following the same method, all samples from each specific area (e.g., area A from all steaks cut according to method I) were collected in one sterile Whirl-Pak stomacher bag

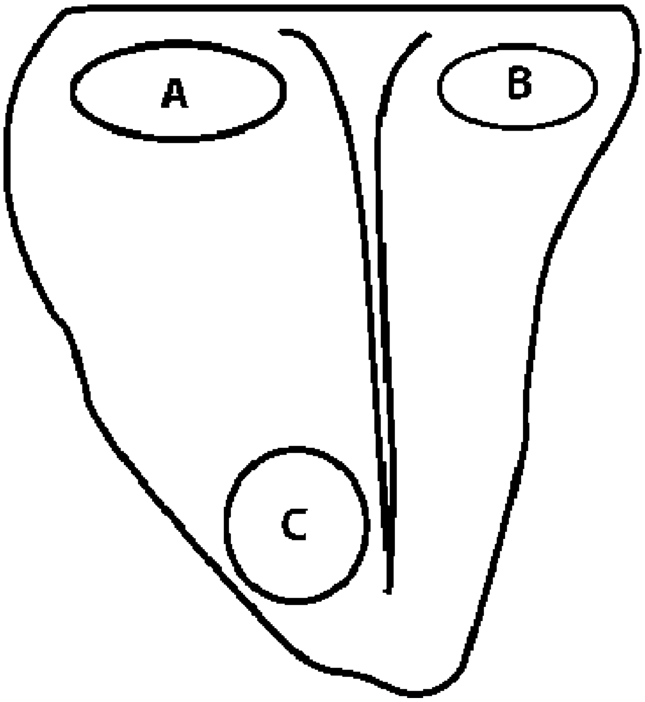

FIGURE 2. *Diagrammatic representation of the three areas (A, B, and C) excised from steaks and ground for immunohistochemical staining.*

(Nasco Co., Fort Atkinson, Wis.). The composite samples were ground together with a Kitchen Aid Professional 5 Stand Mixer (Kitchen Aid, St. Joseph, Mich.). The homogenized samples were each placed in sterile stomacher bags for immunohistochemical staining. The mixer and attachments were thoroughly cleaned with warm water (ca. 55°C) and mild soap (ICN Biomedicals) between each composite sample. In addition, ground steak samples for each area were sampled by stabbing into the homogenate with a sterile, premoistened cotton-tipped swabs and tested for NST by the GFAP-ELISA as previously mentioned.

Preparation of blocks and slides from ground steak samples. Meat product taken from three different areas of each ground steak sample was used to create three blocks (approximately 25 by 20 by 5 mm) of tissue. Tissue blocks were immersed in 10% neutral-buffered formalin (Thermo Electron Corporation, Louisville, Colo.) solution and fixed for up to 24 h before immersion in paraffin wax. Each block was cut into 4- to 6-μm-thick sections with a microtome (Polysciences, Inc., Warrington, Pa.), and the sections were floated on a warm (55°C) water bath. One section from each tissue block was placed onto a positively charged glass slide and dried overnight for GFAP immunohistochemical staining. Dorsal root ganglia and two brain titer slides were also prepared as outlined above for controls, with three pieces of human brain tissue (cerebellum, cerebral cortex, and deeper tissue including putamen) per slide. Test samples, positive and negative controls, and dorsal root ganglion slides were immersed in xylene five times to remove paraffin and then rehydrated twice with 100 and 95% ethanol, respectively.

Hematoxylin and eosin staining of samples. After deparaffinization and rehydration, the slides were immersed in Gill's hematoxylin (Polysciences) solution for 1 min and rinsed in 1% acetic acid, followed by water. Slides were then rinsed in Scott's Tap Water (Pathtech, Victoria, Australia), followed by water. Finally, slides were rinsed in 95% ethanol and immersed in eosin (Polysciences) solution for 30 s.

Immunohistochemical staining for GFAP from ground steak samples. After deparaffinization and rehydration, antigen retrieval Citra buffer (BioGenex Laboratories, San Ramon, Calif.) was used to perform antigen retrieval according to the manufacturer's instructions. Following a rinse with Tris-buffered saline with added Tween 20 (TBST), the diluted monoclonal anti-GFAP antibody (BioGenex) was applied to all sample slides and one control slide (positive control) and then incubated at room temperature (about 23°C) for 30 min. Slides were rinsed a second time in TBST, and the secondary antibody (biotinylated goat anti-mouse, Biocare Medical, Walnut Creek, Calif.) was applied to all slides, including the first control slide (positive control) and the second control slide (negative control). All slides were incubated at room temperature (about 23°C) for 20 min; they were then rinsed with TBST buffer and incubated at room temperature (about 23°C) for 20 min in alkaline phosphatase–labeled streptavidin (BioGenex). Fast red substrate (BioGenex) was applied following a rinse in TBST and incubated for 5 min at room temperature (about 23°C). All slides were rinsed with distilled water, counterstained with Gill's hematoxylin for 15 s, and then rinsed with 1% acetic acid and tap water, respectively. Three drops of Crystal Mount Aqueous/Dry Mounting Media (Biomeda Corp., Foster City, Calif.) were applied to each tissue section, which was then drained, dried for 30 min, and observed under a microscope. Evaluation of positive staining in samples was performed by a veterinary pathologist, and the presence of GFAP in this study was defined by a subjective score according to the following scale: 0—no staining, 1—minimal staining, 2—mild staining, 3—moderate staining, and 4—strong staining.

RESULTS AND DISCUSSION

The goal of this study was to determine which of three cutting methods would minimize the transfer of NST to steaks during the preparation of bone-in short loins. Evaluation of the cutting methods found that the NST contamination at levels exceeding the detection limit of the test was observed only along surfaces of the vertebral column cutting line. Regardless of the type of sample analyzed (vertebral column cutting line surfaces, steak surfaces, or cutting blade surface), only three samples yielded concentrations of $\geq$0.10% NST/100 cm^2 (the detection limit of the test). These samples were (i) the excised dorsal root ganglion (which was tested to confirm the GFAP concentration that was detectable by the ELISA); (ii) the swab sample of the caudal (region III) vertebral surface prior to vertebral column bone removal; and (iii) the swab sample of the cranial (region I) vertebral surface after vertebral column bone removal (Table 1). After the vertebral column bones had been removed (cutting method I), meat on the vertebral column cut surface had detectable NST contamination levels, while steak surfaces produced during further cutting according to method I did not exhibit detectable contamination (Table 2).

In all positive control slides, immunohistochemical staining of astrocytes was easily observed (score 4), whereas neurons did not stain at all (score 0) (data not shown). White matter tracts and astrocytes within white matter were easily observed (score 4), whereas oligodendrocytes appeared not to be stained (score 0). In the dorsal root gan-

TABLE 1. *Nervous system tissue (NST)[a] contamination of three surface regions along the cutting line of the vertebral column bone (vertebral surface region [VR]) and meat portion (meat region [MR]) of bone-in short loins following vertebral column bone removal (generated according to method I)[b]*

Location	No. of samples	Before scrape[c]	After scrape
Vertebral surface region I	4	<0.10[d]	0.22 ± 0.10
Vertebral surface region II	4	<0.10	<0.10
Vertebral surface region III	4	0.10 ± 0.10	<0.10
Meat region I	4	<0.10	<0.10
Meat region II	4	<0.10	<0.10
Meat region III	4	<0.10	<0.10

[a] NST, mean % NST/100 cm^2 ± standard deviation.
[b] Scrape sample of entire vertebral surface region along the cutting line, <0.10% NST/100 cm^2; scrape sample of entire meat region along the cutting line, <0.10% NST/100 cm^2; mean $R^2 = 0.9777$ over two replications; dorsal root ganglion ($n = 1$), 0.94% ± 0.18% NST/100 cm^2.
[c] After the vertebral column bone was removed, the cutting line surfaces were physically scraped to remove tissue debris.
[d] The detection limit for NST in meat with the RidaScreen test kit was <0.10%.

glion dissected from the vertebral column, hematoxylin and eosin staining revealed clear evidence of ganglion cells, surrounded by satellite cells as well as several myelinated nerve tracts. Ganglion cells did not stain (score 0), whereas satellite cells were easily observed (score 4). Within the myelinated nerve tracts, Schwann cells were easily observed (score 4), whereas axon cylinders and myelin sheaths did not stain (score 0), although occasional sheaths had fine punctuate staining (score 1). The majority of steak surface samples contained pieces of normal skeletal muscle bundles, surrounded by loose connective tissue, containing adipocytes, small blood vessels, and occasional myelinated and nonmyelinated nerve tracts. The myocytes generally did not stain (score 0), although in some sections, they contained occasional punctuate granules (considered nonspecific staining) that had minimal staining (score 2). Small myelinated and nonmyelinated nerves did not stain (score 0); however, some large myelinated nerves did have moderate staining (score 3). Immunohistochemical staining of ground tissue excised from steak surfaces revealed that regardless of the cutting method, areas in the immediate vicinity of the meat surface region (Fig. 1) ranged from no staining to moderate staining (Table 3). The most likely explanation for this observation is that positive staining in this area was of peripheral nerves (i.e., dorsal and ventral root nerves) rather than the CNS. Furthermore, analysis of the ground steak swabs with the commercial test kit resulted in values of 0.137 to 0.201% NST (Table 3), most likely because of the presence of peripheral nerves in the muscle tissue. The commercial test kit does not discriminate between CNS and peripheral nervous system tissue since it detects the presence of GFAP, a protein present in both tissues although at much lower levels in peripheral nervous system tissue. By homogenizing a composite sample of 24 excised whole-muscle samples, the likelihood of detecting GFAP associated with the peripheral nervous system was increased when compared to surface swabbing a steak. Thus, although GFAP was detected in the ground sample, it is highly unlikely that it was a result of CNS cross-contamination due to steak cutting since it was not detected on steak surfaces.

At the sensitivity of the commercial test used, there was no detectable (<0.10%) NST contamination on the cutting blade or steak surfaces (Table 2). Thus, the cutting methods were similar in that none of the methods resulted in transfer of NST above 0.10% NST/100 cm^2. The protocols applied in this study were representative of industry practices, and results indicate that transfer of NST during steak cutting, if any, may be at levels lower than 0.10% NST/100 cm^2. Thus, any future experiments or use of test-

TABLE 2. *Nervous system tissue (NST)[a] contamination of steak and cutting blade surfaces from steaks cut from bone-in short loins according to three methods*

Cutting method	No. of samples	% NST/100 cm^2 on steak surface	% NST on cutting blade	R^2 value[b]
I. Vertebral column bones were removed and then steaks were cut from the vertebral column side to flank side of loin	24	<0.10[c]	<0.10	0.9777
II. Steaks were cut from vertebral column side to the flank side of loin prior to removal of vertebral column bones	24	<0.10	<0.10	0.9232
III. Steaks were cut from the flank side to the vertebral column side of loin prior to removal of vertebral column bones	24	<0.10	<0.10	0.9881

[a] NST, mean % NST ± standard deviation.
[b] R^2 values averaged over two replications.
[c] The detection limit for NST in meat with the RidaScreen test kit was <0.10%.

TABLE 3. *Presence[a] of nervous system tissue (NST) by GFAP-ELISA and immunohistochemical (IHC) staining for GFAP in samples excised and ground from three areas (A, B, and C) of steaks cut from bone-in short loins according to three methods (I, II, and III)*

Cutting method	Area	% NST	IHC score	IHC description
I	A	0.146	0–2	None to mild
I	B	0.172	0	None
I	C	0.163	0	None
II	A	0.141	0–2	None to mild
II	B	0.156	0–3	None to moderate
II	C	0.137	0	None
III	A	0.201	1–3	Minimal to moderate
III	B	0.181	0–3	None to moderate
III	C	0.168	0	None

[a] Presence as defined by this study is a subjective score assigned by an individual examining slides according to the following scale: 0—no staining, 1—minimal staining, 2—mild staining, 3—moderate staining, 4—marked staining.

ing in the industry to evaluate SRM removal strategies should consider the use of more sensitive detection methods to detect SRM contamination of beef products.

Regulatory agencies have recommended that meat processors who fabricate bone-in loin subprimals do so by removing the vertebral column bone before cutting individual steaks. The rationale for such a recommendation is that once the vertebral column bone and associated CNS tissue are separated from the meat portion of the subprimal, the risk of CNS cross-contamination is reduced. In practice, however, the process of cutting the vertebral column bone from the subprimal may be problematic, with the placement of the blade's position through the cut being dependent upon the workers' ability to predict the path of the blade, which is continuously changing from loin to loin and within the loin. Thus, there is a possibility that the blade is inappropriately maneuvered during the cut, resulting in over- or undercutting of the bone. Undercutting during vertebral column bone removal may incompletely separate the vertebral bone, while overcutting during bone removal could result in excessive loss of meat, which would be costly, in the long run, for processors.

The cutting methods evaluated in this study considered the recommended deboning process and alternatives by which steaks are cut from bone-in short loin. The reason for considering the specific alternatives in this study was to evaluate these cutting methods for their relative abilities to minimize NST introduction on resulting steak surfaces.

The results of this study indicate that NST contamination of the cutting blade or steak surfaces during steak cutting, if any, is lower than 0.10% NST/100 cm^2. There was no difference in the potential of the three cutting methods to transfer NST above a 0.10% NST/100 cm^2 contamination level, as measured by the RidaScreen GFAP-ELISA. Furthermore, immunohistochemical evaluation of areas on steak surfaces excised and ground samples indicated that regardless of the cutting method, there was generally no staining to moderate staining, indicating that detectable NST contamination from these samples was most likely due to peripheral nerve detection. On the basis of detection levels $\geq$0.10% NST/100 cm^2, it appears that removal of vertebral column bone as regulated may be accomplished by removal of the vertebral bone either before or after the steaks are cut from the short loin. The practice of first cutting steaks from bone-in loin subprimals and then removing the vertebral column bone may benefit beef processors by reducing the amount of meat loss due to overcutting.

REFERENCES

1. Anil, M. H., S. Love, C. R. Helps, and D. A. Harbour. 2002. Potential for carcass contamination with brain tissue following stunning and slaughter in cattle and sheep. *Food Control* 13:431–436.
2. Garland, T., N. Bauer, and M. Bailey, Jr. 1996. Brain emboli in the lungs of cattle after stunning. *Lancet* 348:610.
3. Helps, C. R., A. V. Fisher, D. A. Harbour, D. H. O'Neill, and A. C. Knight. 2004. Transfer of spinal cord material to subsequent bovine carcasses at splitting. *J. Food Prot.* 67:1921–1926.
4. Helps, C. R., P. Hindell, T. J. Hillman, A. V. Fisher, H. Anil, A. C. Knight, R. T. Whyte, D. H. O'Neill, T. G. Knowles, and D. A. Harbour. 2002. Contamination of beef carcasses by spinal cord tissue during splitting. *Food Control* 13:417–423.
5. Schmidt, G. R., K. L. Hossner, R. S. Yemm, and D. H. Gould. 1999. Potential for disruption of central nervous system tissue in beef cattle by different types of captive bolt stunners. *J. Food Prot.* 62:390–393.
6. Schmidt, G. R., R. S. Yemm, K. D. Childs, J. P. O'Callaghan, and K. L. Hossner. 2001. The detection of central nervous system tissue on beef carcasses and in comminuted beef. *J. Food Prot.* 64:2047–2052.
7. Scott, M. R., R. Will, J. Ironside, H. O. Nguyen, P. Tremblay, S. J. DeArmond, and S. B. Pruisner. 1999. Compelling transgenetic evidence for transmission of bovine spongiform encephalopathy prions to humans. *Proc. Natl. Acad. Sci. USA* 96:15137–15142.