Available online at www.sciencedirect.com
SCIENCE DIRECT
Meat Science 72 (2006) 656–659
MEAT SCIENCE
www.elsevier.com/locate/meatsci

# Central nervous system tissue detection in meat from advanced meat recovery systems

Maha N. Hajmeer [a,*], Dean O. Cliver [a], James L. Marsden [b]

[a] Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, One Shields Avenue, Davis, CA 95616-8743, USA

[b] Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS 66506, USA

Received 7 March 2005; received in revised form 22 September 2005; accepted 22 September 2005

**Abstract**

Three hundred meat samples, recovered from beef neck- and breast-bones using a conventional advanced meat recovery (AMR) system, the de-sinewed minced meat (DMM10) technology, and hand-boning, were collected and tested for presence of central nervous system tissue (CNST) in meat using an ELISA-based test. Samples were collected at two processing facilities (Est. A and B). Sternum meat was the non-CNST reference (control) – it is distant from brain and spinal cord locations on a carcass, with low likelihood of contamination with CNST. Neckbone meat was recovered from bones obtained from carcasses where the spinal cord was removed manually, Est. B, or using a Jarvis circular hydraulic cord remover saw, Est. A. All samples from AMR, DMM, and hand methods showed lower calculated levels of "risk material" than the stated limit of detection (0.1%) of ELISA kit. There was no apparent difference among these, and use of the Jarvis saw had no perceptible advantage.


*Keywords:* Advanced meat recovery systems; Beef; ELISA method; Neural tissue

## 1. Introduction

Advanced meat recovery (AMR) systems are machinery designed to separate edible meat from bone. In 1995, the United States Department of Agriculture's (USDA) definition of meat was amended to include products prepared via AMR systems (USDA, 2002). Under that directive, meat derived via AMR is considered comparable in texture and composition to meat trimmings and hand-deboned products, and no special labeling is required. Additionally, products obtained using such systems must not contain spinal cord tissue because it falls outside the definition of meat. In 2002, USDA issued a revision to their earlier directive under which inspectors at establishments using AMR systems were instructed to take routine regulatory samples to verify that spinal cord is not present in AMR products. This is intended to prevent misbranding of meat products under the Food Safety and Inspection Service regulations.

In addition to misbranding concerns, contamination of beef with central nervous system tissue (CNST) including spinal cord has been a concern from the food safety standpoint because of potential human health implications in relation to bovine spongiform encephalopathy (BSE). Introducing CNST (i.e., brain and spinal cord materials) to the edible portion of the carcass is suspected of increasing the risk of human infection with new variant Creutzfeldt–Jakob disease (vCJD). Processing practices such as AMR systems might introduce CNST to meat products (Bauer, Garland, & Edwards, 1996; BSEEB, 1999; Garland, Bauer, & Bailer, 1996; Schmidt, Hossner, Yemm, & Gould, 1999a, Schmidt, Hossner, Yemm, Gould, & O'Callaghan, 1999b; USDA, 1998). To prevent CNST from being introduced into the human food chain, the European

* Corresponding author. Fax: +1 530 754 7373.
*E-mail address:* mnhajmeer@ucdavis.edu (M.N. Hajmeer).

0309-1740/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2005.09.015

Commission (EC) in 1997 endorsed a proposal to regulate the use of specified risk materials (SRMs) such as brain and spinal cord presenting a BSE risk, and decided that SRM must not to be used in food effective October 1, 2000 (DEFRA, 2005).

The purpose of this study was to evaluate hand-boning and two AMR processing methods (the conventional AMR system and the DMM10 technology, a new AMR system) for presence of neural tissue in meat using a rapid immunological detection method. If found comparable to hand boning in terms of the probability of introducing neural tissue into meat, these mechanical methods can simplify the deboning process of meat. Eventually, this might enhance worker safety by eliminating a hazardous task, while reducing general meat price by improving carcass meat yield.

## 2. Materials and methods

### 2.1. Meat collection and preparation for analysis

Three hundred meat samples recovered from beef neck- and breast-(sternum) bones using a conventional AMR system, the de-sinewed minced meat (DMM10) technology, and manual (hand) boning were collected and transported frozen (~−20 °C) to the Food Safety Laboratory at the University of California, Davis (UCD). Collection of raw materials (i.e., meat samples hand-boned or processed with the conventional AMR or the DMM10) was performed at two processing establishments (Est. A and Est. B). One of the establishments was equipped with a conventional AMR system (Protecon AMR System, model BFD, with Hydrahop; Townsend Protecon-Langen, Des Moines, Iowa). Therefore, the samples set for processing via the AMR technology were prepared at that location. The operational parameters to produce samples using the conventional AMR HydraHop system were a hydraulic pressure set at 165.5 bar, with a 3-s stroke and a cycle time of 21.2 s.

Hand-boning and processing via the DMM10 system were performed at the Meat Laboratory at Iowa State University. With the DMM10 technology, the samples were processed at a pressure of 80 bar and a dwell time (i.e., time during which the pressure is kept on to the bones) of 0.1 s. This meant that as soon as the required set pressure was reached, the pressure was released. Samples longer than ~150 mm were cut to approximately that length with a manually operated band saw. The de-sinewed minced meat samples came out of a secondary filter with a perforation of 2 mm (some samples were produced with a perforation of 3 mm). Samples were packed in individual bags immediately after production, and stored at −18 °C until they were sent for analysis.

Sternum meat served as the non-CNST reference (control), as it is distal from the brain and spinal cord locations on a carcass – with low likelihood of contamination with neural tissue. Neckbone meat was recovered from bones obtained from carcasses where the spinal cord was removed manually (Est. B), or using a Jarvis circular hydraulic spinal cord remover saw (SPC165G, Jarvis Products Corporation, Middletown, CT), at Est. A.

Samples were stored at −70 °C upon receipt at the laboratory. In preparation for testing, the meat selected for analysis was gradually thawed by transferring it from the −70 °C freezer into a −20 °C unit then to 4 °C for 24 h. Detection of neural tissue was performed on thawed samples using a commercial ELISA-based test – Ridascreen™ risk material 10/5 kit (R-biopharm, AG, Darmstadt, Germany). This kit was selected as earlier work at our laboratory illustrated its precision, ease of use, and quick yield of results (Hajmeer, Cliver, & Provost, 2003).

### 2.2. Immunological analysis for detection of neural tissue

Samples were prepared for this analysis by dipping a Dacron® fiber-tipped, sterile swab (Fisher Scientific, Houston, TX) five times into the sample while rotating the swab. The swab, containing approximately 50 mg of meat, was removed, and squeezed in a test tube containing 1 ml sample buffer containing sodium dodecyl sulfate (SDS). Since preparation of samples in SDS extract buffer is not done for traditional ELISAs, it is important that laboratory personnel using this assay exercise proper precautions and be aware of the information on the materials safety data sheet (MSDS).

A 100-μl sample was transferred from each test tube to an assay well. A sufficient number of antibody-coated wells was 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 SC, were provided with the Ridascreen® assay). Enzyme conjugate (50 μl) was added to each well containing sample, and the plate was incubated for 10 min at room temperature (20–25 °C). After incubation, the liquid was poured out to empty the wells, and the micro-well holder tapped upside down thoroughly against absorbent paper, so that the liquid added earlier was completely removed from the wells. The wells were washed with 250 μl washing buffer and emptied as described earlier. A 100-μl volume of substrate/chromogen was added to each well and mixed thoroughly; the plate was incubated for 5 min at room temperature in the dark. The reaction was stopped by adding 100 μl stop solution to each well. Color intensity or optical density (absorbance) was determined by photometric evaluation using a microtiter spectrophotometer (SpectraMAX 340, Molecular Devices Corporation, Sunnyvale, CA) with a filter at 450 nm. Results were interpreted as indicated in the Ridascreen® risk material 10/5 kit information booklet. An analysis of variance was conducted on collected data. A Microsoft® Excel 2000 software was used (Microsoft Corporation, Redmond, WA).

## 3. Results and discussion

No neural material was detected in any meat sample by means of the Ridascreen® risk material kit. The detection limit for this test is 0.1% per manufacturer's instructions, and the percent risk material obtained for all samples fell below this limit (Tables 1 and 2). Six experimental runs were required to account for all samples submitted; data regarding the standard curve for each experimental run are presented in Table 3. A high correlation coefficient (CC) was obtained for the standard curve per run – for a run to be acceptable the CC value must be above 98%. We also tested the sensitivity of the ELISA test, by including positive controls using 0.03 and 0.1% spinal cord (SC) suspension (w/w basis in water), and 0%, 0.025%, 0.05%, and 0.1% SC in meat (w/w). The positive controls were analyzed in conjunction with samples submitted for analysis at our laboratory. Results of these controls were positive for neural tissue (except for 0% SC).

The percent risk material (%RM) detected in all samples tested, regardless of meat source (sternum or neckbones) and meat recovery method (conventional AMR technology, DMM technology, and hand-boning), fell below the detection limit of the ELISA kit, 0.1% (Table 2). The calibration standards provided by the kit manufacturer were said to be a blend of bovine brain (600 ng GFAP/mg) and spinal cord (2000 ng GFAP/mg) Based on that information, the test used has a sensitivity of 0.03% SC versus 0.1% brain (Hajmeer et al., 2003). The test is directed to glial fibrillary acidic protein (GFAP), which our earlier study showed to be more concentrated in spinal cord than in brain (Hajmeer et al., 2003). Assuming that spinal cord was the risk material more likely to occur in these AMR products, it is noteworthy that the test was able to detect as little as 0.025% spinal cord consistently.

Keeping in mind that the %RM for all samples fell below the detection limit, we noted that (1) sternum meat recovered via DMM seems comparable to hand-boned

Table 1
Summary of optical density values obtained for meat samples received and analyzed via the ELISA R-biopharm kit[a]

| Establishment sampled | Meat source (location on beef carcass) | | | | |
|---|---|---|---|---|---|
| | Neckbones (technology[b]) | | | Sternum (technology) | |
| | AMR | DMM | Hand | DMM | Hand |
| A[c] | 0.224 ± 0.061[d]<br>0.154–0.421<br>($n = 25$) | 0.199 ± 0.096<br>0.087–0.468<br>($n = 30$) | 0.284 ± 0.099<br>0.172–0.418<br>($n = 10$) | NA[e] | NA |
| B[f] | NA | 0.215 ± 0.122<br>0.095–0.517<br>($n = 30$) | 0.308 ± 0.089<br>0.174–0.401<br>($n = 10$) | 0.110 ± 0.018<br>0.087–0.166<br>($n = 30$) | 0.213 ± 0.094<br>0.125–0.558<br>($n = 30$) |

[a] Kit detection limit is 0.1% risk material. This is a semi-quantitative test. Values falling below detection limit are considered negative and those above it are positive.
[b] Refers to technology or method used to remove meat from the bones: AMR for the conventional advanced meat recovery system, DMM for desinewed minced meat (the new AMR technology), and Hand for hand-boning.
[c] Establishment using the Jarvis circular saw for spinal cord removal.
[d] Mean ± SD, range (number of samples).
[e] Not available, no samples were collected for analysis.
[f] Establishment not using the Jarvis saw for spinal cord removal.

Table 2
Summary of percent risk materials calculated for meat samples received and analyzed via the ELISA R-biopharm kit[a]

| Establishment sampled | Meat source (location on beef carcass) | | | | |
|---|---|---|---|---|---|
| | Neckbones (technology[b]) | | | Sternum (technology) | |
| | AMR | DMM | Hand | DMM | Hand |
| A[c] | 0.019 ± 0.011[d]<br>0.006–0.055<br>($n = 25$) | 0.019 ± 0.017<br>0.001–0.065<br>($n = 30$) | 0.031 ± 0.022<br>0.007–0.061<br>($n = 10$) | NA[e] | NA |
| B[f] | NA | 0.013 ± 0.004<br>0.006–0.019<br>($n = 30$) | 0.037 ± 0.020<br>0.007–0.071<br>($n = 10$) | 0.010 ± 0.006<br>0.003–0.024<br>($n = 30$) | 0.019 ± 0.019<br>0.001–0.088<br>($n = 30$) |

[a] Kit detection limit is 0.1% risk material. This is a semi-quantitative test. Values falling below detection limit are considered negative and those above it are positive.
[b] Refers to technology or method used to remove meat from the bones: AMR for the conventional advanced meat recovery system, DMM for desinewed minced meat (the new AMR technology), and Hand for handboning.
[c] Establishment using the Jarvis circular saw for spinal cord removal.
[d] Mean ± SD, range (number of samples).
[e] Not available, no samples were collected for analysis.
[f] Establishment not using the Jarvis saw for spinal cord removal.

Table 3
Summary of standard curve information per experimental run

| Run number | Standard | | | | $R^2$ |
|---|---|---|---|---|---|
| | 1 (0%RM[a]) | 2 (0.1%RM) | 3 (0.2%RM) | 4 (0.4%RM) | |
| 1 | 0.065[b] | 0.376 | 0.732 | 1.297 | 0.9978 |
| 2 | 0.090 | 0.695 | 1.238 | 2.292 | 0.9990 |
| 3 | 0.095 | 0.430 | 0.768 | 1.495 | 0.9995 |
| 4 | 0.111 | 0.629 | 1.122 | 2.120 | 0.9999 |
| 5 | 0.136 | 0.641 | 1.084 | 1.997 | 0.9995 |
| 6 | 0.135 | 0.596 | 1.064 | 1.951 | 0.9998 |

[a] Risk material, a mixture of bovine brain and spinal cord provided by the kit manufacturer for calibration.
[b] Optical density.

samples in %RM detected, (2) the %RM detected in neckbone meat recovered via the conventional AMR and the DMM technology were comparable. The calculated %RM values were also lower than those determined for hand-boned meat samples, regardless of the meat processing establishment (Est. A and Est. B), so (3) the %RM detected in neckbone samples obtained from Est. A where the Jarvis circular saw was used for spinal cord removal from the carcasses seems comparable with the %RM determined for samples obtained from Est. B, where the Jarvis saw was not used. Thus, the Jarvis saw does not seem to offer an added advantage in minimizing contamination of the meat with neural material.

## 4. Conclusion

The intense pressure applied to trimmed bones, to extract as much meat as possible from the bones, raised concerns about the AMR methods (conventional or new) from the regulatory, quality, and safety aspects. This is due to the potential for misbranding of meat (if spinal cord is introduced), affecting structural quality of meat, and introduction of bone marrow and neural tissue. Our findings indicate that the impact of the AMR and hand boning methods on introducing neural tissue in meat is comparable. More importantly, all samples tested (using AMR and hand methods) showed lower calculated levels of "risk material" than the stated limit of detection (0.1%) of the test kit (i.e., negative for neural tissue based on ELISA test used).

## Acknowledgments

We thank Townsend Engineering, National Beef and the Livestock of Meat Industry Council at the KSU Foundation for their support. We thank Shahin Fouroutan and Blair Duncan for their technical assistance, as well as the Iowa State University Meats Laboratory.

## References

Bauer, N. E., Garland, T., & Edwards, J. F. (1996). Brain emboli in slaughtered cattle. *Veterinary Pathology, 33*, 660.

BSEEB. (1999). Contamination of beef carcasses with brain tissue. *BSE Enforcement Bulletin, 40*:4. London, UK: Food Standards Agency.

DEFRA. (2005). Available from http://www.defra.gov.uk.

Garland, T., Bauer, N., & Bailer, M. (1996). Brain emboli in the lungs of cattle after stunning. *Lancet, 348*, 610–611.

Hajmeer, M., Cliver, D. O., & Provost, R. (2003). Spinal cord tissue detection in comminuted beef: comparison of two immunological methods. *Meat Science, 65*, 757–763.

Schmidt, G. T., Hossner, K. L., Yemm, S., & Gould, D. H. (1999a). Potential for disruption of central nervous system (CNS) tissue in beef cattle by different types of captive bolt stunners. *Journal of Food Protection, 62*(4), 390–393.

Schmidt, G. T., Hossner, K. L., Yemm, S., Gould, D. H., & O'Callaghan, J. P. (1999b). An enzyme-linked immunosorbent assay for glial fibrillary acidic protein as an indicator of the presence of brain or spinal cord in meat. *Journal of Food Protection, 64*(4), 394–397.

USDA (1998). Proposed rules: meat produced by advanced meat/bone separation machinery and recovery systems. *Federal Register, 63*, 17959–17965.

USDA. (2002). USDA strengthens advanced meat recovery policies. Available from http://www.fsis.usda.gov/OA/news/2002/amrpolicies.htm.