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Contamination of beef carcasses by spinal cord tissue during splitting

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Abstract

In 1989/90, the use of specified bovine offal (SBO) in human food was banned in the UK. Spinal cord, an SBO material, is now removed from beef carcasses post splitting. More recently, following a European Commission Decision introduced on 1st October 2000, regulations in all EU states require the removal of central nervous system (CNS) material from sheep carcasses over 12 months of age and all cattle carcasses. However, in the majority of abattoirs, carcasses are split using a band saw; this often cuts the spinal cord in half along much of its length. This can obviously lead to potential dissemination of CNS material over the carcass and surrounding area resulting in possible contamination with the bovine spongiform encephalopathy (BSE) infective agent. We have used enzyme-linked immunosorbant assays (ELISAs) to detect CNS-specific glial fibrillary acidic protein (GFAP) and S-100β protein. Both assays show the presence of CNS material on the carcass after splitting with a conventional band saw. This contamination was still present after the carcass had been washed or steam-vacuum cleaned. However, significantly less CNS contamination was observed on carcasses whose spinal column had been removed by an experimental oval saw prior to splitting. With further engineering development, this new technique should be capable of removing spinal cord with minimal contamination risk.

Keywords: BSE; vCJD; Spinal cord; Contamination; Experimental oval saw

1. Introduction

Variant Creutzfeldt-Jakob disease (vCJD) or suspected vCJD has affected 85 people to date in the UK (The National Creutzfeldt-Jakob Disease Surveillance Unit, Edinburgh). It has been shown that the transmissible agent responsible for this disorder is indistinguishable from that found in bovine spongiform encephalopathy (BSE) (Collinge, Sidle, Meads, Ironside, & Hill, 1996; Hill et al., 1997; Scott et al., 1999). This finding reinforces the hypothesis that exposure of humans to the BSE agent, presumably through the diet, is the cause of vCJD (Ironside, 1998; Will et al., 1996).

Since the introduction of specified risk material (SRM) regulations, tissues suspected of carrying the BSE infective agent have been banned from the food chain. These tissues include the skull (including the brain and eyes), the tonsils, the spinal cord and the ileum in all EU member states. However, because of the way that cattle are slaughtered it is possible for both brain and spinal cord tissue to contaminate the carcass and, hence, enter the food chain. It has been shown that the use of captive bolt guns to stun cattle causes brain tissue to enter the blood stream and it could, therefore, be disseminated throughout the carcass (Anil et al., 1999; Garland, Bauer, & Bailey, 1996; Schmidt, Hossner, Yemm, & Gould, 1999a). During dressing, beef carcasses are normally split down the vertebral column using a band saw and the cut spinal cord removed from each half of the carcass. Since the cord is often cut along most of its length the possibility exists that central nervous system (CNS) material could be spread over the carcass and the surrounding environment. To address this problem, an experimental saw has been designed to remove a column of vertebral bone encasing the intact spinal cord. In this study, we have quantified the contamination resulting from the use of both saws and have examined the effect

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that steam washing has on reducing contamination when just the normal saw is used. We have used the CNS-specific proteins glial fibrillary acidic protein (GFAP) (Schmidt, Hossner, Yemm, Gould, & O'Callaghan, 1999b) and S-100β (Moore, Perez, & Gehring, 1968) to detect and quantify this contamination.

2. Methods

2.1. Sample collection

Two groups of five beef carcasses were used. The first carcass was split approximately 45 min after slaughter with a Jarvis-Buster V1 band saw equipped with a cold water blade rinse/coolant, with one side of each carcass being spray washed with water and the other side being steam cleaned using a Jarvis Steam Vacuum System (Model CV1). Before splitting the second group, an experimental oval saw was used to remove the spinal column. After splitting, one side of each carcass was spray washed with water.

Samples were collected using $100 \times 100 \times 10$ mm^3 synthetic sponges (Sydney Heath & Son), placed into 20 ml PBS, 0.5% Triton-X-100, mixed and stored at 4°C. 48 h later the samples were aliquoted and frozen at −70°C for long-term storage, prior to being thawed and used in the enzyme-linked immunosorbant assays (ELISA). Samples were collected from five defined areas on the lateral and medial surface of each half of the split carcass (see Fig. 1), yielding 10 samples per carcass side (i.e. 50 samples band saw and water wash, 50 samples band saw and steam wash and 50 samples oval saw and water wash). Swab samples were also taken from polyethylene screens measuring 0.6 m wide by 2.5 m high and mounted at approximately 45° to the slaughter line rail on either side of the saw operator. Samples were also taken from hand held polyethylene screens measuring 0.2 m × 0.3 m, positioned to either side and beneath the saw during splitting and from the operator's apron. A sample of the saw wash water collected in a tray below the carcass was also taken.

Five aerosol samples for each group of carcasses were collected on to 4.7 cm Whatman QMA open face filters at a flow rate of 35 l per min during the splitting of each carcass and for a period of 1 min following completion of the split. Two of the aerosol samples were located at either of the large screens, one above the carcass, one on a hand held lance held close to the saw blade during the splitting process and one mounted on the chest of the operator. An additional aerosol sample using a sampling head designed for measuring inhaled aerosols, also mounted on the chest of the operator, was taken at a flow rate of 2 l per min. Each aerosol sample taken was cumulative over the splitting of each group of 5 carcasses.

2.2. GFAP enzyme-linked immunosorbant assay

A modification of the capture ELISA for GFAP (Schmidt et al., 1999b) was used to detect CNS con-

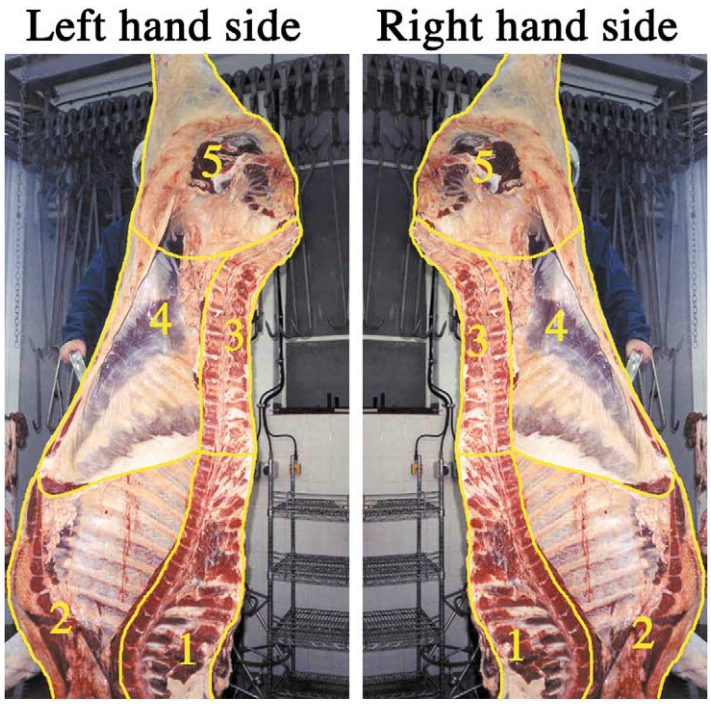

Fig. 1. Schematic diagram of the 5 areas swabbed on the medial surface of each half (left and right) of the split beef carcass. The lateral surface of each half of the carcass was similarly divided for swabbing.

tamination of beef carcasses. ELISA plates were coated with 50 μl of 1:500 dilution rabbit anti-GFAP (Dako) in 50 mM Carbonate–Bicarbonate buffer pH 9.6 (Sigma) and incubated at 37°C for 1 h. The plates were washed with PBS, 0.05% Tween-20 and non-specific protein binding blocked by adding 200 μl PBS/0.05% Tween-20/5% non-fat dried skimmed milk (blocking solution) and rocking at room temperature for 1 h. The plates were again washed and 50 μl of undiluted, 1:2, 1:4 and 1:8 dilutions of sample was added to each well and incubated at room temperature for 90 min with rocking. After a further wash, 50 μl of blocking solution containing 1:500 dilution of mouse anti-GFAP (Roche) was added per well and incubated at room temperature for 1 h with rocking. The plates were washed, and 50 μl of blocking solution containing a 1:2000 dilution of alkaline phosphatase-conjugated donkey anti-mouse IgG (Jackson Immunoresearch Laboratories, USA) was added to each well and incubated as above. After a final wash, the ELISA was developed by adding 50 μl of 1 mg/ml *p*-nitrophenol phosphate to each well and development allowed to proceed at 4°C over 24 h. The plates were then read at 405 nm to obtain the optical density of each well. Each ELISA plate also contained a series of standards generated by serially diluting a 2 ng/μl solution of GFAP (Roche) which allowed the generation of a standard curve and the quantitation of the sample results. Total protein was measured using the BCA kit (Pierce & Warriner).

2.3. Dilution curves for GFAP

GFAP (Roche) was serially diluted 2-fold starting at 1 ng/50 μl to generate a standard curve. Spinal cord material was homogenised in PBS/0.5% Triton-X-100 using a Ribolyser (Hybaid, UK) set on speed 5.0 twice for 20 s each. The subsequent homogenate was centrifuged at 20,000*g* for 5 min and the supernatant analysed for total protein using the BCA kit (Pierce & Warriner). Dilutions of the homogenate were then analysed for GFAP as described above. Muscle was homogenised as described above and a known amount of spinal cord homogenate added and serially diluted in the muscle homogenate. This was then analysed for GFAP as above.

2.4. S-100β enzyme-linked immunosorbant assay

ELISA plates were coated with 200 μl per well of 1 μg/μl rabbit anti-human S-100β (Research Biologicals) in 50 mM Carbonate–Bicarbonate buffer pH 9.6 (Sigma) and stored at 4°C. Prior to use plates were washed four times with PBS, 0.1% Tween-20 and air dried. Bovine S-100β standard (Sigma) was diluted in PBS 0.5% bovine serum albumin (Sigma) in duplicate to final concentrations of 60, 20, 10, 5, 1 and 0 ng/ml. In the case of samples from aerosol samplers the dilution buffer for the standards was PBS/0.1% Triton-X-100. Carcass swab samples were initially diluted 1 in 100 in PBS 0.5% bovine serum albumin (Sigma) and 100 μl added to the plate in duplicate. Aerosol and screen samples were added to the plate in duplicate without dilution. The plates containing samples and standards were incubated at room temperature for 2 h and washed as above.

Rabbit anti-cow S-100 (DAKO) was biotinylated with long arm NHS biotin (Vector Laboratories). The biotin was diluted to 100 μM and the antibody to 1 mg/ml. 24.5 μl of biotin was added to 1 ml antibody and incubated at room temperature for four hours with occasional mixing. The mixture was dialysed overnight in three changes of 0.1 M sodium hydrogen carbonate and the dialysate used in the assay. The biotinylated antibody was diluted 1 in 500 in PBS/0.1% BSA and 100 μl added to each well and incubated at room temperature for one hour and washed as above.

Horseradish peroxidase conjugated to streptavidin (Vector Laboratories) was diluted 1 in 2000 in PBS/0.1% BSA and 100 μl added to each well and incubated at room temperature for 20 min. 30 mg of *o*-phenylenediamine (OPD) (Sigma) was dissolved in 24 ml 0.1 M citric acid phosphate buffer pH 5 and 20 μl hydrogen peroxide added. The plates were washed as above, 100 μl OPD solution added to each well and the plates incubated in the dark for approximately 15 min. The reactions were stopped by adding 100 μl of 2 M sulphuric acid to each well and read at 490 nm using a microplate reader. Samples not having an OD within the standard curve were re-diluted and re-analysed as appropriate.

2.5. Dilution curves for S-100β

Bovine S-100β standard (Sigma) was diluted in PBS 0.5% bovine serum albumin (Sigma) in duplicate to final concentrations of 60, 20, 10, 5, 1 and 0 ng/ml and applied to the ELISA. Spinal cord material was homogenised in PBS/0.5% Triton-X-100 using a Potter homogeniser and serial 10-fold dilutions applied to the ELISA. Muscle was homogenised as described above and was either used alone or mixed with homogenised spinal cord; serial 10-fold dilutions were then applied to the ELISA.

3. Statistics

Differences between levels of contamination on the aprons, the hand held screens and the trays when using the two types of saw were investigated using *t*-tests.

The effect of steam or water washing, when just the band saw was used, was investigated separately from the effect of saw. Thus the data from the 5 carcass sides cut using just the band saw and then water washed were

used twice, once in each analysis. The analyses was carried out using the statistics package MLwiN ver 1.1 (Multilevel Models Project, Institute of Education, University of London) which allowed the hierarchical structure of the data to be modelled (area, within surface, within side, within carcass). For technical reasons, when carrying out the study, carcass side (left/right) had to be confounded with type of saw used. However, every care was taken to ensure that this had no effect on the results.

All counts were transformed to a normal distribution by taking their natural logarithms.

4. Results and discussion

Both GFAP and S-100β have previously been used to specifically detect CNS contamination (Moore et al., 1968; Schmidt et al., 1999b; Love et al., 2000). Fig. 2 shows a typical dilution curve for the GFAP standard used in the ELISA, with a detection limit of about 0.05 ng. The figure also shows dilution curves for spinal cord tissue, spinal cord tissue diluted in muscle homogenate and muscle homogenate alone. It can be seen that spinal cord alone and spinal cord diluted in muscle homogenate have very similar dilution curves, indicating that GFAP from CNS tissue can be detected even in the presence of a large excess of muscle protein. The dilution curve for muscle homogenate alone shows no reactivity in the ELISA. It can be seen that the dilution curves for GFAP, spinal cord and spinal cord diluted in muscle are parallel over much of their range. Fig. 3 shows that the S-100β ELISA gives parallel dilution curves for the S-100β standard, spinal cord alone and spinal cord diluted in muscle homogenate.

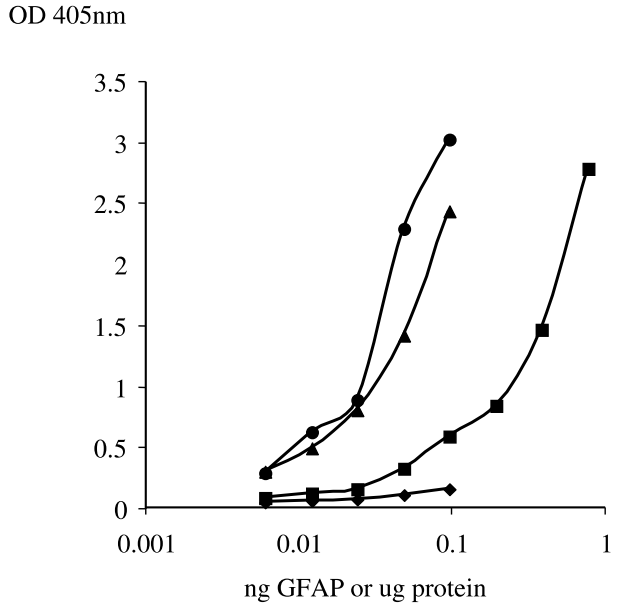

Fig. 2. Standard curve for the GFAP ELISA (■) showing parallel dilutions for spinal cord (●) and muscle spiked with spinal cord (▲). Muscle alone (◆) shows no reactivity in the ELISA.

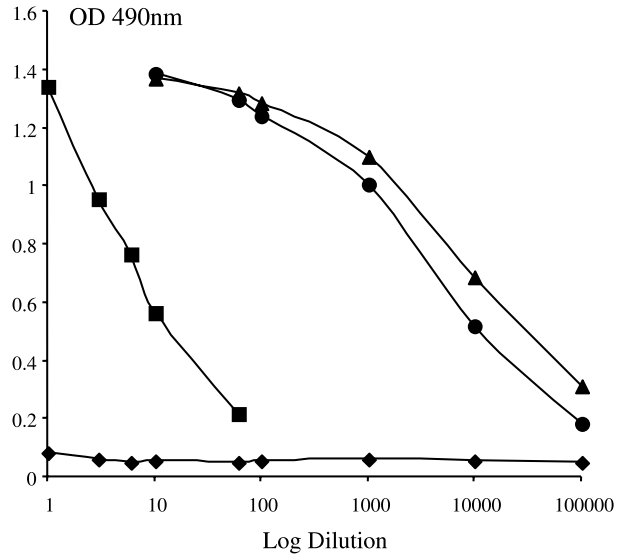

Fig. 3. Standard curve for the S-100β ELISA (■) showing parallel dilutions for spinal cord (●) and muscle spiked with spinal cord (▲). Muscle alone (◆) shows no reactivity in the ELISA.

Figs. 4 and 5 show bar graphs for the detection of S-100β and GFAP from the samples, respectively. From the two graphs it can be seen that the S-100β ELISA assay is more sensitive than the GFAP ELISA, although both show the same overall pattern of contamination. Thus only the S-100β ELISA results were used in the statistical analyses.

The use of the experimental oval saw resulted in significantly less CNS contamination on the hand held screens and on the aprons. There was no difference in the levels of contamination found in the drip trays (Table 1). No CNS contamination was detected in the aerosol samplers or on the other screens around the carcass.

The parameter estimates for the statistical models of the saw data and the cleaning data are given in Table 2. Use of the oval saw reduced contamination on both surfaces and all areas of the carcass by approximately 1 natural log (ln) count. Contamination was higher on the internal surface by approximately 1.5 ln counts, which ever saw was used. Both internally and externally, and which ever saw was used, areas 1 and 3 were most contaminated with approximately 3 ln counts when the band saw was used. Areas 2 and 5 were less contaminated by approximately 1.5 ln counts and area 4, again, on both surfaces, was the least contaminated with 2.75 ln counts less than areas 1 and 3.

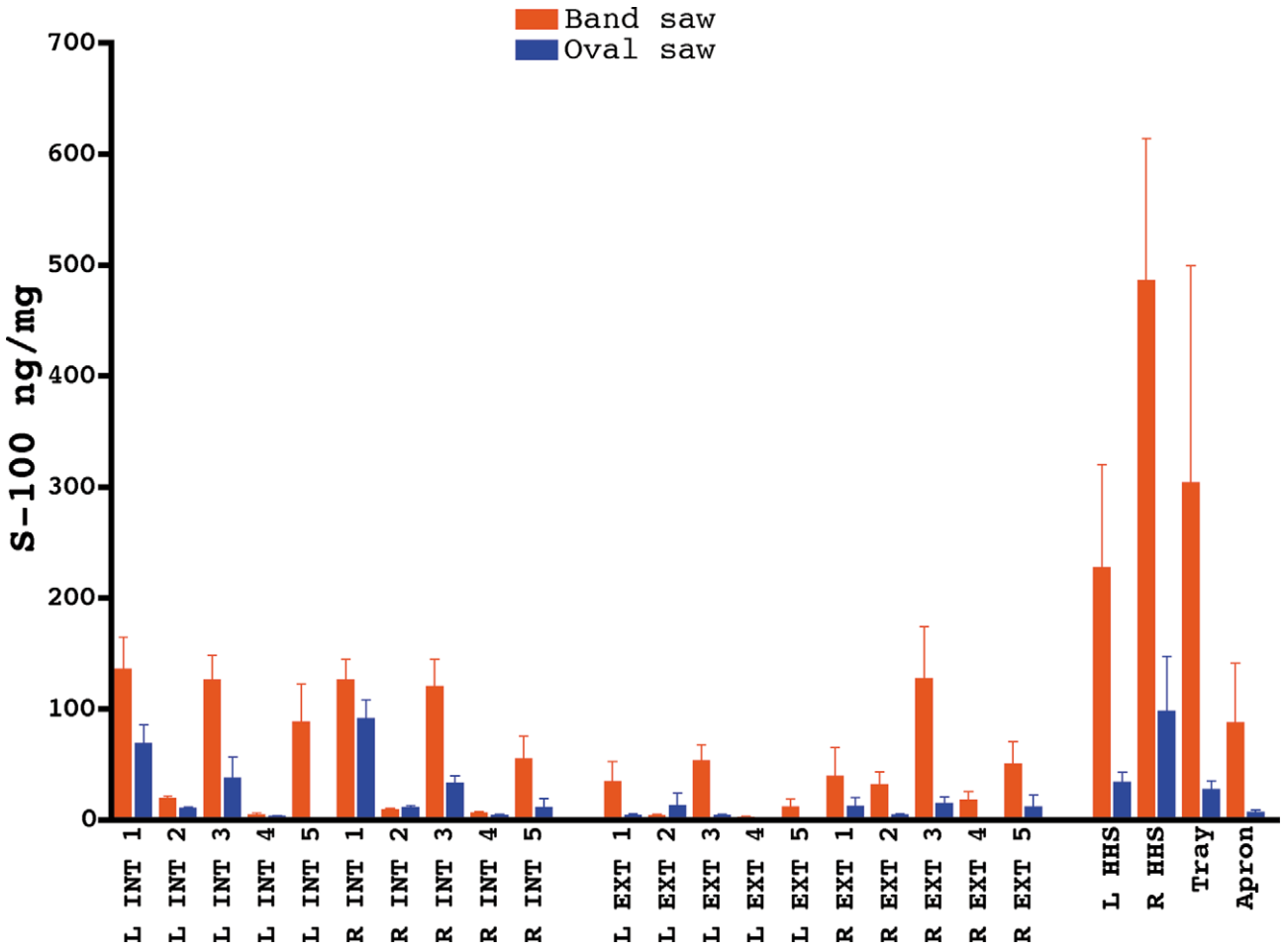

Fig. 4. CNS contamination of beef carcasses after splitting with a Jarvis-Buster V1 band saw (red bars) and an experimental oval saw (blue bars). Areas 1–5 (as defined in Fig. 1) were swabbed and analysed for S-100β. The results are expressed as ng S-100β per mg total protein. L – left half, R – right half, INT – medial surface, EXT – lateral surface, L HHS – left-hand side hand-held screen, R HHS – right-hand side hand-held screen. The operators apron was also swabbed and the tray water was also sampled.

Steam cleaning had no effect of reducing contamination of the internal surface of the carcass and appeared to increase contamination of the external surface by approximately 1.5 ln counts. The pattern of contamination across the five areas of the internal and external surfaces of the carcasses were similar to those described above in the comparison of saws. The apparent increase in contamination of the external surface after steam washing was unexpected but could have been due to transfer of CNS material from the internal surface by the cleaning process.

The results indicate that the majority of the CNS contamination occurs on the internal surfaces of the carcass, along the cut vertebral surface, with lesser contamination in the body cavity. Owing to constraints on the carcass handling facilities it was not possible to remove the entire spinal cord encased in vertebral bone with the oval saw. Hence, at the neck end (area 1) the saw

Table 1
The means (sem) of the ln counts from the S-100β assay and the results of the *t*-tests comparing contamination levels from the use of the two saws on the screens, trays and aprons[a]

$n = 5$	Band saw	Oval saw	t	P	Lower CI	Upper CI
Hand held screen	5.51 (0.543)	3.98 (0.318)	2.433	0.041	0.08	2.98
Tray	4.47 (1.071)	2.90 (0.538)	1.304	0.229	−1.20	4.33
Apron	3.87 (0.600)	1.49 (0.460)	6.473	0.009	0.79	3.96

[a] The lower and upper 95% confidence intervals (CI) for the differences between the mean ln counts are also shown. P is equal to significance.

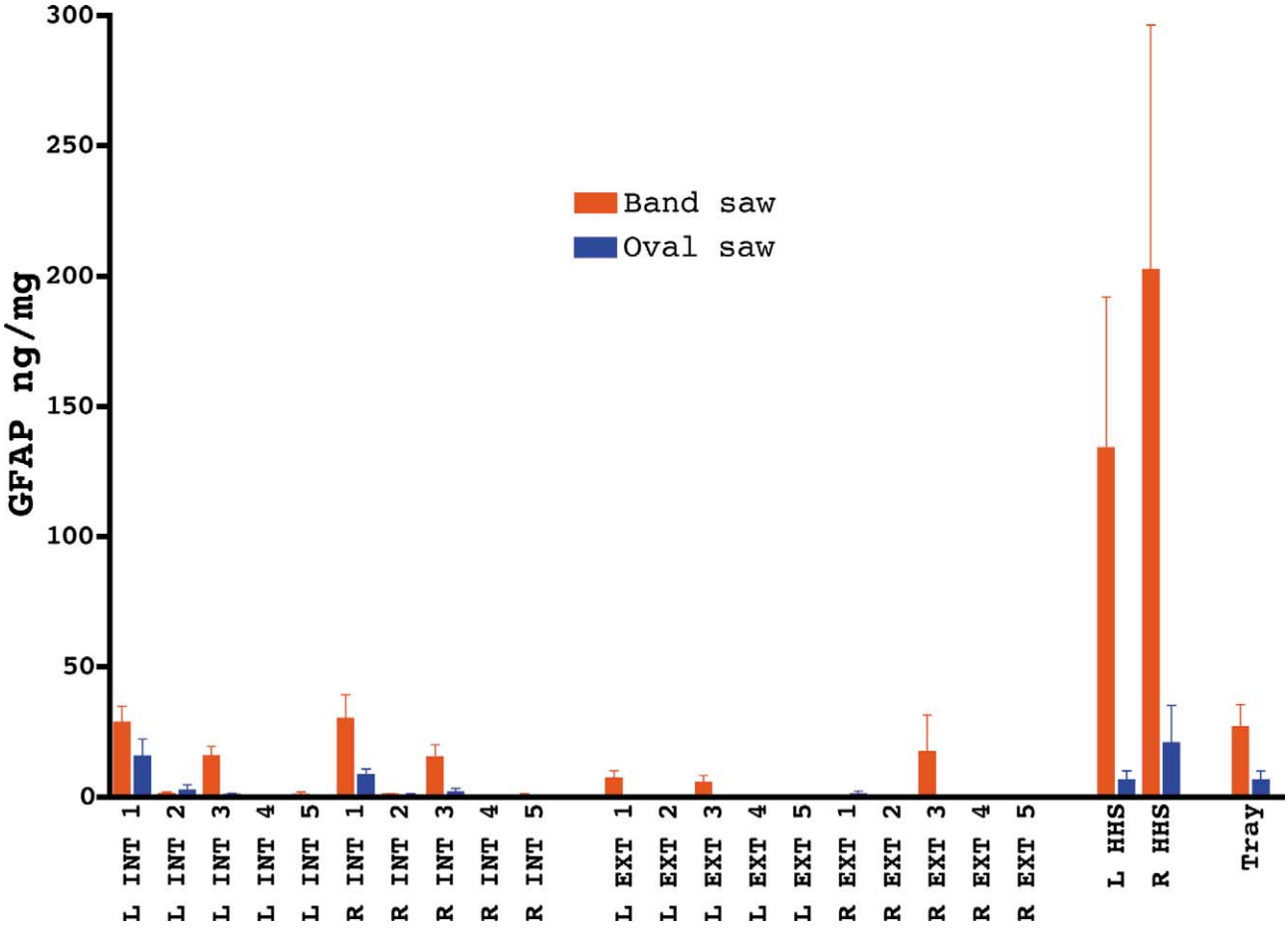

Fig. 5. CNS contamination of beef carcasses after splitting with a Jarvis-Buster V1 band saw (red bars) and an experimental oval saw (blue bars). Areas 1–5 (as defined in Fig. 1) were swabbed and analysed for GFAP. The results are expressed as ng GFAP per mg total protein. L – left half, R – right half, INT – medial surface, EXT – lateral surface, L HHS – left-hand side hand-held screen, R HHS – right-hand side hand-held screen. The tray water was also sampled.

Table 2
Simplified statistical models of the effects of saw, surface and area and cleaning, surface and area on the levels of carcass contamination as ln counts[a]

Saw

$$\ln(\text{count}) = 3.17(0.29) - 0.90(0.26)\ \text{Oval Saw} + 1.49(0.20)\ \text{Internal Surface} - 1.53(0.31)\ \text{Area 2} + 0.12(0.31)\ \text{Area 3} - 2.75(0.31)\ \text{Area 4} - 1.50(0.31)\ \text{Area 5}$$

Cleaning

$$\ln(\text{count}) = 2.83(0.28) + 1.30(0.29)\ \text{Steam} + 1.52(0.28)\ \text{Internal Surface} - 1.46(0.40)\ \text{Steam and Internal Surface} - 1.61(0.28)\ \text{Area 2} + 0.57(0.28)\ \text{Area 3} - 2.44(0.28)\ \text{Area 4} - 0.60(0.28)\ \text{Area 5}$$

[a] The constant in the 'saw' equation is the mean count for area 1 on the external surface when the band saw was used. The constant in the 'cleaning' equation is the mean count for area 1 on the external surface when washed with water. Standard errors of the parameter estimates are given in the brackets. For example the predicted count for the oval saw/external surface/area 3 is: $\ln(\text{count}) = 3.17 - 0.9 + 0.12$.

cut through the spinal cord; the spinal cord remaining in the neck area was split by the band saw when the carcass was split. This probably accounts for the level of CNS contamination still present when the oval saw was used.

This was only a small scale study so the detailed results should be approached with some caution. Nonetheless, as would be expected, the oval saw does appear to be effective in reducing contamination.

5. Conclusions

Since spinal cord is known to harbour the BSE infective agent (PrPsc) (Bradley, 1999) and has been banned from the human food chain, it seems unwise to allow it to be cut in half during the dressing process. We have demonstrated that this practice can disseminate spinal cord material over the carcass, operator and en-

vironment during the splitting process. If the animal being slaughtered has sub-clinical BSE the result will be dispersal of PrPsc over the carcass which, if consumed by humans, may lead to the development of vCJD.

Use of the oval saw resulted in significantly less CNS contamination, and when the engineering development is completed the technique should allow complete removal of the spinal cord, with reduced risk of contamination.

Acknowledgements

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