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Dissemination of central nervous system tissue from the brain and spinal cord of cattle after captive bolt stunning and carcass splitting

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Abstract

In the absence of reliable live animal tests for the presence of BSE in cattle, a number of measures have been applied to exclude specified risk materials (SRM) from the human food chain. However, concerns remain that current practices in the stunning and slaughter of cattle may disseminate central nervous system (CNS) tissue to meat and meat contact surfaces. The objective of this study was to establish the particular risks of CNS tissue dissemination associated with captive bolt stunning and carcass splitting. The study applied enzyme linked immunosorbent assays (ELISAs) in the detection and quantification of two CNS proteins, syntaxin 1b and GFAP. The study observed extensive dispersal of both CNS proteins onto equipment, beef hide and personnel. These results demonstrate that despite the rigorous application of current SRM control policies, normal slaughter practices continue to present significant opportunities for CNS material including BSE prion present in the CNS of any sub-clinically infected cattle to contaminate meat entering the human food chain.

Keywords: Central nervous system (CNS); Abattoir; Captive bolt stunning; Carcass splitting

1. Introduction

Shortly after the identification of Bovine Spongiform Encephalopathy (BSE) in cattle, concerns emerged that this disease could be transmitted to humans contacting or consuming beef and beef products (Brown, Will, Bradley, Asher, & Detwiler, 2001). Such concerns have been exacerbated by confirmation that the infectious agent, a prion isolated from BSE-infected cattle, shares a number of characteristic biological and structural features with the infectious agent of variant Creutzfeldt-Jakob disease (vCJD) in humans (Brown et al., 2001; Bruce et al., 1997; Collinge, Sidle, Meads, Ironside, & Hill, 1996; Scott et al., 1999). Particular risks are associated with those elements of the bovine central nervous system (CNS) which are most closely linked with BSE i.e. the brain and spinal cord, and their potential for contamination of abattoir workers, carcass meat and the abattoir environment during the slaughter of BSE infected animals.

Current practices in the stunning, slaughter and butchery of cattle have led to concerns regarding the possible dispersion of CNS material to edible meat (Anil et al., 1999, 2001; Anil, Love, Helps, & Harbour, 2002; Bauer, Garland, & Edwards, 1996; Buncic, McKinstry, Reid, & Anil, 2002; Daly, Prendergast, Sheridan, Blair, & McDowell, 2002; Garland, Bauer and Bailey, 1996; Grandin, 1997; Helps et al., 2002; Love, Anil, Williams, & Shand, 1999; Love et al., 2000; Schmidt, Hossner, Yemm, & Gould, 1999). Current slaughter methods may allow brain and/or spinal cord tissue to contaminate the carcass and enter the food chain (Helps et al., 2002). Embolic brain tissue in the lungs of cattle has been reported following stunning with a captive bolt gun (CBG) (Bauer et al., 1996; Garland et al., 1996). The use of pneumatically operated air injection penetrative CBGs (Schmidt, Hossner, Yemm, & Gould, 1999) or conventional CBGs, in combination with pithing, have been implicated in the disruption of CNS tissue in beef cattle and its subsequent deposition in other organs (Anil et al., 1999). Consequently such procedures are no longer recommended (European Commission Decision, 00/418/EC [http://europa.eu.int/

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eur-lex/en/lif/dat/2000/en_300D0418.html]). The bovine heart continues to pump for several minutes following the use of a CBG, during which time any CNS material that enters the jugular venous blood may be disseminated throughout the body (Anil et al., 2001). This has important implications for food safety if the brain tissue of stunned cattle is infected with the prion responsible for BSE (Prusiner, 1991).

In response to the BSE crisis, consumer expectations and manufacturing practices with respect to the use of CNS tissue in meat products have been drastically changed in recent years (Almond & Pattison, 1997; Dealler & Lacey, 1990; Lücker, Eigenbrodt, Wenisch, Failing, Leiser, & Bülte, 1999). To limit the transmission of BSE prion, by preventing the transfer of SRM, it is essential to be able to monitor and prevent the movement of brain and spinal cord material on carcass meat, and more generally within the abattoir environment. One approach is to track the spread of one or more CNS specific proteins, as markers of more general dissemination of CNS tissues.

The cells of the brain contain several proteins that are unique for the nervous system, including syntaxin 1b, a membrane bound protein, which constitutes 1% of total brain protein (Bennett, Calakos, & Scheller, 1992), and Glial Fibrillary Acidic Protein (GFAP), which makes up 10% total spinal cord protein (Boon, 1990; Lücker, Eigenbrodt, Wenisch, Leiser, & Bülte, 2000; Rosengren, Wikkelsø, & Hagberg 1994; Scott et al., 1999).

The objective of this study was to determine the effect of CB stunning and carcass splitting on the contamination of carcass meat, operatives, equipment and the abattoir environment with CNS material during commercial beef slaughter. Syntaxin 1b (Anil et al., 1999) and GFAP (Schmidt, Hossner, Yemm, Gould, & O'Callaghan, 1999) were used to detect and quantify this contamination.

2. Materials and methods

2.1. Sample collection

Samples were collected in an Irish commercial beef abattoir, during three visits between August 2001 and January 2002. Samples were taken at a number of stages of slaughter, dressing, chilling and boning of animals (Fig. 1) and examined for the presence of Syntaxin and/or GFAP. A total of 100 swab samples were taken at each location (except where otherwise indicated):

2.2. Sample locations

During slaughter:

(A) the CBG bolt immediately after withdrawal from each [stunned] animal,

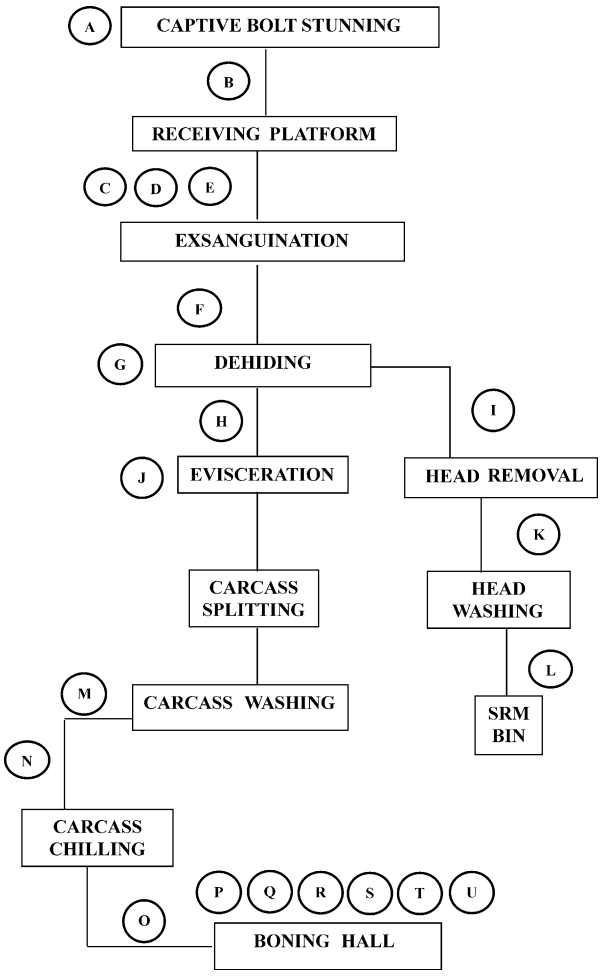

Fig. 1. Sampling points along the slaughter line (A–N), after chilling (O) and within the boning hall (P–U) of a commercial beef abattoir.

(B) the receiving platform after contact with the head of each animal,
(C) the CB aperture area, immediately after hoisting and exsanguination of each animal,
(D) (swabs were secured over) each CB aperture for 25 min immediately after hoisting and exsanguination (remaining in place until dehiding),
(E) (swabs were secured over) the CB aperture of 12 animals immediately after hoisting and exsanguination. Three of these swabs were removed within 30 s, and three swabs were removed every 5 min thereafter (up to 55 min). This was carried out in triplicate over three consecutive days,
(F) the knife (F1) and apron (F2) of the operative who cut and pulled the hide back from the brisket area of each carcass before dehiding,
(G) the floor adjoining the dehiding machine after the removal of each hide,
(H) the CB aperture immediately after the removal of each hide,
(I) 100 samples from each of the hands (I1) and apron

(I2) of the operative who had just removed the head of each animal,
(J) the hands of the operative who sawed each brisket bone before evisceration,
(K) samples (100 × 20 ml) of water draining from the exterior of each head during the washing process,
(L) 100 samples from each of the hands (L1) and apron (L2) of the operative placing each washed head into the SRM receiving bin,
(M) the first 25 cm of the spinal cord channel (see Fig. 2) of matched carcass sides, before (100 [M1]) and after (100 [M2]) manual spray washing (each carcass washed for approximately 30 s with water [40 °C] containing 0.1 ppm chlorine, by an operative on a rise and fall stand), and
(N) the hands of the operative who transferred each washed carcass into the chillers.

After chilling:

(O) the entire surfaces of four areas (see Fig. 2) on matching sides of chilled carcasses.

During boning:

(P) 100 samples from each of the apron (P1) and knife (J2) of the operative after removing each rib cage,
(Q) 100 samples from each of the apron (Q1) and knife (Q2) after removing each striploin, and
(R) each (detached) striploin on the conveyor belt to packaging.

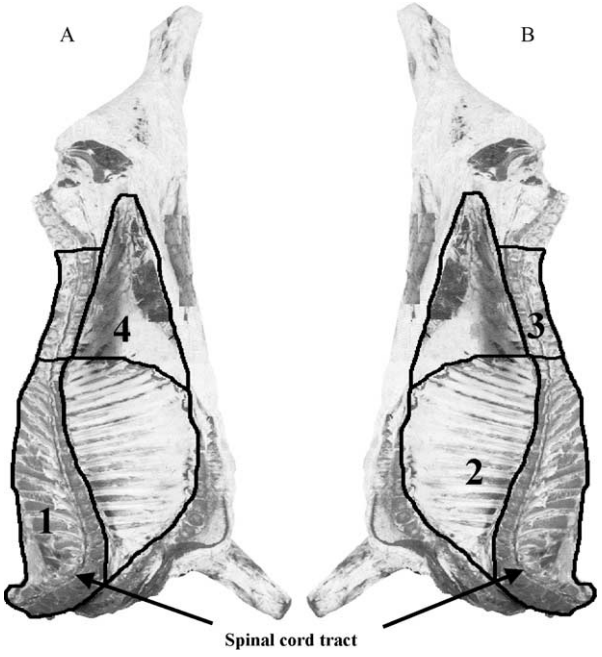

Fig. 2. Schematic diagram of the spinal cord channel (25 cm) swabbed on matching carcass sides along with the four surface areas swabbed on matching carcass sides (of different carcasses) after overnight chilling.

Swabs (3) were taken during each of three abattoir visits, from;

- the cutting surface of the saw used to split the carcass into fore and hindquarters within the boning hall (S),
- the working surface of the boning hall tables (T), and
- the working surface of the boning hall conveyor belt (U).

At three stages of abattoir operations, i.e.

- 1–2 h before commencement of slaughter operations ($t_{-1/2}$),
- Between 2 and 3 h after commencement of slaughter operations ($t_{-2/3}$), and
- Between 6 and 7 h after commencement of slaughter operations ($t_{-6/7}$).

2.3. Sampling and protein extraction procedures

Swab samples were recovered by the method of Helps et al. (2002). Briefly, each site was aseptically swabbed with a 100 × 100 × 10 mm dry sponge (Sydney Heath & Son Ltd, Staffordshire, England), and each charged swab inserted into a 150 ml Sterilin container (Bibby Sterilin, UK), containing 20 ml phosphate buffered saline (PBS) + 0.5% Triton X-100 (Sigma).

Head wash water samples (20 ml) were supplemented with Triton X-100, to a final concentration of 0.5% (v/v).

Samples were shaken, transported to the laboratory, and stored at 4 °C for 48 h to facilitate solubilisation of recovered proteins. Protein extracts from swab samples were manually aseptically expressed into 20 ml Sterilin bottles, stored at 4 °C, and analysed within 5 days.

2.4. Syntaxin 1b enzyme-linked immunosorbant assay

2.4.1. Preparation of calibration curve

Syntaxin 1b content of protein extracts was assessed by a modification of the syntaxin 1b capture ELISA (Anil et al., 1999), using a calibration curve prepared with purified recombinant syntaxin 1b standard (Department of Clinical Veterinary Science, Bristol University, Bristol, UK), serially diluted in PBS/0.5% Triton X-100, to final concentrations of 200, 180, 150, 100, 60, 20, 10, 2, 0.2, 0.02 pg μl^{-1}. Flat bottomed ELISA plates (Cell-Star, Greiner) were coated with 50 µl per well of mouse monoclonal anti-syntaxin antibody, incubated overnight at 4 °C, washed and blocked. Plates were then incubated at 37 °C for 1 h, emptied, blotted, and 50 µl standards were added to each well. Treated plates were incubated at 37 °C for 2 h and

washed. A 50 μl aliquot of blocking solution containing rabbit anti-syntaxin polyclonal antibody was added to each well, and the plates were incubated at 37 °C for 2 h, and washed. A 50 μl aliquot of blocking solution containing alkaline phosphatase-conjugated donkey anti-rabbit immunoglobulin G (IgG) was added to each well, and plates were incubated at 37 °C for two hours. Plates were washed four times in PBS/0.05% Tween-20 and twice in PBS, treated with 50 μl of 4 mg ml^{-1} p-nitrophenol phosphate (Sigma) per well, and the reaction allowed to proceed for 30 min at room temperature. The reaction was stopped by the addition of 50 μl 0.4 N NaOH (Analar) per well, and the optical density (OD) at 410 nm of each well was read using a MRX Microplate reader. Data obtained were used to prepare a calibration curve of OD versus syntaxin 1b concentration (ng) (Fig. 3a).

2.5. Sample analysis

Samples were processed as described above, except that 50 μl of sample was added to monoclonal anti-syntaxin washed, blotted plates.

2.6. GFAP enzyme-linked immunosorbant assay

The method of Schmidt, Hossner, Yemm, Gould, and O'Callaghan (1999) was applied, without modification, to detect GFAP in protein extracts, using bovine GFAP standard (American Research Products, Boston, MA) dissolved in distilled water and serially diluted to final concentrations of 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125 pg μl^{-1} in PBS/0.5% Triton X-100. Data obtained were used to prepare a calibration curve of OD versus GFAP concentration (ng) (Fig. 3b).

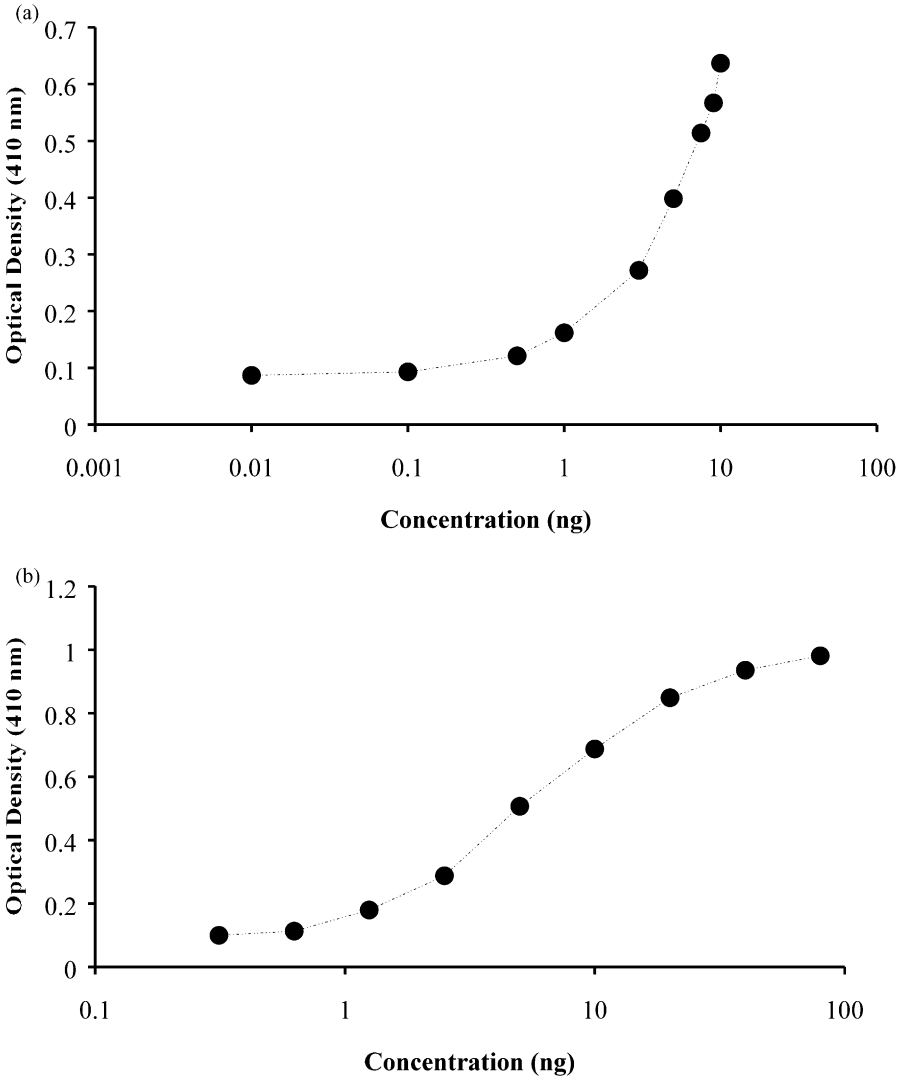

Fig. 3. Calibration curves for (a) syntaxin 1B ELISA and (b) GFAP ELISA.

2.7. Total protein

Total protein contents of protein extracts were determined by the bicinchoninic acid (BCA™) protein assay using a bovine serum albumin (BSA) standard (Pierce, Rockford, USA).

2.8. Disposal of brain and spinal cord material

All test materials, including swabs, protein extracts, brain and spinal cord material were sterilised by autoclaving at 133 °C for 20 mins, and disposed of in line with specified risk material (SRM) handling regulations, through the onsite industrial development unit.

2.9. Statistical analysis

ANOVA was applied to data and statistical tests were performed with log transformations. All counts were increased by 0.001, to allow the inclusion of zero counts in such transformations. This process gave zero counts a value of −3.00 on the log scale, while making no appreciable difference to the log values of all other (nonzero) counts (Daly et al., 2002). The statistical package used was Genstat 5 (Rothamsted Experimental Station, Harpenden, United Kingdom).

3. Results

Table 1 presents the concentrations of syntaxin 1b and GFAP ($\log_{10}$ ng mg^{-1} of total protein), from swab and wash water samples at specified locations i.e. A–D, G, H, K and M, in/on slaughter line samples/surfaces. Significantly higher concentrations of syntaxin 1b were recovered from samples from the captive bolt, the floor of the receiving platform, and the area around the CB aperture immediately after stunning, than from samples from head wash water, and the spinal cord channel before and after carcass washing ($P<0.001$).

A different pattern of relative concentrations was observed in relation to GFAP, i.e. significantly higher concentrations ($P<0.001$) of GFAP were recovered from the area around the CB aperture after dehiding, and the sampled areas of the spinal cord channel, than from locations A–D, G or K. GFAP concentrations in samples taken immediately after animal hoisting [onto the process line], or immediately before dehiding, were significantly lower than in samples taken (immediately) after dehiding ($P<0.001$).

Table 2 presents the concentrations of each CNS protein recovered from swabs secured over the CB aperture, immediately after hoisting and exsanguination (location E). Leakage of brain tissues as demonstrated by the detection of both marker proteins, continued for at least 55 min. Syntaxin 1b was observed 5 min after slaughter and reached a final concentration of 1.51 ng mg^{-1} at 55 mins. GFAP was detected immediately after slaughter and reached a final concentration of −0.18 ng mg^{-1} at 55 min.

Table 3 shows the concentrations of syntaxin 1b and GFAP ($\log_{10}$ ng mg^{-1} of total protein), from the hands (I1) and apron (I2) of the operative who removed the bovine head and from the operative who transferred them to the SRM bin at location L of the slaughter line. Significantly higher concentrations of each protein was recovered from aprons (I2) than from hands (I1) ($P<0.001$).

The recoveries of GFAP from the hands and aprons of operatives working in non CNS associated aspects of the slaughter process, i.e. dehiding, brisket sawing, and

Table 1
Concentrations of syntaxin 1b and GFAP ($\log_{10}$ ng mg^{-1} of total protein) in/on slaughter line samples/surfaces

Sample site (location)	Syntaxin 1b	GFAP	S.E.D. (d.f. = 99)
Captive bolt after stunning (A)	−0.10	−1.27	0.30
Floor of receiving platform (B)	1.23	−2.00	0.19
Bolt aperture after carcass hoisting (C)	0.73	−0.84	0.24
Bolt aperture 25 min after carcass hoisting (D)	0.31	−0.51	0.23
Floor below dehiding machine (G)	−1.79	−0.25	0.21
Bolt aperture after dehiding (I)	1.46	0.87	0.19
Head wash water (K)	−2.75	−1.16	0.18
Spinal cord channel [neck] before wash (M)	−2.81	1.01	0.14
Spinal cord channel [neck] after wash (M)	−1.91	0.84	0.15
SED (df)	0.24 (891)	0.24 (891)	

S.E.D., standard error of differences between means; d.f., degrees of freedom.

Table 2
Concentrations of syntaxin 1b and GFAP ($\log_{10}$ ng mg^{-1} of total protein) from swabs over the CB aperture at location E of the slaughter line

Time (min)	Syntaxin 1b	GFAP	S.E.D. (d.f. = 44)
0	ND	−1.60	0.82
5	1.05	1.97	0.82
10	1.21	1.40	0.82
15	1.50	−0.14	0.82
20	1.40	2.14	0.82
25	1.47	0.64	0.82
30	2.84	ND	0.82
35	−0.10	ND	0.82
40	1.37	ND	0.82
45	−0.51	ND	0.82
50	0.36	ND	0.82
55	1.51	−0.18	0.82
S.E.D. (d.f.)	0.51 (22)	1.04 (22)	

S.E.D., standard error of differences between means; d.f., degrees of freedom. ND, not detected.

Table 3
Concentrations of syntaxin 1b and GFAP (log_{10} ng mg^{-1} of total protein), from hands and aprons of operatives removing/disposing of bovine heads

Process (code)	Sample site (code)	Syntaxin 1b	GFAP	S.E.D. (d.f. = 99)
Head removal (I)	Hands of operative (I1)	−0.59	−1.67	0.22
	Apron of operative (I2)	0.44	−0.20	0.18
Transfer of head to SRM bin (L)	Hands of operative (L1)	−2.34	−1.86	0.28
	Apron of operative (L2)	0.54	−0.54	0.31
S.E.D. (d.f.)		0.24 (396)	0.27 (396)	

S.E.D., standard error of differences between means; d.f., degrees of freedom.

Table 4
Concentrations of GFAP (log_{10} ng mg^{-1} of total protein), from operatives working in non CNS associated slaughter processes

Process (code)	Sample site (code)	GFAP	No. positive	Range
Before dehiding (F)	Apron of operative (F1)	ND	0%	–
	Knife of operative (F2)	ND	0%	–
Brisket sawing (J)	Hands of operative	−2.83	4%	4.37–139.07
Chill loading (N)	Hands of operative	−2.8	5%	17.19–92.09

ND, not detected.

chill loading (F1, F2, J and N) are shown in Table 4. GFAP was not recovered from samples taken from the knife (F1) or apron (F2) of the operative preparing the hide for removal. However, GFAP was recovered from 4% of samples taken from the hands of the operative using the brisket saw (J), at concentrations ranging from 4 to 139 ng mg^{-1} of total protein. GFAP was also recovered from 5% of samples taken from the hands of the operative loading washed carcasses into the chiller (N), at concentrations ranging from 17 to 92 ng mg^{-1} of total protein.

Table 5 shows the recovery of GFAP ng mg^{-1} from chilled carcasses (O). GFAP was detected in samples from areas 1, 2 and 3 of the carcass, but not in samples from area 4. GFAP concentrations in samples from area 2 were significantly lower than in samples from areas 1 ($P < 0.05$) and 3 ($P < 0.01$).

Table 5
Concentrations of GFAP (log_{10} ng mg^{-1} of total protein) on chilled carcasses (location O)

Sample site	GFAP
Area 1 of carcass	−1.95
Area 2 of carcass	−2.41
Area 3 of carcass	−1.73
Area 4 of carcass	ND
S.E.D. (d.f.)	0.23 (396)

S.E.D., standard error of differences between means; d.f., degrees of freedom; ND, not detected.

Table 6
Concentrations of GFAP (log_{10} ng mg^{-1} of total protein), on the operatives and meat in the boning hall

Process (code)	Sample site (code)	GFAP
Rib cage removal (P)	Apron of operative (P1)	−0.32
	Knife of operative (P2)	−2.66
Striploin removal (Q)	Apron of operative (Q1)	−2.17
	Knife of operative (Q2)	−1.65
Meat being processed (R)	Striploin	−1.69
S.E.D. (d.f.)		0.30 (495)

S.E.D., standard error of differences between means; d.f., degrees of freedom.

The concentrations of GFAP on operative's aprons/knives, and meat being processed in the boning hall, boning hall equipment and surfaces before, during, and at the end of boning operations (locations P–R), are shown in Table 6. Significantly higher concentrations ($P < 0.001$) of GFAP were recovered from the apron of the operative who removed the rib cage (P1) than was recovered on his knife (P2). This pattern (i.e. P1 and P2) was not mirrored in relation to striploin removal, where no significant differences were observed between the concentrations of GFAP on knives and aprons. There were no significant differences between the concentration of GFAP on striploin beef cuts, and the concentration of GFAP on the apron or knife of the operative who removed these cuts from the carcasses.

The changes in concentrations of GFAP on boning hall surfaces during the overall slaughter cycle are presented in Table 7. GFAP was not detected on any boning hall equipment or surfaces prior to commencement of operations. GFAP was detected after 3 h of boning operations, but only on the boning hall table surfaces, at concentrations of 0.87 and 1.12 ng mg^{-1} of total protein. After 6 h of boning hall operations, GFAP was detected on all equipment and surfaces examined.

Table 7
Concentrations of GFAP (log_{10} ng mg^{-1} of total protein), on boning equipment and surfaces before and during boning hall operations

Site (code)	Before operations	2–3 h after start of operations	6–7 h after start of operations	S.E.D. (d.f. = 16)
Saw (S)	ND	ND	0.48	0.88
Table rib cage (T)	ND	0.87	1.9	0.88
Table striploin (T)	ND	1.12	0.29	0.88
Conveyor belt (U)	ND	ND	0.89	0.88
S.E.D. (d.f.)	0.79 (21)	0.79 (21)	0.79 (21)	

S.E.D., standard error of differences between means; d.f., degrees of freedom; ND, not detected.

4. Discussion

This study confirmed the use of syntaxin 1b and GFAP as indicators of CNS contamination during commercial stunning, slaughter and dressing. Both components could be easily recovered and quantified from a wide range of sample locations within the abattoir environment, and were stable in protein extracts for up to 1 week (results not shown). The BSE prion is, at 30 kDa (Prusiner, 1991), of similar size to syntaxin 1b [35 kDa] (Bennet et al., 1992) and GFAP [50 kDa] (Lücker et al., 2000). Such similarities in size, and stability, coupled with previous reports that the syntaxin 1b (Anil et al., 1999; Love et al., 2000) and the GFAP (Schmidt, Hossner, Yemm, Gould, & O'Callaghan, 1999) ELISAs are extremely sensitive and specific for CNS tissue, suggest that these proteins provide accurate methods to track the dissemination of CNS tissues, including BSE prion within abattoirs.

This study follows current convention (Helps et al., 2002; Lücker et al., 2000; Schmidt, Yemm, Childs, O'Callaghan, & Hossner 2002), in reporting the concentrations of syntaxin 1b and GFAP in ng/mg protein/total sample weight, rather than in absolute terms (i.e. in ng). In general, this approach facilitates comparison with previous reports, but could present difficulties in circumstances where there were significant differences between the proportions of target protein and total protein within a set of samples. Such differences are unlikely where most of the protein present is CNS protein, i.e. relatively pure brain tissue. This study dealt with samples recovered from a range of locations, some of which could have been subject to intermittent contamination with a range of proteinaceous materials, mainly blood. To ensure accurate comparison between such samples in terms of ng/mg protein, the study calculated the proportions of each target protein (syntaxin 1b or GFAP) within the total protein of each sample (results not presented). The study established that the dilution effect with blood in all samples was not significant and did not affect the calculation of the concentrations of syntaxin 1b and GFAP, confirming that results could be meaningfully presented as ng/mg. However, care should be taken in those future studies which report results in these proportionate terms, to ensure that samples share reasonably consistent target to total protein ratios.

Syntaxin 1b was present in higher concentrations than GFAP, on the CB, receiving platform for stunned animals and in material leaking from the CBG apertures of the bovine heads. This was to be expected, as syntaxin 1b occurs in 50- to 100-fold higher concentrations than GFAP in brain (Anil et al., 1999; Love et al., 2000) and most activities related to captive bolt stunning are involved in disruption and possible dissemination of brain rather than spinal cord materials. However, in more general terms, these observations confirm the significant risks of direct and indirect dissemination of brain tissue, during and immediately after stunning. Contamination of the CB is particularly undesirable as, once contaminated, it has the potential to spread CNS material from animal to animal and thus transfer any prion present in one animal, into other animals. Other agents, i.e. bacteria have been previously noted as transferring by this route (Buncic et al., 2002; Mackey & Derrick, 1979).

The observed release of CNS proteins from the CBG aperture during post stunning processing demonstrates the potential for the prion to leak onto the slaughter floor, with subsequent dissemination to other areas of the abattoir by the movement of personnel, equipment, etc. The study noted that prior to decapitation, brain material leaking through the CB aperture contaminated the surrounding area of hide, with opportunities to contaminate much of the bleeding area and equipment. The study also recovered higher CNS contamination from the CB apertures after dehiding than from other sites along the slaughter line. This raises a number of concerns, including the possibility that such continuing leakage of CNS material may spread to carcass surfaces. Similarly, the risks associated with such leakage are increased by the vigorous physical shaking of the animal's head and the occasional contact with the floor adjoining the dehiding machine during hide removal. In many cases this impact was noted as sufficient to dislodge visible fragments of CNS tissue through the CB aperture, and to generate aerosols of CNS material. Such release of CNS material, in a variety of forms, during the early stages of processing is likely to lead to significant, direct and indirect, widespread contamination to carcass meat, equipment and operatives. The detection of both CNS proteins from the head wash water after decapitation suggests that this process, and the contaminated water it produces could splash, drip or contribute to aerosolisation of contaminated CNS material. This could contaminate abattoir workers and the environment and equipment in the immediate area of these activities. More importantly, as the disease related form of the prion protein is relatively resistant to degradation, losing infectivity rather slowly in the environment (Gale, Young, Stanfield, & Oakes, 1998), the possibility of CNS, SRM and/or prions being washed into, and persisting in, abattoir and wider drainage systems is a cause for concern.

This study detected the target CNS specific proteins on the hands and aprons of the operatives who decapitated the carcasses and the operatives who transferred the head to the SRM bin. Such detection increases concerns about the wider dissemination of SRM during head processing, and indicates that carcass washing is not an effective method in removing disseminated spinal cord protein from carcasses. The data suggests that

carcass washing may do little more than spread contaminated material into previously uncontaminated areas. These concerns are exacerbated by the observation of the target proteins on the hands of the operative transferring the carcasses to the chills after washing. Such observations demonstrate that previously uncontaminated carcasses could become contaminated with CNS tissue from the operative's hands. Such widespread detection of both CNS specific proteins, at the major sites along the slaughter line, demonstrates that CNS contamination is likely to be spread widely along the slaughter line.

This study detected the spread of CNS tissues from the spinal cord onto other sites on the carcass, and noted that such contamination persisted after the gross removal of SRM (including the spinal cord). Contamination was higher on areas 1 and 3, probably because of their proximity to the "sawing line" at carcass splitting. Less contamination was observed in one of the more distal areas of the carcass, i.e. area 2, and no contamination was observed in area 4). These results are in agreement with Helps et al. (2002), who also observed greatest CNS contamination on areas 1 and 3. This study confirmed the carcass splitting saw as posing a particular risk in terms of cross contamination of carcasses, equipment, surfaces, and operatives, as there are no current commercially viable procedures for the removal of proteins from this equipment. The infectious agent of BSE is extremely resistant to inactivation by standard decontamination procedures (Taylor, 1989; Taylor et al., 1994), and it is not practical to decontaminate equipment between successive animals on a moving slaughter line (Buncic et al., 2002). These operational constraints on effective equipment decontamination during slaughter, increase the risks of transfer of the BSE prion from an animal to one or more subsequently processed carcasses.

The results of this present study suggest that current slaughter conditions and procedures may result in widespread dissemination of SRM within abattoir environments, contaminating equipment, surfaces, operatives, and carcasses destined for human consumption. Should animals suffering from sub-clinical BSE enter such slaughter processes, it is likely that the BSE prion would be subject to similar dissemination. This would result in contaminating operatives, equipment, waste water, and processed carcasses. It is clear that the impact of such wide spread dissemination to the health of operatives and meat consumers, will be significantly modulated by dose response models, the (low) frequency of slaughter of sub-clinically infected BSE animals and hygienic practices. However, it is clearly unsatisfactory that CNS material which is recognised as posing some degree of risk to operatives and consumers, is being widely circulated within abattoirs. It is also likely that such circulation of SRM during slaughter and processing is reducing the overall effectiveness of the considerable efforts being made to control SRM (and by association prion) contamination on carcasses and derived meats.

This study observed the ubiquity, albeit on occasions only in trace amounts, of CNS tissue in the abattoir environment, demonstrating a likely route of spread of BSE prion protein present in infected cattle passing through conventional slaughter processes. Procedures are in place to prevent, or significantly constrain, BSE positive animals from entering the food chain. However, in the light of the identified possible risks, such entry could pose, it may be necessary to derive alternative and/or additional means of preventing such frequent and wide ranging release of CNS material during slaughter. Such developments could usefully consider non penetrative stunning methods, to limit dissemination of CNS material from the skull, along with alternative means of spinal cord removal. In this regard a special oval saw is being developed to remove the spinal cord intact from beef carcasses. This should significantly reduce the risk of carcass contamination from this source (Helps et al., 2002; Anon, 2002). Such changes may well pose a number of operational and commercial difficulties, but are necessary as elements of the application of the "precautionary principle" in the protection and reassurance of consumers in relation to the control of BSE.

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