FINAL REPORT

Project RMIS No.4533

Spread of Brain and Spinal Cord Material during Beef Slaughter

The National Food Centre

RESEARCH & TRAINING FOR THE FOOD INDUSTRY

RESEARCH REPORT NO 54

Contents

SPREAD OF BRAIN AND SPINAL CORD MATERIAL DURING BEEF SLAUGHTER

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INTRODUCTION

Emboli of brain tissue in the lungs have been reported in cases following severe head injury (McMillan 1956) and birth trauma in people (Hauck *et al* 1990) and in cattle following stunning (Bauer 1996; Garland 1996). This has important implications for food safety if the brain tissue of stunned cattle is infected with the prion responsible for bovine spongiform encephalopathy (BSE), (Prusiner 1991). BSE emerged following changes in the rendering process in the early 1980s which allowed the aetiologic agent to survive, contaminate protein feed supplement and infect cattle (Brown *et al.* 2001). Within a short period of time after the first case of BSE, concerns were expressed regarding the transmission of BSE to humans and the likelihood of infection from consumption of beef and beef products. This has become increasingly significant with the discovery of the link between BSE in cattle and variant Creutzfeldt-Jakob disease (vCJD) in man (Bruce *et al* 1997).

Current stunning practices in the slaughter of cattle have led to concerns regarding the possible dispersion of infected brain and spinal cord material to edible meat tissue and cross contamination in the abattoir environment. In particular the use of pneumatically operated air injection penetrative captive bolt guns (Schmidt *et al* 1999a) and conventional captive bolt guns, followed by pithing, have been implicated in the disruption of central nervous system (CNS) tissue in beef cattle and its subsequent deposition in other organs (Anil *et al* 1999). However, current slaughter practice, using penetrative cartridge fired captive bolt guns, may induce disruption of brain tissues, and spread into the bovine circulatory system, leading to the dispersion of CNS tissues (including prion proteins) throughout the derived carcass. There are also concerns that slaughter processes may disperse infected brain and spinal cord material into the slaughterhouse environment.

Experiments were designed to assess the occurrence and extent of the dispersal of brain and spinal cord on meat and in the abattoir environment following stunning, slaughter and dressing. A bacterium was used to model the spread of brain and spinal cord in cattle following slaughter and dressing, as the infectious agent itself could not be used. It was anticipated that the bacterium would model the pathway that infected brain material would

follow after stunning and thus be representative of how the prion would be dispersed.

The objectives of this project were:

- To model the effects of commercial captive bolt stunning procedures on the movement of CNS material within slaughtered animals, the abattoir environment and personnel using a marker strain of Pseudomonas fluorescens (antibiotic resistant).
- To determine the levels of two CNS specific proteins, syntaxin and glial fibrillary acidic protein (GFAP), on carcass meat, in the abattoir environment and on personnel following commercial stunning, slaughter and dressing.

The other research partners were: The University of Bristol (UOB) UK, Association pour le developpppment de l'institut de la viande (ADIV) France, Silsoe Research Institute (SRI) UK and the Meat and Livestock Commission (MLC) UK.

Their objectives were:

- To develop protein antigen - based ELISAs to assess the level of cross contamination with CNS material (UOB).
- To develop vacuum equipment for removal of the CNS from unsplit carcasses or during splitting (ADIV).
- To develop standardised sampling methods for CNS material deposited on carcass meat, abattoir and cutting room structures and equipment (SRI).
- To produce guidelines on best practice to avoid CNS contamination (MLC).

MODELLING THE SPREAD OF BRAIN (CNS) TISSUE DURING STUNNING AND CARCASS DRESSING.

Inoculation of marker organism:

Animals were placed in a stunning box and shot with a cartridge fired captive bolt pistol (Cash special 22 calibre pistol). The marker organism, a non-pathogenic Ps. fluorescens strain was introduced into cattle brains through the captive bolt entry aperture after stunning by injection at two different inoculum levels, 6.0 and 9.0 log 10 cfu (one million and one billion bacteria per millilitre, respectively). In total, 1 ml of each inoculum was introduced using a 10 cm syringe. After inoculation, the aperture caused by the captive bolt was plugged with a sterile rubber bung, the surrounding hide washed with 10% sodium hypochlorite and the animals exsanguinated, dehided, eviscerated, split into sides, washed and weighed, following normal commercial dressing procedures. In all, 12 inoculated animals and two control animals were used. Blood taken at the time of exsanguination (sticking) and tissues/organs taken during carcass dressing were tested for the presence of the organism. The Trapezius cervicis (neck) muscle, lymph node, lungs, spleen, liver, kidney and subsamples of spinal cord, taken from six different regions within the vertebral column as outlined in Figure 1, were examined. The hands and knife of the operative and the saw used at carcass splitting were monitored for the presence of the organism.

Recovery of the marker organism :

Blood samples were serially diluted (1:10, v/v) in 9 ml maximum recovery diluent (MRD) and 1 ml aliquots were directly spread plated onto pseudomonas specific (CFC) agar plates and allowed to dry for 10 min. The plates were incubated for 2 h at 30°C and then overlaid with 0.21 ml of a filter sterilised stock solution of nalidixic acid at 1 mg ml-1, to achieve a final concentration of 10µg ml-1 nalidixic acid within the CFC agar. These plates were incubated for 48 h at 30°C, and examined. Tissue samples were treated exactly as outlined for the blood with the exception that a 25g sample of each organ/muscle was aseptically removed after surface flaming, transferred into

sterile stomacher bags, (Seward, London, UK), and stomached for 120s with 225 ml of tryptone soya broth. Wet and dry swabs from the exsanguination knife, the hands of operatives and the carcass splitting saw, were mixed thoroughly by vortexing for 10 s, diluted in MRD and treated as described above. The identity of presumptive isolates of the marker organism was confirmed by biochemical tests.

RESULTS

The marker organism was detected in samples of blood, organs and the musculature of inoculated animals. In general, higher counts of bacteria were recovered at the higher inoculum level. Exceptions to this were the blood sampled at 30s and the spleen (Figures 2 and 3). When examining the counts from different tissues and organs, numbers in the lymph node, lung, liver and spinal cord tissue were significantly higher at the higher inoculum ($P < 0.05$). The section of spinal cord closest to the brain (section six in Fig. 1), was the only area of the spinal cord where the organism was recovered. All other sections were negative. The organism was recovered in the neck muscle at mean counts of 0.58 log10 cfu g-1. The lowest recovery of the bacterium was in the lungs and liver following inoculation at the lower inoculation level.

The bacterium was recovered on the hands of operatives and on the knife used at exsanguination and on the saw used for carcass splitting (Fig 4). Lower numbers of the bacterium were recovered on the operatives' hands than on the equipment used.

DISCUSSION

The data showed that a marker organism inoculated into the brain of cattle during or after stunning could subsequently be detected in the blood, musculature and a number of organs, including the spinal cord. The concentration of inoculum used was significant, in general, the higher the concentration of bacteria inoculated, the more were recovered.

The spread of brain tissue as a result of stunning is now well established and has been demonstrated in cattle by a number of workers (Anil *et al* 1999;

Bauer *et al* 1996; Garland *et al* 1996). All these studies report the presence of emboli in the lungs of cattle after stunning, using a variety of captive bolt guns. In the present study the marker organism was found in the lungs of some of the animals tested, presumably transferred to the lungs via the bloodstream (Mackey and Derrick, 1979). The marker organism was also detected in the blood of animals during exsanguination. In studies by Love et al. (2000) and Anil et al. (1999) the presence of brain emboli and CNS tissue proteins was demonstrated in jugular venous blood 30s after stunning. Blood clots located in the hearts of cattle after stunning were also found to contain spinal cord fragments (Schmidt et al 1999a).

The above data suggest that a marker organism inoculated into the brain of cattle could be used to model the transfer of CNS tissue in the bloodstream and the deposition of such material in the lungs. It was also demonstrated that the organism could be found in a number of other organs throughout the body, and in muscle.

The contamination of the exsanguination knife, the operators' hands and the saw were in keeping with the observed contamination of the blood and the spinal column of the animal. The contamination of these is of special importance, since they all have the potential to spread contamination from one carcass to the next.

The effect of stunning in cattle on the subsequent appearance of CNS materials in the lungs and blood of animals and on equipment used for slaughter was indicated by the spread of the marker organism. It showed a wider spread to other organs than was previously observed for CNS tissue or proteins, suggesting that material present in or introduced into the CNS of cattle during captive bolt stunning, may become widely dispersed in the abattoir and within the derived carcasses including meat entering the human food chain.

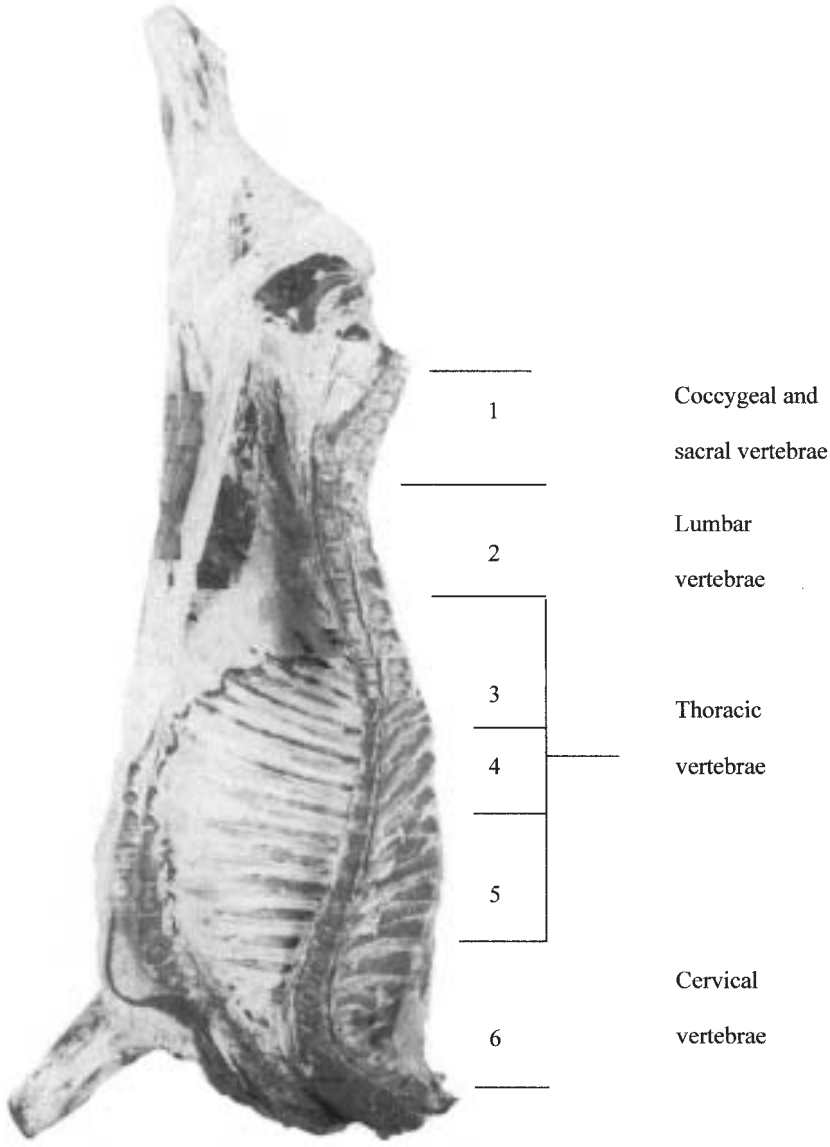

Figure 1: The median sagittal plane of a bovine carcass showing the six sampling sections for the spinal cord and each of the five vertebral divisions.

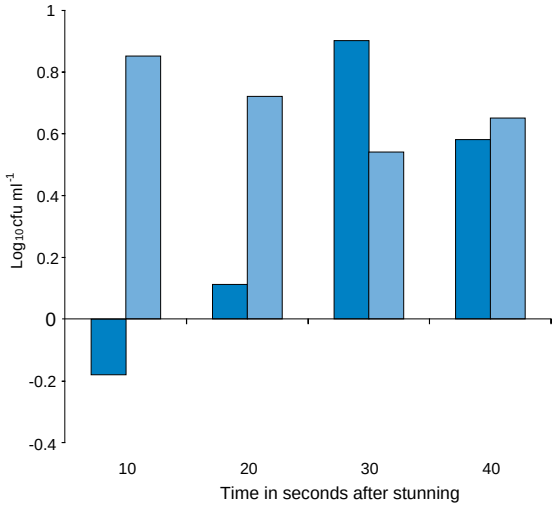

Figure 2: The effect of inoculation of cattle brains after stunning with 10^6 (■) and 10^9 (■) bacterial cells of Ps. fluorescens on the subsequent appearance of the bacterium in blood samples taken at different time intervals during exsanguination.

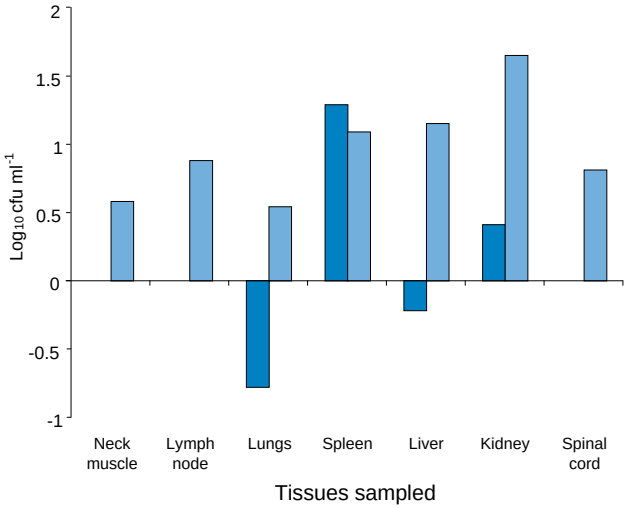

Figure 3: The effect of inoculation of cattle brains after stunning with 10^6 (■) and 10^9 (■) cells of Ps. fluorescens on the subsequent appearance of the bacterium in carcass tissue.

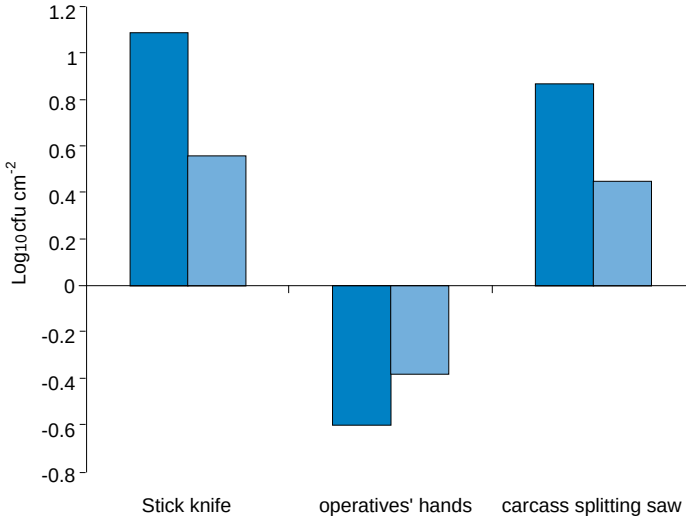

Figure 4: The effect of inoculation of cattle brains after stunning with 10^6 (■) and 10^9 (■) cells of *Ps. fluorescens* on the subsequent detection of the bacterium on equipment and personnel. Values below zero on the log scale represent less than 10 cells per cm^2 of surface sampled.

DETECTION OF TWO CNS SPECIFIC PROTEINS ON CARCASS MEAT, IN THE ABATTOIR ENVIRONMENT AND ON PERSONNEL.

Central nervous system (CNS) tissue, that is brain and spinal cord, poses a risk to abattoir workers, carcass meat and the abattoir environment if the animals slaughtered are infected with BSE. Therefore the ability to track brain and spinal cord material on carcass meat or in the abattoir environment would permit us to ascertain whether critical areas of the slaughter process should be classified as potential risks for BSE.

Two proteins which are specific to the CNS have been identified. Syntaxin 1B, a membrane bound protein, is brain specific, making up 1% of total brain protein; glial fibrillary acidic protein (GFAP), a major constituent of non-neural cells, is CNS specific, making up 10% total spinal cord protein (Bennet *et al*, 1992, Eng, 1985). These proteins are detectable from samples using an

enzyme linked immunosorbent assay (ELISA), which is a quantitative method for the detection of specific proteins. Antibodies are used to concentrate and bind specific proteins in samples which are then detectable by colorimetric or fluorescent signals. ELISA's for the identification of syntaxin 1B (Anil *et al*, 1999) and GFAP (Schmidt *et al*, 1999b) were used on samples collected in three Irish commercial beef abattoirs.

In the three abattoirs, the hide around the captive bolt aperture was swabbed after stunning and carcasses were sampled at the following stages: before (BCW) and after carcass washing (ACW) in areas 1- 4 (Fig 5), and before and after spinal cord (SC) tract washing.

Samples were taken from the retractable bolt of the captive bolt gun, the saw used at carcass splitting and the hands and apron of the operative who decapitated the animals. Samples were also taken from the apron of the operative who split the carcass.

RESULTS

There was dispersal of brain proteins onto equipment, beef hide and personnel during stunning and slaughter. Syntaxin 1B was detected in different concentrations on the captive bolt gun, stunning aperture, and personnel in all three abattoirs investigated. The highest concentration for syntaxin 1B was in abattoir three with lower levels detected in the other abattoirs (Fig 6).

The results also indicate that there was dispersal of CNS proteins onto meat, equipment and personnel as a result of splitting of the spinal column during beef carcass dressing. GFAP was detected on carcasses, equipment and personnel in all three abattoirs investigated. In general, the highest concentrations of GFAP on carcasses were at sites one and three along the vertebrae (Fig. 1), before and after washing. In abattoir one GFAP was detected on carcass meat and equipment and personnel employed at carcass splitting. Abattoir one and two had the highest levels of GFAP, whilst in abattoir three trace amounts were detected on carcass meat only (Fig 7).

DISCUSSION

The results indicate that there is dispersal of brain and spinal cord material onto carcass meat, abattoir personnel and equipment used during commercial stunning, slaughter and dressing.

The contamination of the captive bolt gun is important as it has the potential to spread CNS material from animal to animal and thus from infected to non-infected animals. In particular brain material leaks out from the shooting aperture onto the area of hide around the aperture on the head, thereby potentially contaminating the entire bleeding area. Brain protein was also found on the hands and aprons of the operatives who decapitated the carcasses indicating that brain tissue is spread widely along the slaughter line.

Spinal cord tissue is also a source of infectious material. During carcass splitting, areas closest to the vertebrae had the greatest risk of contamination by spinal cord material from the action of the saw. Indeed the saw itself poses a risk for cross contamination as there is no effective decontamination procedure available for the removal of proteins from equipment. This is a problem for most of the equipment used in stunning, slaughter and dressing. Routinely, carcasses are washed before entering the chills. Washing was not an effective method for cleaning carcasses, as in most cases, residual spinal cord protein concentration was not reduced. In some cases washing served only to spread the material on the carcass, thereby contaminating previously uncontaminated areas. As expected the highest level of spinal cord protein was found along the spinal cord tract and again washing was not an efficient cleaning method.

Unlike bacterial contamination, cooking is not an effective barrier to protein contamination on meat, as prion proteins are not denatured by normal cooking temperatures of meat. Therefore, in the abattoir emphasis should be placed on preventing CNS tissue from spreading onto meat where it can enter the food chain or onto equipment where abattoir workers are exposed to this material.

The other partners in this project developed an ELISA method for the detection of syntaxin 1B and using this method, detected syntaxin 1B in the

jugular venous blood of an animal that had been stunned with a pneumatically operated captive bolt gun (Love et al, 2000). Emboli of CNS tissue were also detected in the jugular venous blood of two of fifteen sheep stunned with a conventional cartridge operated captive bolt gun and in two of fifteen sheep stunned with a pneumatically activated gun (Anil et al, 2001). Three methods were developed that have the potential to reduce the level of contamination of carcasses with spinal cord: 1) an oval saw, which removes the entire spinal column before splitting. A prototype for sheep has been used successfully. A prototype for beef has been developed and shown to reduce CNS contamination of carcasses. 2) cord removal by suction prior to splitting with a conventional saw is potentially beneficial. A system for vacuum removal of the spinal cord has been tested in cattle, but its effectiveness in reducing CNS contamination requires investigation by CNS assay. 3) Hot-boning of the unsplit carcass. More development and evaluation work is required on these methods.

CONCLUSIONS

- A marker organism inoculated into the brains of cattle could be used to model the transfer of CNS tissue in blood, organs and musculature.
- The marker organism was recovered from operatives' hands and equipment showing that contamination could be spread from one carcass to the next.
- The concentration of inoculum used was significant, the higher the inoculum the greater the level of contamination.
- Central nervous system material was found on the carcasses, the workers, and on the abattoir equipment during standard commercial slaughter of cattle.
- Traces of brain and spinal cord tissues were detected on exposed surfaces on the killing line.
- The penetrating bolt on the stunning gun and the carcass splitting saw were contaminated with central nervous system material, thus making them likely sources of cross-contamination from infected to non-infected carcasses.
- Routine washing of carcasses did not remove contaminating central nervous system materials.
- The contamination of the abattoir environment with traces of central nervous system tissue demonstrated the route of spread of the BSE infectious agent, the prion protein, from infected cattle during conventional slaughter

In addition, the French and British research Partners in this project concluded the following:

- The use of a pneumatically activated captive bolt gun with air injection leads to neural emboli in both sheep and cattle.

- Pithing can lead to neural embolism.
- Neural emboli have been detected in sheep but not in cattle following stunning with penetrating captive bolt gun (without air injection).
- Use of electrical or non-penetrating captive bolt stunning does not cause neural embolism.
- In the UK, cattle more than 30 months old and animals entering the food chain are treated differently within a single abattoir, but it is undesirable to slaughter both categories of cattle in a single establishment as there is a risk of cross contamination.
- The major source of CNS contamination on the carcass is from splitting the spinal column. Most of the CNS material is spread on the medial cut surface of the carcass in the vicinity of the vertebral column.
- Washing with water or steam vacuuming is not effective in removing CNS material from the carcass.
- There is no evidence of aerosolisation of CNS material during splitting of the carcass, although projecta do occur and contaminate the area around the splitting station and may fall on the operator's clothing.
- The primal cuts, forerib and striploin receive the highest level of cord material during splitting. The level of contamination of these primals is greatly reduced after de-boning.
- There is preliminary evidence that splitting with a reciprocating saw causes lower levels of contamination of the carcass than with a band saw.

RECOMMENDATIONS TO INDUSTRY

Materials from cattle which may contain prions including the spinal cord, are referred to as specified risk material (SRM) and must be completely removed, permanently stained and sent directly to a specially dedicated rendering plant. The stages at which these are involved are stunning, head removal, carcass splitting and spinal cord removal, (Bolton et al, 2000).

To reduce or eliminate the contamination of carcass meat or operatives or abattoir environment with brain material the use of a non-penetrative concussion or penetrative captive bolt system is recommended for stunning. Penetrative captive bolt air compression (i.e. pneumatic) stunning is not recommended. Pithing in combination with stunning, which is practiced in 70% of UK operations, has also been shown to disperse brain tissue and should not be used. In addition to the above we recommend that electrical stunning or non-penetrative captive bolt guns should be used instead of penetrating bolts. Where penetrating captive bolt guns continue to be used, the blood, heart, lungs and visceral organs should be designated as SRM.

Carcass splitting using conventional techniques results in contamination of the carcass with SRM. An effective means of destroying the prion if present, needs to be developed. Carcass splitting is not a CCP as there is no control available other than ensuring that the correct procedures are carried out. Procedures must be in place to ensure the complete removal of the spinal cord and correct disposal in bins provided. A standard operating procedure (SOP) should be drawn up, displayed and enforced in every abattoir. In addition to the above, reduction of the dispersal of spinal cord material may also be achieved by developing carcass splitting saws or equipment which remove the spinal cord intact or which cut the carcass in such a way as to reduce contamination levels.

Penetrating captive bolt guns, either cartridge or pneumatically operated, should not be used for sheep and consideration should be given to phasing out their use for cattle. The ban on pithing should be maintained, electrical and non-penetrating captive bolt stunning equipment in use presently do not always correctly stun the animal, so improvements in the equipment are required to resolve welfare issues.

LIST OF PUBLICATIONS FROM THIS PROJECT

Daly, D.J., Prendergast, D.M. and Sheridan, J.J. 1998. Possible routes of cross-contamination of brain tissue onto carcass meat and abattoir environment during slaughter. The National Food Centre, Annual Research Report p:57-58.

Daly, D.J., Prendergast, D.M. and Sheridan, J.J. 1999. Contamination of carcass meat and the abattoir environment with central nervous system material during butchery. The National Food Centre, Annual Research Report p:47.

Daly, D.J., Prendergast, D.M. and Sheridan, J.J. 1999. Contamination of carcass meat and the abattoir environment with central nervous system material after captive bolt stunning and butchery in cattle. The National Food Centre Annual Research Report p:48-49

Daly, D.J., Prendergast, D.M., Moriarty, E. and Sheridan, J.J. 2000. Contamination of carcass meat and the abattoir environment with central nervous system material. *Irish Journal of Agriculture and Food Research*, **39** (1) :156.

Daly, D.J., Prendergast, D.M., Moriarty, E. and Sheridan, J.J. 2000. Detection of central nervous system (CNS) specific proteins using an enzyme linked immunosorbant assay and immunofluorescent microscopy. Proceedings: *Emerging issues in food safety*, University College, Cork, p: 14.

Daly, D.J., Prendergast, D.M. and Sheridan, J.J. 2000. Detection of central nervous system (CNS) tissue on carcass meat. The National Food Centre, Annual Research Report p:46-47

Daly, D.J., Prendergast, D.M. and Sheridan, J.J. 2000. Detection of central nervous system tissue in the abattoir environment. The National Food Centre, Annual Research Report p:47-48

Daly, D.J., Prendergast, D. M. and Sheridan, J.J. 2001. The use of a marker organism to model the spread of central nervous system (CNS) tissue in cattle and the abattoir environment during commercial stunning and carcass dressing. *Applied and Environmental Microbiology* (accepted for publication)

REFERENCES:

Anil, M. H., Love, S., Williams, A., Shand, J., McKinstry, L., Helps, C. R., Waterman-Pearson, A. and Seghatchian J. 1999. Potential contamination of beef carcases with brain tissue at slaughter. *The Veterinary Record* 145 (October 16):462-463.

Bauer N. E., Garland, T. and Edwards J. F . 1996. Brain emboli in slaughtered cattle. *Veterinary Pathology* **33** (5):600-600.

Bennett, M. K., Calakos, N. and Scheller R. H. 1992. Syntaxin: A synaptic protein implicated in docking of synaptic vesicles at presynaptic active zones. *Science* **257:**255-259.

Bolton, D.J., Sheridan J.J., and Doherty, A.M. 2000. HACCP for Irish beef slaughter. Teagasc, The National Food Centre report 2000. P: 18-19.

Brown, P., Will, R.G., Bradley, R., Asher, D.M. and Detwiler L. 2001. Bovine Spongiform Encephalopathy and Variant Creutzfeldt-Jakob Disease:background, evolution, and current concerns. *Emerging Infectious Diseases* **7** (1):6-16.

Bruce, M. E., Will R.G., Ironside, J.W., McConnell, l., Drummond, D., Suttie, A., McCardle, L., Chree, A., Hope, J., Birkett, C., Cousens, S., Fraser, H. and Bostock C. J. 1997.Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature* **389:**498-501.

Eng, L. F. 1985. Glial Fibrillary Acidic protein (GFAP): the major protein of glial intermediate filaments in differentiated astrocytes. *Journal of Neuroimmunology* **8:**203-214.

Garland T., Bauer, N. and Bailey M. 1996. Brain emboli in the lungs of cattle after stunning. *The Lancet* **348:**610-610.

Hauck A. J., Bambara, J.F. and Edwards W. D. 1990. Embolism of brain tissue to the lung in a neonate. *Archives of Pathology and Laboratory Medicine* **114:**217-218.

Love, S., Helps, C.R., Williams, S., Shand, A., McKinstry, J.L., Brown, S.N.,

Harbour, D.A. and Anil M.H. 2000. Methods for detection of haematogenous dissemination of brain tissue after stunning of cattle with captive bolt guns. *Journal of Neuroscience Methods* **99:**53-58.

Mackey B. M. and Derrick C. M. 1979. Contamination of the deep tissues of carcasses by bacteria present on the slaughter instruments or in the gut. *Journal of Applied Bacteriology* **46:**355-366.

McMillan J. B. 1956 Emboli of cerebral tissue in the lungs following severe head injury. *American Journal Of Pathology* **32:**405-415.

Prusiner, S. B. 1991. Molecular biology of prion diseases. *Science* **252** (5012):1515-1522.

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

Schmidt, G. R., Hossner, K.L., Yemm, R.S., Gould, D.H. and 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* **62** (4):394-397.

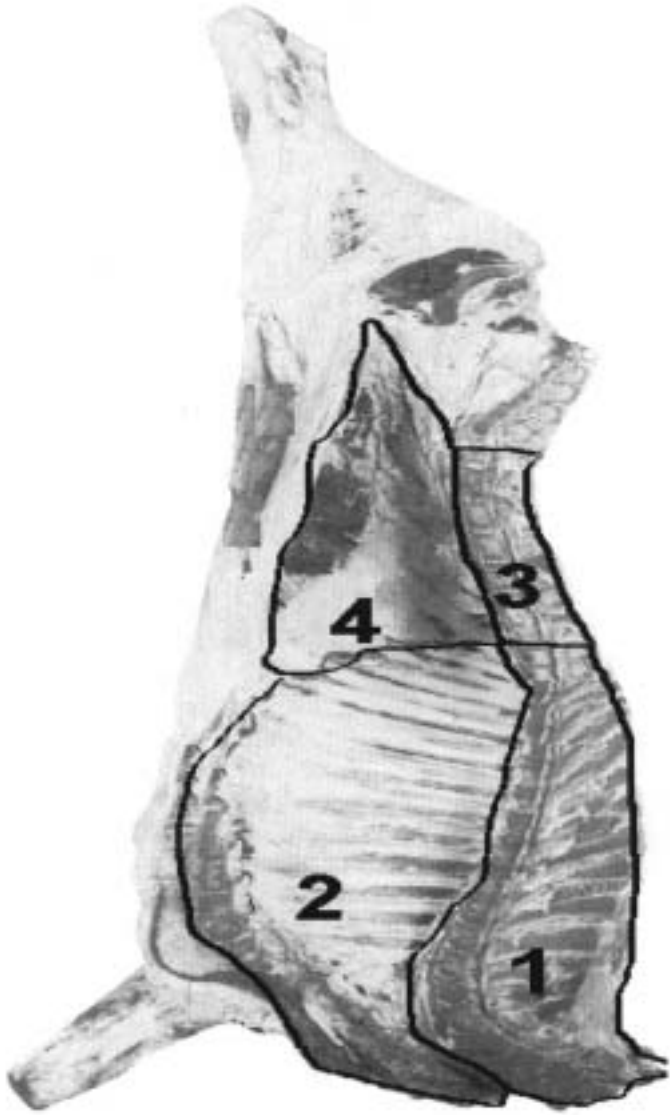

Figure 5: Sampling sites on the carcass for the detection of GFAP protein, which is specific to the central nervous system.

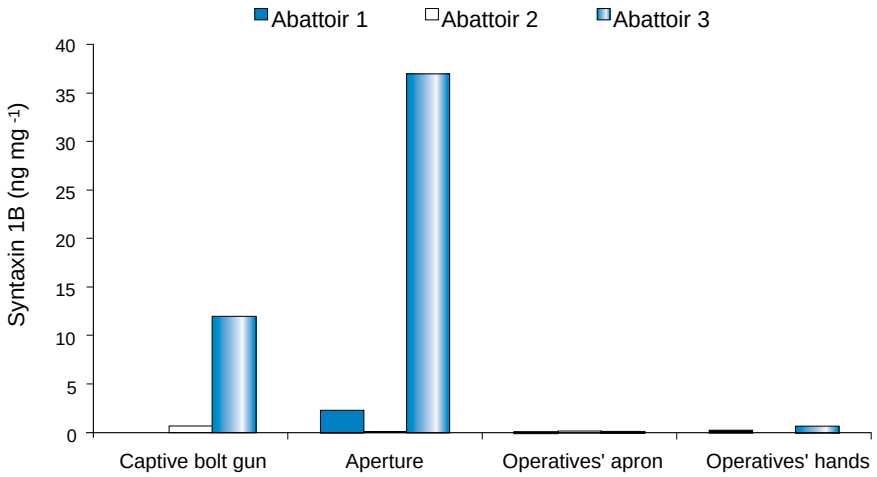

Figure 6: The concentration of brain protein syntaxin 1B on the captive bolt gun, beef hide at the stunning aperture and on the operatives' aprons and hands in three abattoirs.

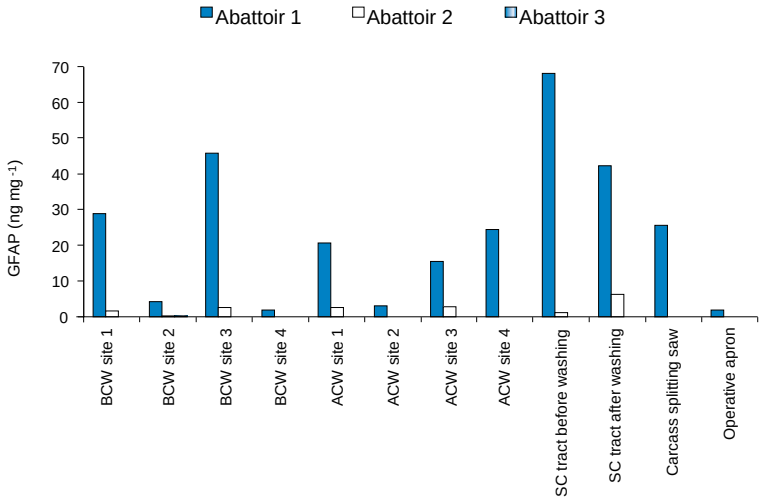

Figure 7: The concentration of central nervous system protein (GFAP) on beef carcasses, equipment and personnel in three abattoirs.
BCW: before carcass washing; ACW: after carcass washing; SC: spinal cord.

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