THE PREVALENCE AND POTENTIAL FOR DISSEMINATION OF NEURAL EMBOLISM IN CATTLE AND SHEEP FOLLOWING THE USE OF MECHANICAL STUNNING METHODS CURRENTLY USED IN THE UK

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Background

The emergence of the BSE and establishment of its link with vCJD in the UK and in several other countries has initiated a review of all practices in the meat industry that may have contributed to the emergence and transmission of these diseases. It is known that brain and spinal cord tissues carry the highest burden of infectivity in a BSE infected animal and for this reason these tissues along with certain other high risk tissues are designated specified risk material (SRM). All such tissues must be excluded from those parts of the carcass intended for consumption. The possibility that sheep may also harbour the BSE agent has led to the application of equally stringent controls on sheep as for cattle.

The use of captive bolt guns is the most popular stunning method employed in cattle and a considerable percentage of sheep are stunned by the same technique in the UK.

The first reported discovery of neural tissue fragments in the pulmonary arteries of some cattle after the use of a air-injection pneumatically activated captive bolt gun called into question the continued use of these devices in light of the BSE epidemic (Garland, Bauer & Bailey, 1996). Further studies aimed at specifically detecting disseminated CNS tissue in blood have demonstrated that a pneumatically operated gun alone caused dissemination of brain tissue and neural embolism could also occur in cattle after use of conventional captive bolt guns followed by pithing. In addition it has been shown that the use of both cartridge activated and pneumatic type captive bolt guns can result in neural embolism in sheep without the need for additional procedures (Anil et al. 1999, 2001).

In light of these findings, there is now a need to carry out in-depth studies to assess and establish the risk of neural embolism posed by current mechanical stunning methods and in addition to investigate the potential for contamination of edible tissues of the carcass.

Objectives

In this study we have attempted to determine the prevalence of neural embolism in both cattle and sheep following the use of types of captive bolt gun currently used on these species in the UK. Secondly we have investigated the potential for dissemination of such neural emboli within the carcass during the process of stunning and slaughter.

Prevalence of neural embolism in cattle following the use of the captive bolt gun

Methods and materials

Two groups of one hundred cattle each were randomly chosen from animals at a OTMS abattoir in Bristol, UK and were sampled for the presence of CNS tissue in the venous return from the head using a previously described technique (Anil et al. 1999, 2001, 2002).

One group of animals were stunned with a penetrative cartridge activated captive bolt gun (Cowpuncher, Cash 8000, Accles & Shelvoke) while the remaining one hundred animals were stunned with a non-penetrating cartridge activated captive bolt gun (Cash Magnum Knocker, Accles & Shelvoke). The Cowpuncher gun is commonly used throughout the UK in cattle abattoirs and the Magnum Knocker is the currently available non-penetrating gun.

The blood samples collected were tested for the presence of CNS tissue by an ELISA test for the CNS specific protein GFAP. In addition a proportion of samples from each animal was also analysed by a combination of microscopy and immunocytochemistry in which neural tissue emboli identified by microscopy were confirmed by staining with the CNS specific proteins S-100 and neurofilament protein (Love et al. 2000).

Results

Table 1. Prevalence of neural embolism in cattle

CBG	*n*	*GFAP ELISA*	*Immunocytochemistry*	*Total confirmed positives*
Penetrating CBG	100	2	3	4
Non-penetrating CBG	100	0	2	2

The prevalence of neural embolism in cattle following the use of a penetrative captive bolt gun was 4 per cent with a 95 per cent confidence interval from 2 to 7.7 per cent (Table 1). The prevalence of neural embolism in cattle following the use of a non-penetrative captive bolt gun was 2 per cent with a confidence interval from 0.6 to 7 per cent.

Discussion

The study has demonstrated that embolism of neural tissue can occur following the use of both penetrative and non-penetrative captive bolt guns without the additional trauma caused by procedures such as pithing. In addition the study has suggested the likely prevalence of neural tissue embolism in the UK at the present time and also gives an insight into the level of historical contamination that may have occurred.

Both assays used have been demonstrated to be effective methods of detection of neural emboli in blood however the small sampling volume employed by both tests explains the less than perfect agreement between the results obtained by each test. It is possible that a small number of positive samples may have been overlooked. Extensive bench testing of the GFAP ELISA has shown the limit of detection of brain tissue in blood to be at least 78µg/ml. The immunocytochemistry assay has been well validated by previous studies (Love et al. 2000) and in all control samples proved to be completely reliable.

Prevalence of neural embolism in sheep following the use of the captive bolt gun

Methods and materials

Two hundred animals were sampled for the presence of neural tissue fragments in the jugular venous return after the use of a captive bolt stunning device. Half of these animals had been stunned using a cartridge activated penetrating CBG (Cox MK.9 Accles & Shelvoke) while the remaining one hundred animals were stunned by the use of a pneumatically activated penetrating CBG (air pressure of 100psi).

The manner of sampling, processing and testing for neural tissue followed that used in the cattle study (see above) and previous work (Anil et al. 2001).

Results

Table 1. Prevalence of neural embolism in sheep

CBG	*n*	*GFAP ELISA*	*Immunocytochemistry*	*Total confirmed positives*
Cartridge activated	100	17	13	22
Pneumatically activated	100	12	7	13

The prevalence of neural embolism in sheep following the use of the cartridge activated, penetrating captive bolt gun was 22 per cent with a 95 per cent confidence interval from 15 to 31.1 per cent (Table 2).

The prevalence of neural embolism in sheep following the use of the pneumatically activated, penetrating captive bolt gun was 13 per cent with a 95 per cent confidence interval from 7.8 to 21 per cent.

Discussion

Although naturally occurring BSE has not been identified in any sheep in the UK to date, the high prevalence of neural embolism in this species following the use of these stunning methods must raise questions of the safety of captive bolt stunning in sheep. It is probable that the high prevalence found in comparison to that found in the cattle study relates to the greater brain damage inflicted by the bolt in this species. A further study is planned in which this question will be investigated.

The dissemination of neural emboli in the sheep carcass after the use of CBG stunning

Methods and materials

Small volumes of brain tissue were introduced into the jugular veins of anaesthetised sheep while simultaneously sampling the aortic blood and stunning by the use of a pneumatically activated captive bolt gun. The brain tissue suspension injected had previously been harvested from the bolthole of a fresh carcass following the use of the same gun. Aortic blood samples were collected at one minute intervals until blood flow ceased as a result of circulatory arrest. All samples were analysed for the presence of CNS tissue by GFAP ELISA and by a combination of microscopy and immunocytochemistry.

Results

CNS tissue was detected in the blood samples of six of eleven animals by GFAP ELISA and in two of eleven animals by microscopy and immunocytochemistry.

In addition in five of the positive animals CNS tissue was detected within the first minute of sampling.

Discussion

Neural emboli have previously been detected in jugular blood samples after stunning by CBG (Anil et al. 1999, 2001, 2002). Such neural emboli have not previously been demonstrated to penetrate the pulmonary capillary system to enter the aortic systemic circulation. This is clearly a critical determinant of the risk that stunning by captive bolt gun may disseminate PrP^{sc} throughout the carcass. This study has demonstrated that such passage through the lungs can occur by neural tissue fragments up to 20μm in diameter.

In addition we have found that such neural emboli may penetrate the lungs within one minute of being found in the jugular return. This finding is important in the context of stunning and slaughter as it suggests that there may be sufficient time for dissemination of emboli in the carcass before blood loss and circulatory arrest.

The present study has confirmed the potential for embolisation of particulate neural tissue through the pulmonary vasculature and has provided information on the timing of such an event.

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