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Potential for carcass contamination with brain tissue following stunning and slaughter in cattle and sheep

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Abstract

The risk associated with the use of captive bolt guns (CBGs) in dissemination of brain tissue during preslaughter stunning has been investigated. Such dissemination poses a threat to public health in relation to possible slaughter of animals with preclinical BSE. Fragments of brain tissue were detected in the jugular venous blood of cattle and sheep slaughtered after the use of a pneumatically operated or a conventional cartridge-operated penetrating CBG. The results indicate that there is a risk of haematogenous dissemination of central nervous system (CNS) tissue with the use of penetrating CBGs. However, use of a non-penetrating CBG in either species neither caused any embolism nor did electrical stunning in sheep. We recommend further study of procedures for the stunning and slaughter of ruminants in abattoirs.

Keywords: Bovine spongiform encephalopathy; Variant Creutzfeldt–Jakob disease; Captive bolt guns; Risk assessment; Embolism; Cytoblocks; Syntaxin 1-B

1. Introduction

Concern for public safety following the BSE outbreak and the appearance of cases of vCJD has prompted review of current methods for the stunning and slaughter of cattle and sheep. The use of current stunning procedures on an animal with BSE could, at least theoretically, contaminate the carcass with prions. Potential contamination could occur by haematogenous dissemination of brain tissue during the stunning procedure or by dispersal of spinal cord material during the splitting of beef carcasses. Projects to determine whether central nervous system (CNS) material could be detected in edible parts of the carcasses are in progress in the UK. This paper concerns the results of the investigation into haematogenous dissemination of brain tissue.

Most cattle and a substantial proportion of sheep (38% in the UK, MHS, 1997) are stunned with a penetrating captive bolt gun (CBG) prior to slaughter. The use of CBGs may damage intracranial blood vessels and dislodge brain tissue. The heart continues pumping for several minutes following the use of a CBG, during which time any CNS material that enters the jugular venous blood could be disseminated throughout the body. The report by Garland, Bauer, and Bailey (1996) of brain tissue in the lungs of slaughtered cattle first led to concern that the use of CBGs could result in haematogenous dissemination of infective brain material. The validity of these findings was, however, questioned (Taylor, 1996) and similar studies carried out on 210 cattle in UK abattoirs failed to confirm the occurrence of pulmonary embolism of brain tissue (Munro, 1997). In view of the relatively crude and insensitive procedures that were used for detecting brain tissue in those studies, further research was commissioned by the Ministry of Agriculture, Food and Fisheries in the United Kingdom, to assess the risk that current methods for the stunning and slaughter of cattle and sheep might cause embolic dissemination of CNS material into edible parts of the carcass.

In two projects, different CBGs and slaughter procedures were investigated, to evaluate whether these caused the entry of brain tissue into the jugular venous blood in cattle and sheep. In addition, electrical stunning was also tested in sheep. The preliminary results of the cattle study have been published (Anil et al., 1999).

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2. Materials and methods

2.1. Animals

Cattle. The studies were conducted on a total of 60 slaughter cattle. Anaesthesia was induced with intravenous ketamine (12 mg kg^{-1} body weight) and xylazine (0.6 mg kg^{-1} body weight) and maintained with a continuous infusion of a mixture of xylazine (0.01%), ketamine (0.1%) in guaiphenesin (5%) at a rate of 20 ml min^{-1} per animal. This mixture was used because it has been shown to maintain cardiovascular stability due to the opposing actions on blood pressure of xylazine and ketamine. Foley catheters were introduced into both jugular veins, a jugular venous blood sample was taken, the catheters were then inflated and the animal immediately stunned with one of the several CBGs. Cattle were allocated to six treatment groups:

- Group 1 (15 cattle): jugular vein catheters inserted, animals stunned with a pneumatically activated penetrating CBG (no pithing required due to air injection into spinal canal (Hantover, USA));
- Group 2 (5 cattle): aortic catheters inserted, stunned with a pneumatically activated penetrating CBG (Hantover, USA);
- Group 3 (15 cattle): jugular vein catheters inserted, stunned with a cartridge-operated conventional penetrating CBG, known as cow puncher (Accles and Shelvoke, UK) followed by pithing;
- Group 4 (5 cattle): aortic catheters inserted, stunned with a cartridge-operated penetrating conventional CBG (cow puncher, Accles and Shelvoke, UK);
- Group 5 (15 cattle): jugular vein catheters inserted, stunned by a non-penetrating cartridge-operated (therefore no pithing) CBG, known as cash knocker (Accles and Shelvoke, UK);
- Group 6 (5 cattle): aortic catheters inserted, non-penetrating cartridge-operated CBG (cash knocker, Accles and Shelvoke, UK).

For 60 s after the stunning, all of the blood draining from the jugular catheters was collected. Blood was collected into six 500 ml citrated bottles, each bottle receiving the jugular venous output over a 10 s period. The animal was then exsanguinated. A small sample of blood (<10 ml) was removed from each bottle for subsequent ELISA tests. The remainder was kept for microscopy and immunocytochemistry (see below).

Sheep. The studies were carried out in 60 sheep that had been sent for culling. Intravenous catheters were placed in the animals' jugular vein for the injection of anaesthetic agent. Prior to slaughter, animals were anaesthetised and maintained with intravenous ketamine (12 mg kg^{-1} body weight) and xylazine (0.4 mg kg^{-1} body weight).

Following the induction of anaesthesia, either Foley catheters were introduced into the jugular veins or PVC catheters were advanced into the aorta via the carotid arteries, a pre-stun blood sample was taken, then the Foley catheters, if fitted, were inflated and the animal was immediately stunned. Sheep were allocated to six treatment groups:

- Group 1 (15 sheep): jugular vein catheters inserted, animals stunned with a pneumatically activated penetrating CBG (Cash Ramrod, Accles and Shelvoke, UK);
- Group 2 (5 sheep): aortic catheters inserted, stunned with a pneumatically activated penetrating CBG;
- Group 3 (15 sheep): jugular vein catheters inserted, stunned with a cartridge-operated conventional penetrating CBG (Temple Cox Mark X, Accles and Shelvoke, UK);
- Group 4 (5 sheep): aortic catheters inserted, stunned with a cartridge-operated penetrating conventional CBG;
- Group 5 (15 sheep): jugular vein catheters inserted, electrical stunning, head only, by application of 250 V for 3 s;
- Group 6 (5 sheep): aortic catheters inserted, electrical stunning, head only, by application of 250 V for 3 s.

For 60 s after stunning, all of the blood draining from the jugular veins (in those sheep with jugular venous catheters) was collected into bottles with citrate anticoagulant, each bottle receiving the entire jugular venous output over a 10 s period. A small aortic sample (10 ml) was taken from each animal with an arterial catheter. After sample collection, each animal was exsanguinated. Blood samples were processed and prepared for later analysis as described before.

We used two previously validated methods to look for CNS tissue in blood: immunocytochemistry on sections of buffy-coat Cytoblocks for S-100β protein, and capture ELISA for syntaxin 1-B. Neither of these CNS proteins is normally found in the blood. The details of the techniques employed and their validation have previously been reported (Anil et al., 1999; Love et al., 2000).

3. Results

3.1. Cattle studies

Multiple fragments of brain tissue were detected in the jugular venous blood of 4 of the 15 cattle slaughtered after use of a pneumatically operated penetrating CBG (Hantover, USA). The fragments were detected by microscopy and immunocytochemistry of buffy-coat material and also by syntaxin 1-B ELISA of whole blood. There was perfect concordance between the identifica-

tion of CNS tissue in sections stained with haematoxylin and eosin and that in adjacent sections immunostained for S100β protein, although the smaller fragments of CNS tissue were much more readily identified in the latter preparations, which contained many immunolabelled fragments of varying size, some less than 5 μm in diameter (Fig. 1). No CNS tissue was identified in any of the other samples, including those obtained immediately before the stunning of the animals that showed evidence of jugular venous neuroembolism after stunning nor from any aortic blood sample. Some of the samples from the other animals did, however, contain fragments of skin or catheter tubing.

Jugular venous blood from one other of the 15 animals slaughtered after use of a pneumatically operated CBG yielded positive results in the syntaxin 1-B ELISA, although brain material was not detected by microscopy in the blood sample from this animal. Brain tissue was also found in the venous blood of 1 of the 16 cattle slaughtered after use of a conventional cartridge-operated penetrating CBG with subsequent pithing; the brain tissue was detected on microscopy and by immunocytochemistry for S100β protein, and the blood contained markedly elevated levels of syntaxin 1-B. Fig. 2 shows the time course of detection of brain tissue, by capture ELISA for syntaxin, in sequentially sampled jugular venous blood taken from animals after the use of a pneumatically operated CBG or a conventional CBG followed by pithing. No brain tissue or elevation of syntaxin 1-B was detected in the blood from the 15 cattle slaughtered after the use of a conventional CBG without pithing or from 14 slaughtered after the use of a mushroom head, non-penetrating CBG.

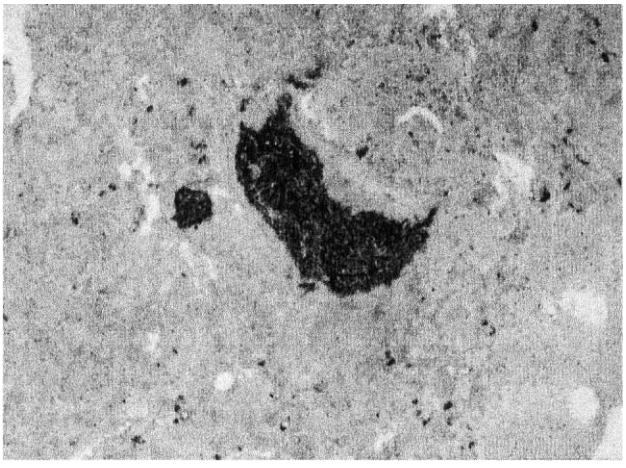

Fig. 1. Section through a Cytoblock of the buffy-coat fraction of a jugular venous blood sample from a cow that had been stunned with a pneumatically operated penetrating CBG. The brown reaction product on immunocytochemistry for S100β protein reveals two relatively large and several smaller fragments of brain tissue within the sample. Reproduced by permission of *Veterinary Record*.

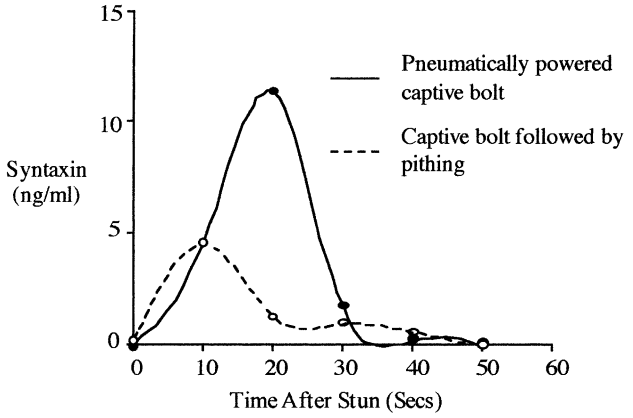

Fig. 2. Venous blood syntaxin 1-B levels in two cattle stunned with a paCBG (solid line) and a conventional CBG followed by pithing (interrupted line). Reproduced by permission of *Veterinary Record*.

3.2. Studies in sheep

CNS tissue was detected in the jugular venous blood of 2 of the 15 sheep that had been stunned with a conventional penetrating CBG and 2 of the 15 that had been stunned with a pneumatically activated penetrating CBG. The embolic brain tissue was again recognisable morphologically in sections through the buffy-coat cytoblocks and its detection was facilitated by immunocytochemistry for S100β protein. Many fragments of varying size, some less than 5 μm in diameter were again observed. Figs. 3 and 4 show the time-course of detection of brain tissue, by capture ELISA for syntaxin, in sequential samples of jugular venous blood taken from sheep after the use of a pneumatically activated penetrating CBG and a conventional cartridge activated CBG, respectively. Electrical stunning of a further 15 sheep did not cause detectable jugular venous embolism

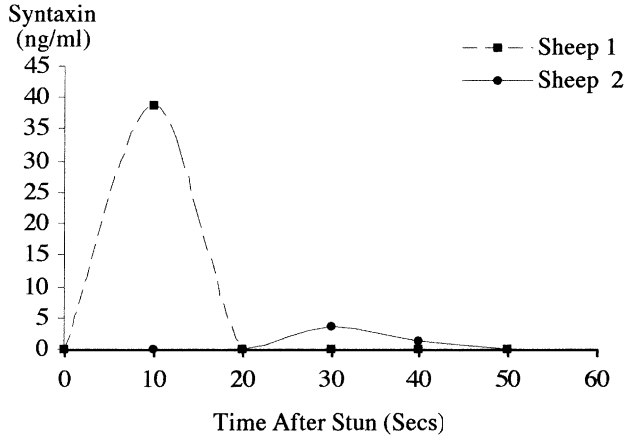

Fig. 3. Venous blood syntaxin 1-B levels in sheep stunned with a pneumatically activated penetrating CBG. Reproduced by permission of *Veterinary Record*.

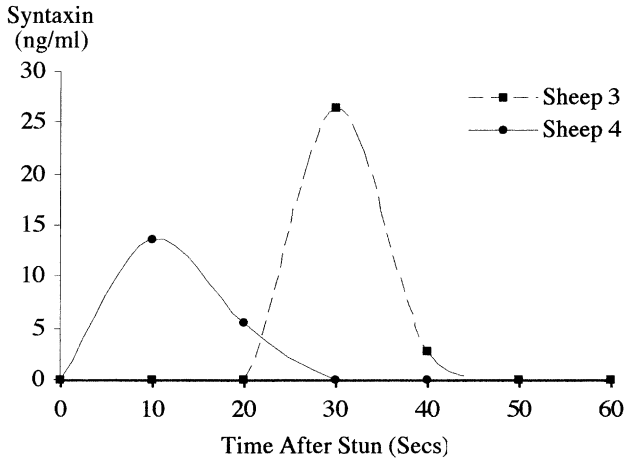

Fig. 4. Venous blood syntaxin 1-B levels in sheep stunned with a cartridge-operated penetrating CBG. Reproduced by permission of *Veterinary Record*.

of CNS tissue. CNS tissue was not detected in any of the 10 ml arterial samples that were analysed.

4. Discussion

Several methods have been used to identify proteins of presumed CNS origin in the peripheral blood, particularly after head injury or stroke. However, none of these methods has been shown to be entirely specific and none distinguishes between the entry of CNS tissue or simply of the selected neural proteins into the blood. In view of the possible embolic dissemination of non-CNS tissues, such as skin, muscle and bone marrow, after penetrating CBG stunning, it was important to devise a method of assessing the risk of embolism that allowed unequivocal characterisation of the embolic material. We believe that the concentration of embolic brain tissue within Cytoblocks of buffy coat material allows this characterisation, by a combination of morphological examination and multiple immunocytochemical stains on adjacent sections through the same tissue fragment. The technique has a further advantage for risk assessment in relation to BSE, in that one can make direct measurements of the size of the embolic fragments and thereby obtain an indication of the possibility of their being able to pass through the pulmonary capillary bed and into the systemic circulation. In the present study we have found antibody to S100β protein to be particularly useful for identifying CNS tissue immunocytochemically. S100β is a calcium binding protein that is abundantly but not exclusively expressed in nervous tissue (Barger & Van Eldik, 1992) and other antibodies or combinations of antibodies could be used to obtain more detailed characterisation of embolic material.

The results of the morphological and immunocytochemical approach to detecting embolic CNS material were complemented by the capture ELISA for syntaxin 1-B. The assay has proven to be a sensitive means of detecting this presynaptic protein within the blood. We have previously shown that the assay is not affected by contamination with muscle and that a positive result cannot be attributed to the presence of other tissues such as liver, lung or bone marrow within the specimen (Anil et al., 1999; Love et al., 2000). Comparison of ELISA readings obtained from homogenates of brain and spinal cord tissue shows the concentration of syntaxin to be approximately 50- to 100-fold lower in cord than brain (Anil et al., 1999; Love et al., 2000). The levels of syntaxin in intercostal muscle, psoas muscle, midflank muscle, dorsal line muscle, liver, lung and sternal marrow are below the threshold for detection with this assay.

The close concordance between the results of the ELISA and microscopy provides reassurance as to their specificity. The fact that a single animal yielded a blood sample after stunning that was positive for syntaxin but was not found to contain fragments of CNS tissue on microscopy and immunocytochemistry may reflect the greater sensitivity of the syntaxin assay. It is, in addition, possible that stunning may occasionally cause the leakage of neural proteins across the blood-brain barrier without actual embolisation of intact tissue fragments. Breakdown of the blood-brain barrier has certainly been demonstrated after experimental fluid-percussion brain injury, which does not cause physical disruption of blood vessels and would not be expected to lead to embolism of tissue fragments (Tanno, Nockels, Pitts, & Noble, 1992).

Although our primary aim was to develop a rational and reliable approach to assessment of the risk embolic dissemination of CNS material during the stunning and slaughter of cattle and sheep, the techniques may well find use in other contexts, such as in assessing the severity of brain damage after head injury in man.

The demonstration of a causal relationship between the agent of BSE and vCJD (Ironside, 1998), and the report of brain emboli in the lungs of cattle slaughtered with a paCBG (Garland et al., 1996) caused concern that the removal of specified risk materials from slaughtered animals may not be sufficient to remove completely the risk of transmission of BSE prions to humans through meat consumption. These results confirm that there is a risk of embolic dissemination of brain tissue with the use of the pneumatically operated air injection gun and show, in addition, that neuroembolism can also occur with use of a conventional penetrating CBG followed by pithing. This practice, used in 70% of UK abattoirs (Meat Hygiene Service, 1997), is now banned in the whole of the European Union. The emboli are detectable in jugular venous blood within 30 s of stun-

ning and will already have passed into and, possibly, through the lungs before exsanguination is carried out (at about 90 s). These findings are in keeping with observations of embolic brain tissue in the lungs of human victims of head injury (McMillan, 1956; Hatfield & Challa, 1980) and of elevated neuron-specific enolase and BB creatine kinase in the serum (Skogseid, Nordby, Urdal, Paus, & Lilleaas, 1992). The question as to whether or not emboli reach the arterial circulation and are deposited in edible tissues needs further detailed investigation. Although in this study CNS tissue was not detected in any aortic blood sample, the number of animals tested was small and only 10 ml of blood was taken for testing. It is noteworthy that the showers of embolic brain tissue include many fragments of sufficiently small size to be capable, in principle, of passing through the pulmonary capillary bed.

When penetrating captive bolt stunning (CBS) is used, the bolt trajectory causes considerable damage. We have, in a preliminary investigation, examined brains of several cattle stunned with a penetrating captive bolt (cow puncher, Accles and Shelvoke). We estimate that an average of 10 g of brain tissue (out of a total of 450 g) can be dislodged (unpublished results). This figure represents, approximately, the maximum potential load of prion-contaminated tissue that might be disseminated haematogenously as a result of using a CBG on an animal with BSE. Maignien, Lasmezas, Beringue, Dormont, and Deslys (1999) reported infectivity of $10^{9.3}$ LD_{50} per g of brain tissue from C57BL/6 mice infected with the 4PBI BSE strain. This figure represents the LD_{50} dose for infection by intracerebral injection. However, it has been calculated that the dose required for oral transmission is considerably higher than that needed for transmission by intracerebral inoculation. Prusiner, Cochran, and Alpers (1985) and Kimberlin and Walker (1989) reported that between 10^5 and 10^9 times as much infectivity is required for the oral route of infection. On the basis of these criteria, we have calculated that between 50 μg and 500 mg of brain tissue should be sufficient for transmission of infection by the oral route. Therefore, 10 g of dislodged brain tissue may represent between 20 and 20,000 units of infectivity.

Although most sheep for slaughter are electrically stunned, CBS is still widely used in the UK. According to the Meat Hygiene Service (1997), CBS is used in as many as 38% of sheep. There are two principal types of CBG in popular use: cartridge-operated (i.e., conventional) and pneumatically operated guns. The former has its bolt activated by the explosion of a blank cartridge, whereas the latter is connected to an airline supplying pressure between 90 and 120 psi to eject the bolt. The cartridge size and air pressure can be adjusted according to the size and age of animals. Although the sheep used in this study were old and had thicker skulls than is the case for younger slaughter lambs, we used relatively modest 1.5 grain cartridges and 100 psi pressures for stunning the sheep, as would be used for the stunning of younger animals. Therefore, the finding of embolic CNS tissue in jugular venous blood cannot be attributed to the use of excessive stunning force.

The positive samples were taken from the jugular vein draining the head. The emboli would almost certainly reach the lungs. This was already demonstrated in cattle by Garland et al. (1996) and our results are also in keeping with the demonstration of embolic brain tissue in the lungs of humans who had received head injury (McMillan, 1956; Hatfield & Challa, 1980). Since some of the emboli in the present study were smaller than the calibre of most capillaries, it is likely that some of the embolic tissue can pass through the pulmonary vasculature and through the left side of the heart to the visceral and other edible parts. We found no evidence of emboli in the arterial blood that we took from the aorta but this probably reflects the small size of the blood samples and the restricted timing of the sampling. Clearly, more detailed investigations are required to determine whether or not emboli reach the systemic arterial circulation and the edible parts of the carcass.

These present results indicate that CBS, widely used in the UK, can cause haematogenous dissemination of CBG tissue and has the potential for contamination of sheep carcasses. The possible infection of sheep with BSE is a cause for concern (Bone, 2000). Although, scrapie, the prevalent prion disease in sheep is not known to carry any risk to man, we do not yet know whether or not its clinical and pathological manifestations are distinguishable from BSE in sheep. The incubation period of prion diseases such as scrapie and experimentally induced BSE appears to be shorter in sheep than in cattle. Foster, Hope, and Fraser (1993) showed that the incubation period of BSE in sheep after oral inoculation was from 440 days. Therefore, CNS infectivity may be present in animals around one year of age, when carcasses are most likely to be used for human consumption.

In conclusion, the results confirm the risk of haematogenous dissemination of CNS tissue with the use of the pneumatically operated gun and show, in addition, that neuroembolism can also occur when use of a conventional penetrating CBG is followed by pithing. However, the question as to whether or not emboli can reach arterial circulation and are deposited in edible tissues needs further study. Future investigations are planned to determine the risk of neural embolism associated with different methods of stunning. The possible dissemination of CNS tissue to the edible parts of the carcass will also be examined in more detail. In the interim, a review of procedures for the stunning and slaughter of ruminants in UK abattoirs may be appropriate.

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