ELSEVIER

Food Control 13 (2002) 425–430

FOOD CONTROL

www.elsevier.com/locate/foodcont

Spread of microbial contamination associated with penetrative captive bolt stunning of food animals

S. Buncic *, J. McKinstry, C.-A. Reid, M.H. Anil

Department of Clinical Veterinary Science, University of Bristol, Division of Food Animal Science, Langford, Bristol BS40 5DU, UK

Received 4 April 2001; received in revised form 25 May 2001; accepted 29 May 2001

Abstract

To determine whether penetrative stunning of animals can result in internal and/or external microbial contamination of meat, experimental animals (lambs) were inoculated with a marker organisms (nalidixic acid resistant *Escherichia coli* K12 or *Pseudomonas fluorescens*) into the brain through the stun wound immediately after stunning by a cartridge-operated, penetrative captive bolt pistol. After the animals were slaughtered and dressed, the marker organisms were found, on average, in blood and liver of 90% animals, lungs and spleen of 80%, lymph nodes of 30%, in deep muscle of 20% and on carcass surface of 50% of brain-inoculated animals. When the pistol which had been used to stun one brain-inoculated lamb was used to stun consecutive, non-inoculated lambs, the marker organisms were found, on average, in stun wounds of 100%, in blood of 30% and on the carcass surface of 40% consecutively stunned animals. No marker organisms were detected in their muscle or organs. When the stun spot on the surface of the head of some lambs was inoculated with marker organism and subsequently stunned through the same spot, the marker organism was detected in the air, on hands, and aprons of the worker in the stunning area. Overall, the results from this study indicate that penetrative stunning of food animals can carry risks of internal and/or external microbial contamination of edible tissues and organs.

Keywords: Stunning; Meat contamination; Pathogens; Lambs

1. Introduction

The coat of live animals is contaminated with pathogenic bacteria relatively frequently (Elder et al., 2000; Reid, Avery, Small, & Buncic, 2000) and is the principal source of carcass contamination (Mackey & Roberts, 1993; Bell, 1997; Biss & Hathaway, 1995; Gill & Baker, 1998; Duffy, LeValley, Belk, Sofos, & Smith, 2000). Therefore, any operations during slaughter and dressing of food animals that involve penetration of the contaminated skin, such as when making initial cuts for skinning, carry a risk of introducing pathogenic bacteria from the skin onto/into edible parts of the animal. Also, it has been demonstrated that bacterial contamination of edible tissues, via blood circulation, can occur if contaminated knife is used for sticking (Jensen, 1945; Mackey & Derrick, 1979; Labadie, Gouet, & Fournaud, 1977). Consequently, sterilization of sticking knives in hot water before sticking has become a regulatory requirement. Other examples include penetration of skin (and the scull) during penetrative stunning of cattle and sheep. Concern has been expressed about the potential human health risks associated with pneumatic penetrative stunning of bovines, in case of dissemination of BSE agents from the brain of infected animals to edible tissues via blood circulation. Initial reports indicated that brain tissue emboli can be found in the lungs of slaughtered cattle (Garland, Bauer, & Bailey, 1996), while other reports questioned the validity of those findings (Munro, 1997; Taylor, 1996). More recent reports confirmed a possibility of dissemination of central nervous tissue material associated with penetrative stunning of cattle (Anil et al., 1999, 2001; Love et al., 2000; Schmidt, Hossner, Yemm, & Gould, 1999), and consequently the use of penetrative captive bolt air compression (i.e. pneumatic) stunning is not recommended. However, the question remains whether any penetrative stunning of animals, even if non-pneumatic, can result in the spread of pathogenic bacteria from the animal coat or the brain throughout the body of the same animal, or from one

* Corresponding author. Tel.: +44-117-928-9410; fax: +44-117-928-9324.

E-mail address: sava.buncic@bris.ac.uk (S. Buncic).

0956-7135/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0956-7135(01)00054-8

slaughtered animal to others. In an earlier study, the marker bacteria that had been heavily inoculated on bolt of an penetrative captive bolt pistol were subsequently found in spleens, but not in muscles, of stunned animals (Mackey & Derrick, 1979). However, this observation was based on a relatively small number of animals and, also, the study did not address the issues of stunning-associated spread of the contamination to the abattoir environment and/or to consecutively slaughtered animals. Since even contamination of meat with a single cell of, for example, *Escherichia coli* O157 is considered as a health risk, and since penetrative stunning pistols are not sterilized between animals, microbial meat safety implications of penetrative stunning need to be further investigated.

Therefore, the aim of the present study was to determine whether, and how, penetrative stunning of animals can result in internal and/or external contamination of edible tissues and organs.

2. Materials and methods

2.1. Marker microorganisms

Non-pathogenic *E. coli* K12, as well as *Pseudomonas fluorescens* ATCC 13525 (obtained from The National Food Centre, Dublin) were used as the marker organisms. Experimental animals were contaminated with pure undiluted cultures of the organisms: nalidixic acid resistant *E. coli* K12 grown overnight at 37°C in 10 ml volumes of heart infusion (HI) broth (Lab M, Bury, UK; Lab 49) and nalidixic acid resistant *Ps. fluorescens* grown overnight at 30°C in Tryptose soya broth (TSB). In each of the two final broth cultures, the count of the marker organism was approximately 9 $\log_{10}$ CFU/ml.

2.2. Stunning of animals

All lambs were stunned in a usual manner, by using a penetrative cartridge-operated captive bolt pistol (Accles and Shelvoke).

2.3. Treatments of animals

Total of 31 lambs were divided into four groups: A, B, C, and D.

2.3.1. Group A – Investigation of spread of the penetrative stunning-associated contamination within the same animal

Ten sheep were divided in two subgroups (five sheep each). Within the first subgroup (A1), each animal was stunned by penetrative captive bolt pistol and immediately inoculated with *E. coli* K12 by injecting 2 ml of the broth culture through the stun wound into the brain. Within the second subgroup (A2), the same treatment was repeated but using *Ps. fluorescens* for the inoculation. After stunning and inoculation, all animals were slaughtered and dressed in a normal manner, and the presence of inoculated organisms was determined in samples of tissues and organs (see below).

2.3.2. Group B – Investigation of spread of the penetrative stunning-associated contamination to the environment

Five sheep were externally contaminated by swabbing the stun spot area (approximately 4 cm^2) on the head with a cotton swab (Jumbo swabs; Medical Wireand Equipment, Bath, Corsham, UK; 104J) soaked in the broth culture organism, and immediately stunning the animals through the contaminated spot. Subsequently, using 50 cm^2 templates and double (wet/dry) swabbing technique, swabs were taken from: (a) aprons, hands and nose cavity of the slaughtermen involved with the stunning of inoculated animals; and (b) the pelt of the stunned animals (leg, breast and shoulder), and (c) from the carcasses of the animals after slaughter and de-pelting. The air, around the stunning site, was sampled by four portable air sampling units (Patent No. 8819423.8, Burkand Manufacturing, Woodcock Hill Industrial Estate, Richmanworth, Hertfordshire WD3 1PJ), each loaded with plates (90 mm diameter) of MacConkey agar no. 3 (Oxoid CM 115) containing 200 ppm nalidixic acid (Sigma N8878). The units were placed 30 mm above floor level to the right and left of each animal's head at distances of 0.5 and 1.0 m. Each unit sampled 100 l of air over a 5-min period starting at the moment of stunning. In parallel, a settle plate method was used by placing one horizontal exposure plate of MacConkey agar no. 3 (containing 200 ppm nalidixic acid) at each of the same four sites (see above) during the same 5 min period.

2.3.3. Group C – Investigation of spread of the penetrative stunning-associated contamination from one animal to consecutively stunned animals

Twelve sheep were divided in two subgroups (six sheep each). Within the first subgroup (C1), one sheep was stunned by penetrative captive bolt pistol and immediately inoculated with *E. coli* K12 by injecting 2 ml of the broth culture through the stun wound into the brain. Stunning was repeated through the same stun wound of the same animal. The same stunning same pistol was used to stun five consecutive sheep (which were not inoculated) without any cleaning/decontamination. Within the second subgroup of sheep (C2), the same treatment was repeated but using *Ps. fluorescens* broth culture for the inoculation. All animals were slaughtered and dressed in a normal manner, and the presence of inoculated

marker organisms was determined in aseptically collected samples of tissues and organs (see below).

2.3.4. Group D – control animals

Four lambs served as a control. They were not inoculated with marker organisms, but were stunned, slaughtered and dressed before animals from groups A, B and C, in an identical manner. Samples of tissues and organs were examined for the presence of the marker organisms (see below).

2.4. Sampling of slaughtered animals

Samples of blood (25 ml) were collected from each animal as mid-stream exsanguination samples into sterile screw-capped bottles containing 500 units of heparin and examined by enrichment and direct plating methods (see below). Portions of liver, spleen, *M. longissimus dorsii*, lung, kidney and whole lymph node (*Lnn illiacus medialis*) were taken from each animal and placed into sterile plastic bags. In the laboratory, the surface of each sample was decontaminated by submerging in boiling water for 2 s. Preliminary experiments confirmed that this procedure was effective in eliminating any marker organisms present on the sample surface. Using sterile scissors and forceps, a piece (10 g) was cut from the cores of each sample and placed in a stomacher bag, weighted, chopped and homogenized with 50 ml maximum recovery diluent (MRD) (Lab M, Bury, UK; Lab103) by stomaching for 1 min.

2.5. Determination of E. coli K12 in samples

2.5.1. Enrichment method

Tissue sample homogenate or blood sample (25 ml amounts) (see above) was added to 225 ml volumes of single strength HI broth, and incubated for 18–24 h at 37°C. The swabs were broken off into 10 ml volumes of single strength HI broths and incubated at 37°C for 18–24 h. From each enrichment broth, loopfuls (0.01 ml) were subsequently streaked onto MacConkey agar No. 3 containing 200 ppm nalidixic acid. The agar plates were then incubated at 37°C for 24 h.

2.5.2. Direct plating method

Each tissue or blood sample homogenate was diluted tenfold in MRD. The swabs were broken off into 10 ml volumes of MRD with beads, homogenated by vortexing for 1 min, and diluted tenfold in MRD. From each homogenate/dilution, 0.2 ml volumes were plated in duplicate onto MacConkey agar No. 3 containing 200 ppm nalidixic acid. These plates, as well as plates used for air sampling (see above), were incubated at 37°C for 24 h and typical *E. coli* K12 colonies counted.

2.6. Determination of Ps. fluorescens ATCC 13525 in samples

2.6.1. Enrichment method

Tissue sample homogenate or blood sample (25 ml amounts) (see above) was added to 225 ml volumes of single strength TSB broth and incubated for 18–24 h at 30°C. The swabs were broken off into 10 ml volumes of single strength TSB broth and incubated for 18–24 h at 30°C. After enrichment, loopfuls (0.01 ml) were streaked onto Pseudomonas CFC selective agar (Oxoid CM559) containing a supplement (Oxoid SR103) and 10 μg ml^{-1} nalidixic acid. The plates were incubated at 30°C for 48 h and examined under UV light.

2.6.2. Direct plating method

Each tissue or blood sample homogenate was diluted tenfold in MRD. The swabs were broken off into 10 ml volumes of MRD with beads, homogenated by vortexing for 1 min, and diluted tenfold in MRD. From each homogenate/dilution, 0.2 ml volumes were plated onto Pseudomonas CFC selective agar (Oxoid CM559) containing a supplement (Oxoid SR103) and 10 μg ml^{-1} nalidixic acid. The plates were incubated at 30°C for 3 days and typical *Ps. fluorescens* colonies counted under UV light.

3. Results and discussion

3.1. Spread of penetrative stunning-associated contamination within individual animal

As it was previously described, a group of 10 lambs were stunned and immediately inoculated into the brain by a marker organism, half of them with *E. coli* while another half with *Ps. fluorescens*. The results of the subsequent microbiological examination (Fig. 1) clearly

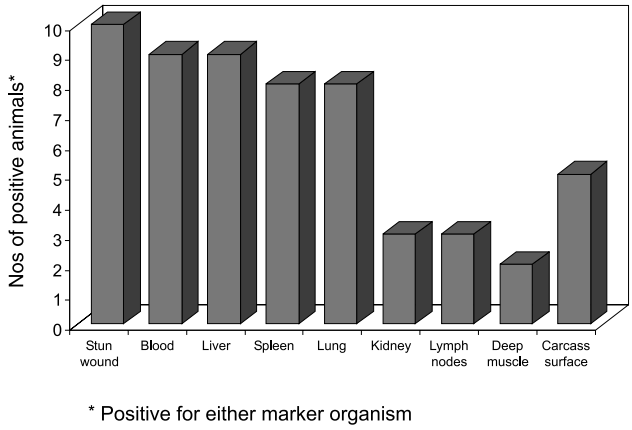

Fig. 1. Spread of the marker organisms (*E. coli* and/or *Ps. fluorescence*) in 10 stunned and brain-inoculated lambs.

demonstrate that microbial contamination associated with penetrative stunning of sheep can be spread from the brain to the edible parts of the same animal, including muscles, via blood circulation. The stunning-associated contamination of muscles observed in the present study is in disagreement with results of Mackey and Derrick (1979) who did not find any marker bacteria in muscles following stunning of sheep by inoculated stunning pistol. This difference is probably due to different experimental conditions, and particularly to the fact that these authors inoculated a "paste" of pelleted bacterial cells onto the bolt before its penetration through the skull, while in the present study liquid bacterial broth culture was inoculated post-stunning directly into the brain. It could be assumed that the "uptake" of bacteria into severed brain blood vessels is higher, and distribution of bacteria within body circulatory system is more homogenous, if bacterial inoculum is in liquid form rather than in "solid" form. Nevertheless, in the present study, positive detection (by enrichment method) and/or total counts (by plate method) of the marker organisms in edible parts of these animals varied considerably between individual animals, between types of tissues/organs and between types of the marker organisms (results not shown). These variations could be contributed to several variable experimental factors including: (a) differences in stunning-associated damages of brain blood vessels between individual animals, which could cause different counts of the bacteria to enter the blood circulation, (b) differences in post-stunning rate and/or duration of heart activity between individual animals, which could cause different transfer of the bacteria through the animal bodies, and (c) differences in volume/mass of blood, tissues and organs between individual animals, which could result in different "dilution" factors for the counts of the marker organism.

Nevertheless, when the results for all inoculated lambs in this group are considered, regardless of the type of the marker organism used, it is clear that the blood and the liver were most commonly contaminated (in 90% animals, each), followed by the lungs and spleen (in 80% animals, each). In contrast, the marker organisms were found less frequently in lymph nodes (in 30% animals), deep muscles (in 20% animals) and on carcass surface (in 50% animals). Possible explanations for this include higher volumes of blood supply in, and/or less complete blood elimination from, internal organs during exsanguination, as compared with muscles. Consequently, muscles normally containing little residual blood (Warriss & Rhodes, 1977) would have contained very low counts of the marker organism, in some animals below the detection limit of the methods used. On the other hand, some literature data indicate that blood and edible tissues can exhibit certain bactericidal effects even post-mortem (Labadie et al., 1977; Gill, 1979; Gill, Penney, & Wauters, 1981). However, due to the lack of detailed information on exact extents of these effects in different tissues of different animal species, and/or against different bacterial species over different periods of time, it is not possible to assess whether, and to what extent, such antimicrobial factors influenced the results of present study.

3.2. Spread of penetrative stunning-associated contamination to the environment

The results indicate that surface contamination of the stun spot in lambs stunned by penetrative stunning method can result in significant spread of the microorganism throughout the environment (Table 1). It is likely that the initial transfer route for this spread was aerosols created at the moment of the bolt penetrative of the contaminated skin i.e. the skull of the animal, as the marker organism was found in one-third of the air samples from the area. It has been demonstrated that the air, in general, can serve as a vector for spread of significant levels of microorganisms in abattoirs (Worfel, Sofos, Smith, & Scmidt, 1996). Understandably, the slaughterman conducting the stunning became surface contaminated (hands, apron) with the marker organism. Although he was inhaling the air in this area, the marker organism was not detected inside his nose.

The marker organisms were also found on the pelt of the stunned animals. The most likely sources of these markers were the air, contaminated slaughterman's hands, or both. As expected, the contamination of both the environment and the pelt resulted in surface contamination of some of the carcasses (50%) during depelting of these animals (Fig. 1). This is in agreement with previous studies which indicated that a major source of carcass contamination is associated with skinning operations (Biss & Hathaway, 1995; Gill & Baker, 1998; Duffy et al., 2000).

3.3. Spread of penetrative stunning-associated contamination from one animal to consecutive animals

To investigate the potential for the transfer of microbial contamination between animals via a contaminated penetrative stunning pistol, a stunned lamb inoculated with a marker organism into the brain was re-stunned and the stunning pistol was used to stun several consecutive lambs, without any cleaning. When results from the two identical experiments (but with different marker organisms) are considered, it is clear that a contaminated stunning pistol can serve as a vector for the transfer of microbial contamination between animals (Fig. 2). When an average of the two experiments is taken, the microorganism present in the brain of the first animal can be transferred to the stun wounds of 100%, to the blood of 30%, and to the carcass surface of 40% of consecutively stunned animals by the

Table 1
Spread of penetrating stunning-associated contamination to the environment

Environmental samples taken during stunning of five inoculated sheep		Samples (%) positive for the marker organism
Air	Samples ($n = 20$) taken 0.5 m from the stunned animals	10
	Samples ($n = 20$) taken 1.0 m from the stunned animal	30
Pelt of the sheep	Swabs ($n = 10$) from leg	–
	Swabs ($n = 10$) from breast	20
	Swabs ($n = 10$) from shoulder	40
The slaughterman sampled after stunning of each sheep	Swabs from hands ($n = 10$)	80
	Swabs from apron ($n = 10$)	40
	Swabs from nose cavity ($n = 10$)	–

same contaminated pistol. The finding of the marker organisms in blood of consecutive animals confirmed the role of the internal route of contamination.

However, in spite of being present (in low numbers) in the blood of some of these consecutive animals, the marker organism was not found in their tissues and organs. This is probably the consequence of a significant, further "dilution" of the marker organism cells spread throughout the body mass of these animals. Ultimately, this probably resulted in a decrease of the bacterial counts per g of the tissues/organs, such that levels dropped below the detection limit.

When taken that levels of bacteria internally contaminating edible tissues may correlate to the number of bacterial cells introduced to the brain during penetrative stunning, an additional factor should be taken into account. Namely, the penetrative stunning pistol is always contaminated, visibly or invisibly, with a certain amount of organic material from the skin/brain of stunned animals. As the stunning pistol is never cleaned or sterilized under commercial conditions during normal working hours, the potential for growth of pathogenic bacteria in or on the gun should also be considered. There is no doubt that, the higher the level of bacteria present on the penetrative parts of the gun, the higher the associated meat safety risks.

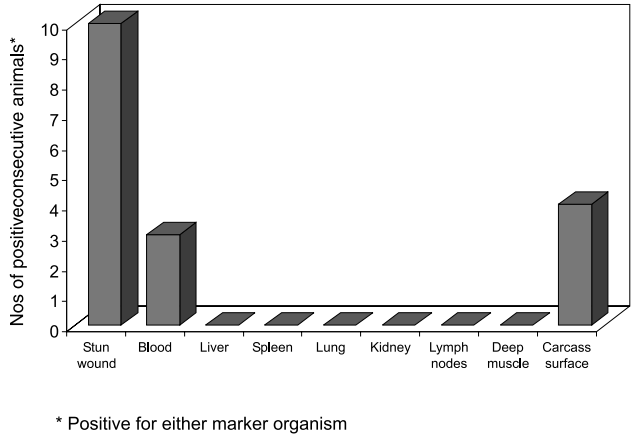

Fig. 2. Spread of the marker organisms (*E. coli* and/or *Ps. fluorescence*) from one brain-inoculated lamb to consecutively stunned, non-inoculated lambs via penetrative stunning pistol.

3.4. Meat safety implications of the results

The present study demonstrated that use of contaminated penetrative captive bolt pistol for stunning of food animals can result in bacterial contamination of edible tissues (both organs and muscles). However, relatively high levels of bacteria experimentally inoculated into the brain do not necessarily reflect actual (probably lower) levels of bacterial contamination of stunning pistol under commercial conditions. Therefore, the present study provides a proof that microbial meat safety risks associated with penetrative stunning of food animals exist, but the results do not enable quantification of these risks.

However, even if a very low number of stunning-associated bacteria in edible tissues are expected, it should be kept in mind that in the case of some pathogens (e.g. *E. coli* O157), even contamination of meat with a single cell is considered a health risk. This concern is further enhanced by the fact that health risks posed by pathogenic bacterial cells present in deep parts of a meat cut (due to internal contamination via penetrative stunning) can be higher than those of the bacterial cells present on the meat surface. Namely, the meat surface is normally cooked to a higher temperatures than the meat center (e.g. in case of beefsteak) so any pathogens in the center have a better chance to survive. On the other hand, one could argue that very low levels of bacteria can be eliminated from meat by its natural antimicrobial mechanisms. However, available information on bactericidal effects of meat relate to marker and/or spoilage microorganisms (Labadie et al., 1977; Gill, 1979; Gill et al., 1981) rather than to pathogens and, also, meat safety systems cannot rely on these effects due to their inherent high variability. Also, it could be speculated that some modern meat technologies, e.g. carcass electrostimulation-hot boning-immediate freezing regimes, would have significantly shortened time available for these effects to take place.

Penetrative stunning pistols are not sterilized between animals normally, and it is unlikely that it would have been practical under commercial conditions. Therefore, risks of spreading harmful agents between animals via penetrative stunning have to be considered as significant. These risks are not limited only to the BSE agent, but also include: (a) enteric pathogens (e.g. *E. coli* O157) that can contaminate the stunning spot on animals head due to the close proximity of animals during transport or in the lairage; and/or (b) brain-infection organisms (e.g. *L. monocytogenes*).

Therefore, although further research would be necessary to assess the actual extent of penetrative stunning-associated microbial contamination of meat in commercial abattoirs, the results of the present study indicate that even non-pneumatic, cartridge-operated penetrative stunning is not satisfactory from a meat safety perspective.

Acknowledgements

The study was partially funded by the EU (project REMCORD).

References

Anil, M. H., Love, S., Williams, S., Shand, A., McKinstry, J. L., Helps, C. R., Waterman-pearson, A., Seghatchian, J., & Harbour, D. A. (1999). Potential contamination of beef carcasses with brain tissue at slaughter. *Veterinary Record*, *145*, 460–462.

Anil, M. H., Love, S., Helps, C. R., McKinstry, J. L., Brown, S. N., Philips, A., Williams, S., Shand, A., Bakirel, T., & Harbour, D. A. (2001). Jugular venous emboli of brain tissue induced in sheep by use of captive bolt guns. *Veterinary Record*, *148*, 619–620.

Bell, R. G. (1997). Distribution and sources of microbial contamination of beef carcasses. *Journal of Applied Microbiology*, *82*, 292–300.

Biss, M. E., & Hathaway, S. C. (1995). Microbiological and visible contamination of lamb carcasses according to preslaughter presentation status: implications for HACCP. *Journal of Food Protection*, *58*, 776–783.

Duffy, E. A., LeValley, S. B., Belk, K. E., Sofos, J. N., & Smith, G. C. (2000). Pre-harvest management practices, good manufacturing practices during harvest, and microbiological quality of lamb carcasses. *Dairy, Food and Environmental Sanitation*, *20*, 753–762.

Elder, R. O., Keen, J. E., Siragusa, G. R., Barkocy-Gallagher, G. A., Koohmaraie, M, & Laegreid, W. W. (2000). Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *PNAS*, *97*, 2999–3003.

Garland, T., Bauer, N., & Bailey, Jr. M., (1996). Brain emboli in the lungs of cattle after stunning. *Lancet*, *348*, 610.

Gill, C. O. (1979). A review: intrinsic bacteria in meat. *Journal of Applied Bacteriology*, *47*, 367–378.

Gill, C. O., Penney, N., & Wauters, A. M. (1981). Survival of clostridial spores in animal tissues. *Applied and Environmental Microbiology*, *41*, 90–92.

Gill, C. O., & Baker, L. P. (1998). Assessment of the hygienic performance of a sheep carcass dressing process. *Journal of Food Protection*, *140*, 570–574.

Jensen, L. B. (1945). *Microbiology of meat* (2nd ed.). Champaign II: Garrand Press.

Labadie, J., Gouet, Ph., & Fournaud, J. (1977). Blood poisoning at slaughter and their consequences. *Zbl. Bakt. Hyg., I. Abt. Orig. B*, *164*, 397–411.

Love, S., Helps, C. R., Williams, S., Shand, A., McKinstry, J. L., Brown, S. N., Harbour, D. A., & 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., & Derrick, C. M. (1979). Contamination of deep tissues of carcasses by bacteria present on the slaughter instruments or in the gut. *Journal of Applied Bacteriology*, *46*, 355–366.

Mackey, B. M., & Roberts, T. A. (1993). Improving slaughter hygiene using HACCP and monitoring. *Fleischwirtschaft*, *75*, 58–61.

Munro, R. (1997). Neural tissue embolism in cattle. *Veterinary Record*, *140*, 536.

Reid, C.-A., Avery, S., Small, A., & Buncic, S. (2000). Presence of food-borne pathogens on cattle hides. In *The food safety objective conference: Public health, HACCP and science*, Georgetown, 4–5 December 2000.

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

Taylor, K. C. (1996). Brain emboli in the lungs of cattle. *Lancet*, *348*, 749.

Warriss, P. D., & Rhodes, D. N. (1977). Haemoglobin concentrations in beef. *Journal of Sciences in Food and Agriculture*, *28*, 931.

Worfel, R., Sofos, J. N., Smith, G. C., & Scmidt, G. R. (1996). Airbone bacterial contamination in beef slaughtering-dressing plants with different layouts. *Dairy, Food and Environmental Sanitation*, *16*, 440–443.