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Presence of food-borne pathogens on cattle hides

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Abstract

The hide of cattle is known to be a source for the microbial contamination of beef, with microorganisms transferred onto the carcass from the hide, during the slaughter and dressing processes. To assess the potential risk of carcass contamination from food-borne bacterial pathogens on cattle hides, a study was carried out involving 90 beef cattle in the South-West of England to determine the prevalence of these microorganisms. A one-pass swab technique was carried out to sample a measured area on the rump, flank and brisket of each animal. These swabs were processed in the laboratory to determine the prevalence of *E. coli* O157, *Salmonella* spp., and *Campylobacter* spp. on these hide areas. The most contaminated area was the brisket with one in five animals testing positive for *E. coli* O157 (22.2% prevalence on average) and approximately one in 10 animals testing positive for *Salmonella* spp. (10.0% prevalence on average). The least contaminated area on the cattle hides was the rump area (3.3% prevalence for *E. coli* O157, 2.2% prevalence for *Salmonella* spp.). *Campylobacter* spp. was not isolated from any samples taken from the 90 cattle studied. The results of this study indicate that the brisket area on the hide of cattle most frequently carries food-borne pathogens and is therefore most likely to lead to cross-contamination of beef during the de-hiding process.

Keywords: Carcass contamination; Food-borne pathogens; Cattle hides

1. Introduction

The contamination of beef during the slaughter and processing of carcasses is a major risk for subsequent food-borne infection in humans. The most dominant pathogens involved i.e. *E. coli* O157, *Salmonella* spp., and *Campylobacter* spp., are carried in the guts of cattle and shed in the faeces of the animals (Chapman, Siddons, Cerdan Malo, & Harkin, 1997; Fedorka-Cray, Dargatz, Thomas, & Gray, 1998; Wesley et al., 2000). The prevalence of these pathogens in cattle has been extensively studied in recent years. Typical prevalence rates for *E. coli* O157 in cattle, of between 1.0% and 27.8% (up to 68% in heifers), have been reported (Chapman et al., 1997; Cízek et al., 1999; Elder et al., 2000; Hancock, Besser, Rice, Herriott, & Tarr, 1997; Hancock, Besser, & Rice, 1998; McDonough et al., 2000; Mechie, Chapman, & Siddons, 1997), with prevalence rates reported for *Salmonella* spp. and *Campylobacter* spp. of 5.5% and 5.0–53.0%, respectively (Fedorka-Cray et al., 1998; Wesley et al., 2000). Prevalence rates of these pathogens, however, are subject to influences such as seasonal variation (Hancock et al., 1997; Mechie et al., 1997; Wesley et al., 2000).

Numerous factors interact to affect the level of hide contamination on animals presented for slaughter (Davies, Hadley, Stosic, & Webster, 2000) which can then affect the microbiological content of the resulting carcass. The degree of visible contamination on the hide has been shown to affect the degree of subsequent contamination of the resultant carcass (McEvoy et al., 2000; Newton, Harrison, & Wauters, 1978; Ridell & Korkeala, 1993). However, a visibly clean hide may not necessarily be pathogen free, and may still result in a potential hazard for cross-contamination of the resulting carcass. Elder et al. (2000) reported 10.7% incidence of *E. coli* O157 on cattle hides in USA. It is likely that the contamination can be spread from such contaminated animals to other animals during transport and lairaging, either directly via body contact (particularly flank and rump areas), or indirectly via contact with contaminated floors/surfaces (particularly brisket area), or both. Subsequently, major risks of hide-to-carcass transfer of the

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microbial contamination exists. The contributing factors during de-hiding include making initial cuts through the skin (particularly at brisket area), alternate use of the same hand for handling the hide itself and the carcass surface, and roll-back of the hide during the process (Bell, 1997; Hudson, Mead, & Hinton, 1998).

The aim of this study was to evaluate the potential risk of hide-to-carcass contamination during the slaughter of cattle in the summer months, by determining the prevalence of the major food-borne pathogens *E. coli* O157, *Salmonella* spp. and *Campylobacter* spp., on the rump, flank and brisket of cattle hides immediately after slaughter.

2. Materials and methods

2.1. Survey of cattle

Three abattoirs within the South-West of England, dealing with beef cattle, were sampled once each, within the months of July–August. At each sampling visit, 30 randomly selected beef cattle (grass-fed, average slaughter weight 500 kg) were sampled from the rump, flank and brisket areas, immediately after bleeding out but prior to de-hiding, using a one-pass swab technique.

2.2. Sampling procedure

General sponge cloths measuring 20×18 were purchased from a local supermarket and cut in half widthwise. Each sponge piece (swab) was folded in half and placed in a sterile stomacher bag, with the folded edge facing the bag opening. Each swab was moistened immediately prior to use with maximum recovery diluent (MRD; Oxoid; 10 ml). The bottom of each swab was grasped through the stomacher bag, and the stomacher bag pulled over the hand, to reveal the folded edge of the swab. A metal template with an internal area measuring 10×10 cm was sterilised using alcohol and held onto the area of the cattle hide to be swabbed. The folded end of the swab was pressed onto the template area and passed from left to right in one motion. The stomacher bag was then pulled back over the hand, encasing the swab inside. All swabs were kept on ice and transported back to the laboratory within 2 h of collection.

2.3. Microbiological analysis

2.3.1. Processing of swabs

In the laboratory the swabs were processed by adding 90 ml MRD to each stomacher bag, and repeatedly squeezed by hand for 1 min to release any microorganisms present on the surface. From this 90, 25 ml was added to 225 ml tryptone soya broth base (Oxoid) containing bile salts, K_2HPO_4 (Sigma, 1.5 g/l) and 5 mg/ml novobiocin (Sigma); 25 ml into 225 ml Park Sanders broth (brucella broth, Difco, 28 g/l; sodium citrate, Sigma, 1 g/l; sodium pyruvate, Sigma, 0.25 g/l) containing 1 ml reconstituted Supplement A (Mast Diagnostics) and 11.8 ml lysed horse blood (Oxoid); and 10 ml into 240 ml buffered peptone water (Oxoid), for the enrichment of *E. coli* O157, *Campylobacter* spp. and *Salmonella* spp., respectively.

2.3.2. Isolation of E. coli O157

After enrichment for 24 h at 41.5°C, the isolation of *E. coli* O157 was carried out using the immunomagnetic separation (IMS) technique (Chapman, Wright, & Siddons, 1994; Wright, Chapman, & Siddons, 1994). Samples were plated onto sorbitol MacConkey agar containing cefixime (Dynal, 25 μl/500 ml) and potassium tellurite (Dynal, 25 μl/500 ml) (CT) and sorbitol MacConkey agar containing cefixime (Dynal, 25 μl/500 ml) and rhamnose (Sigma; 2.5 g/500 ml) (CR). Plates were incubated for 24 h at 37°C, with colourless colonies and colonies with dark pink centres picked and plated onto plate count agar for further examination. Suspect colonies were confirmed by latex agglutination using an *E. coli* O157 test kit (Oxoid).

2.3.3. Isolation of Campylobacter spp.

The Park Sanders broths were enriched for 4 h at 32°C, and 2 ml reconstituted Supplement B added (Mast Diagnostics). The broths were incubated at 37°C for 2 h, and then moved to 42°C for 40–42 h. After incubation, colonies were streaked onto mCCDA (Oxoid) plates containing selective supplement SR155E (Oxoid) and nutritive blood gelatine plates (4.75 g beef extract (Oxoid), 4.75 g bacteriological peptone (Oxoid), 2.38 g sodium chloride (Sigma), 6.40 g agar technical (Oxoid), 475 ml distilled water – sterilised by autoclaving at 121°C for 15 min, 1 vial SR084 supplement (Oxoid) and 25 ml lysed horse blood (Oxoid) added prior to pouring). Plates were incubated for 48 h at 37°C and 2–5 d at 42°C, respectively, under the Campygen gas system (Oxoid). Suspect colonies were confirmed by catalase, oxidase, Gram staining and negative growth at 25°C.

2.3.4. Isolation of Salmonella spp.

After enrichment of the buffered peptone water for 16–24 h at 37°C, 0.1 ml was transferred to 10 ml Rappaport Vassiliadis broth (RV, Oxoid) and 10 ml transferred to 100 ml selenite cysteine broth (E & O laboratories). The broths were incubated at 42°C and 37°C, respectively, for 24 h. Aliquots from each broth were spread, in duplicate, onto XLD (Oxoid) and brilliant green agar (BGA, Oxoid) plates, and incubated at 37°C for 24 h. After plating, the selenite cysteine broths were returned to the incubator for further 24 h, and plated out as described above. Suspect colonies on either

XLD or BGA were confirmed using Gram staining, catalase, oxidase and urease tests, poly O and H antisera (Prolab) and Api 20E kits (Biomerieux).

3. Results and discussion

The results of the microbiological examination of hide swabs from commercially slaughtered bovines for the presence of *E. coli* O157, *Salmonella* spp., and *Campylobacter* spp. are shown in Table 1. Swabs were taken from the rump, flank and brisket areas of the hides of 90 cattle within the South-West of England. Samples were taken immediately after stunning and bleeding, before de-hiding, in order to obtain the most relevant results from the perspective of the risk of carcass contamination.

3.1. Variations related to areas of the hides

On average, *Salmonella* spp. and *E. coli* O157 were found on hide on rump area in 2.2% and 3.3%, respectively, of slaughtered bovines. The flank area of the hide was more frequently contaminated with the pathogens, i.e., the incidence of *Salmonella* spp. and *E. coli* O157 on the flank was 4- and 1.25-fold, respectively, higher than on the rump area. This higher incidence of the pathogens on hide on the flank is probably due to the fact that the flank is a horizontally prominent area on the side of cattle, and therefore comes into direct contact with other animals and vertical surfaces (walls, rails, etc.) more easily. However, the results of this study clearly show that brisket is the area on hide most heavily contaminated with food-borne pathogens. On this area of their coat, on average, more than one in five slaughtered bovines carried *E. coli* O157 (22.2%), and one in 10 animals carried *Salmonella* spp. The contamination of the brisket hide area occurs when animals lie down on contaminated ground/floor either on the farm, during transportation, in lairage, and/or within the stunning box. The average incidence of *E. coli* O157 on the brisket hide area in this study is double that (10.7%) recently reported in USA (Elder et al., 2000). Both studies were carried out in the summer months, i.e., peak prevalence season, and both studies also used comparable methods of isolation. The marked differences in the results may be due to a variety of factors which can potentially affect hide contamination, including faecal shedding-, farming systems-, transport-, and lairage-related conditions.

3.2. Variations related to the abattoirs

Incidences of cattle hide contamination with *E. coli* O157 and *Salmonella* spp. differed between individual abattoirs within, approximately, a 2-fold range (Table 1). No attempts to find precise explanations for these differences were made in this study. Nevertheless, it can be assumed that such differences between abattoirs are caused by multiple factors. Firstly, cattle slaughtered at each of the abattoirs studied, originated from different farms that could easily have had differences in the prevalence of pathogen-shedding of animals. Secondly, it is possible that duration and hygienic conditions during farm-to-abattoir transport (and related levels of hide contamination) also differed between cattle slaughtered at individual abattoirs. Thirdly, physical design of lairages, as well as practices and hygienic conditions along unloading-to-stunning areas, differed significantly between the abattoirs. Numerous routes for the potential spread of pathogens from contaminated cattle hides exists. Those which have been identified include animal-to-animal, animal-to-lairage, and environment-to-animal (Bell, 1997; Small, Reid, Avery, & Buncic, 2000). Significant differences in the overall levels of microbial contamination of cattle hides between abattoirs have been observed in a recent study, where total bacterial counts of bacteria on hides ranged between 8.2 and 12.5 log CFU/100 cm^2 (Bacon et al., 2000).

3.3. Variations related to the pathogens

Overall, among the organisms tested, *E. coli* O157 was the most prevalent pathogen found on the cattle hides (28.8% of the animals tested), followed by *Salmonella* spp. (17.7% of the animals tested). Although it is known that healthy cattle are both reservoirs and excretors of *Campylobacter* spp. (Wesley et al., 2000), this pathogen was not found on any animal hide examined. This may be due to differences in faecal shedding of individual pathogens between cattle tested, or differences in survival rates of individual pathogens on hide and/or animal-related environments, or both.

Table 1
Average incidence of *E. coli* O157, *Salmonella* spp. and *Campylobacter* spp. on hide of bovines[a] slaughtered at three abattoirs

	Rump	Flank	Brisket
E. coli O157	3.3% (0–6.6%)	4.4% (3.3–6.6%)	22.2% (16.6–30.0%)
Salmonella spp.	2.2% (0–6.6%)	8.8% (0–16.6%)	10.0% (3.3–13.3%)
Campylobacter spp.	Not detected at any site		

[a] $n = 90$; percentages within parentheses show the lowest and the highest incidences found in individual abattoirs.

Numerous studies have shown that prevalences of pathogens shed from animal faeces can vary significantly, both within or between pathogenic species, e.g., 5–53% for *Campylobacter* spp., 5.5% for *Salmonella* spp. and 1–11% for *E. coli* O157, have been reported (Fedorka-Cray et al., 1998; Hancock et al., 1997; Mechie et al., 1997; Wesley et al., 2000). Elder et al. (2000) stated that good correlation can sometimes be observed on individual cattle between *E. coli* O157 isolation rates between faecal and hide samples. However, data on survival rates of individual microbial pathogens on cattle hide prior to slaughter, under various commercial conditions, are lacking. It is also possible that *Campylobacter*, which are relatively fragile outside the host, may die off more rapidly on a dry hide than *E. coli* O157 and *Salmonella* spp., which could have contributed to the "zero" prevalence found in this study.

3.4. Meat safety implications of the results

In the UK, all cattle are assessed for visible cleanliness before slaughter using a 5-point scoring system (Meat Hygiene Service), with lower scores (1–2) given to visibly clean/dry animals and the higher scores (4–5) given to excessively dirty (mud, faeces) and wet animals. The later groups are banned for slaughter as they are considered to pose a high risk of carcass contamination during dressing. In spite of the fact that all animals included in the current study were considered as reasonably "visibly clean" (scores 1–2), their hides were very frequently contaminated with pathogens, particularly *E. coli* O157.

This study indicates that of the three hide areas examined in the current study, the brisket area may pose the greatest risk for contamination of the resulting carcass, for two reasons. Firstly, the results from this, and previous studies, have shown that it is a frequently contaminated area on the hide (Elder et al., 2000). Secondly, the initial cut line during de-hiding passes centrally through the brisket. The probability of transferring pathogens from hide to carcass during de-hiding has to be assessed as considerable. Contamination at this point may occur via contaminated hands/equipment, the freed hide rolling back onto the carcass (termed "in-rolling"), or both. In the study carried out by Elder et al. (2000), no correlation was shown between the prevalence of *E. coli* O157 contamination on hides and that on the resulting carcasses. In this study, the prevalence of pathogens on the carcasses was higher than that on the hides (Elder et al., 2000). However, there is little doubt that increased pathogen contamination on the hides of incoming cattle results in at least an increased prevalence of pathogens in the abattoir/slaughterline environment, overall. Inevitably, this increases the risks of direct- or cross-contamination of the carcasses (Byrne, Bolton, Sheridan, McDowell, & Blair, 2000). Further research is necessary to determine whether and how meat safety can be improved via elimination/reduction of microbial contamination on hides prior to slaughter, as this approach would address the fundamental causes of meat contamination rather than dealing with the consequences once the meat is contaminated.

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References

Bacon, R. T., Belk, K. E., Sofos, J. N., Clayton, R. P., Reagan, J. O., & Smith, G. C. (2000). Microbial populations on animal hides and beef carcasses at different stages of slaughter in plants employing multiple-sequential interventions for decontamination. *Journal of Food Protection*, *63*, 1080–1086.

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

Byrne, C. M., Bolton, D. J., Sheridan, J. J., McDowell, D. A., & Blair, I. S. (2000). The effects of preslaughter washing on the reduction of *E. coli* O157:H7 transfer from cattle hides to carcasses during slaughter. *Letters in Applied Microbiology*, *30*, 142–145.

Chapman, P. A., Siddons, C. A., Cerdan Malo, A. T., & Harkin, M. A. (1997). A 1- year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. *Epidemiology and Infection*, *119*, 245–250.

Chapman, P. A., Wright, D. J., & Siddons, C. A. (1994). A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from bovine faeces. *Journal of Medical Microbiology*, *40*, 424–427.

Cízek, A., Alexa, P., Literák, I., Hamrík, J., Novák, P., & Smola, J. (1999). Shiga toxin-producing *Escherichia coli* O157 in feedlot cattle and Norwegian rats from a large-scale farm. *Letters in Applied Microbiology*, *28*, 435–439.

Davies, M. H., Hadley, P. J., Stosic, P. J., & Webster, S. D. (2000). Production factors that influence the hygienic condition of finished beef cattle. *Veterinary record*, *146*, 179–183.

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 feaces, hides, and carcasses of beef cattle during processing. *Proceedings of the National Academy of Science*, *97*, 2999–3003.

Fedorka-Cray, P. J., Dargatz, D. A., Thomas, L. A., & Gray, J. T. (1998). Survey of *Salmonella* serotypes in feedlot cattle. *Journal of Food Protection*, *61*, 525–530.

Hancock, D. D., Besser, T. E., & Rice, D. H. (1998). Ecology of *Eschericia coli* O157:H7 in cattle and impact of management practices. In J. B. Kaper, & A. D. O'Brien (Eds.), *Escherichia coli and other shiga toxin producing E. coli strains* (pp. 85–91). Washington, DC: American Society for Microbiology.

Hancock, D. D., Besser, T. E., Rice, D. H., Herriott, D. E., & Tarr, P. I. (1997). A longitudinal study of *E. coli* O157 in fourteen cattle herds. *Epidemiology and Infection*, *118*, 193–195.

Hudson, W. R., Mead, G. C., & Hinton, M. (1998). Assessing abattoir hygiene with a marker organism. *Veterinary Record*, *142*, 542–547.

McDonough, P. L., Rossiter, C. A., Rebhun, R. B., Stehman, S. M., Lein, D. H., & Shin, S. J. (2000). Prevalence of *Escherichia coli* O157:H7 from cull dairy cows in New York state and comparison of culture methods used during preharvest food safety investigations. *Journal of Clinical Microbiology*, *38*, 318–322.

McEvoy, J. M., Doherty, A. M., Finnerty, M., Sheridan, J. J., McGuire, L., Blair, I. S., MacDowell, D. A., & Harrington, D. (2000). The relationship between hide cleanliness and bacterial numbers on beef carcasses at a commercial abattoir. *Letters in Applied Microbiology*, *30*, 390–395.

Mechie, S. C., Chapman, P. A., & Siddons, C. A. (1997). A fifteen month study of *Escherichia coli* O157:H7 in a dairy herd. *Epidemiology and Infection*, *118*, 17–25.

Newton, K. G., Harrison, J. C. L., & Wauters, A. M. (1978). Sources of psychrotrophic bacteria on meat at the abattoir. *Journal of Applied Bacteriology*, *45*, 75–82.

Ridell, J., & Korkeala, H. (1993). Special treatment during slaughter in Finland of cattle carrying an excessive load of dung. *Meat Science*, *35*, 223–228.

Small, A., Reid, C.-A., Avery, S., Buncic, S. (2000). Potential for the spread of food-borne pathogens in the cattle lairage environment. In *Proceedings of the first euroconference on food safety assurance and veterinary public health: Event 1 food safety assurance in the pre-harvest phase* (p. 86). Vienna, Austria.

Wesley, I. V., Wells, S. J., Harmon, K. M., Green, A., Schroeder-Tucker, L., Glover, M., & Siddique, I. (2000). Fecal shedding of *Campylobacter* and *Arcobacter* spp. in dairy cattle. *Applied and Environmental Microbiology*, *66*, 1994–2000.

Wright, D. J., Chapman, P. A., & Siddons, C. A. (1994). Immunomagnetic separation as a sensitive method for isolating *Escherichia coli* O157 from food samples. *Epidemiology and Infection*, *113*, 31–39.