ELSEVIER

Food Control 13 (2002) 405–410

FOOD CONTROL

www.elsevier.com/locate/foodcont

Evaluation of sampling methods to assess the microbiological status of cattle hides

C.-A. Reid [a], S.M. Avery [a], M.L. Hutchison [b], S. Buncic [a,*]

[a] Department of Clinical Veterinary Science, Division of Food Animal Science, University of Bristol, Langford, Bristol BS40 5DU, UK
[b] Microbiology Department, ADAS Woodthorne, Wolverhampton WV6 8TQ, UK

Received 3 September 2001; received in revised form 11 October 2001; accepted 11 October 2001

Abstract

The study focused on the performance of selected sampling methods for recovery of: (a) marker organism (nalidixic acid resistant *E. coli* K12) topically inoculated onto hide of experimental cattle (to achieve $>6\log_{10}$ CFU/cm^2), and (b) naturally occurring microflora from hide of experimental cattle. Recoveries of bacteria by each of five consecutive passes using wet sponge swabbing of the hide was determined, and compared with bacteria remaining on the hair (recovered by taking hair-clippings). These results showed that: (i) the highest recovery ($5.52\log_{10}$ CFU/cm^2) of the topically inoculated marker organism was achieved by the first pass of the wet sponge swabbing technique, (ii) significant levels of the marker organism ($5.15\log_{10}$ CFU/cm^2) remained on the hair even after five consecutive swabbing passes, and (iii) the highest recovery of natural flora (from non-inoculated hide) was achieved by the hair-clipping sampling technique.

Recoveries of both the marker organism (topically inoculated on hide) and the natural flora (non-inoculated hide) by each of the three hide sampling techniques used (single-pass wet swab, wet–dry swab, and electric hair-clipping) were determined. The results showed that: (i) the efficacy of the hide sampling techniques in recovery of topically inoculated marker organism was in the order: wet–dry swabbing > single-pass wet swabbing > hair-clipping, and (ii) the efficacy of the sampling techniques in recovery of natural flora from non-inoculated hide was, in the order of the highest to lowest efficiency; hair-clipping > wet–dry swabbing > single-pass wet swabbing. From a practical perspective of the microbiological monitoring of hide under commercial abattoir conditions, swabbing techniques are more acceptable than clipping techniques. However, further research is necessary to optimise and validate microbiological sampling techniques for animal coats.

Keywords: Hide microbiology; Hide sampling; Dehiding; Foodborne pathogens; Meat

1. Introduction

It is widely recognised that contamination of cattle hides with faecal material leads to contamination of the carcass, via direct or indirect routes, with enteric pathogens during the dehiding process (Sheridan, 1998). Therefore, from a meat safety perspective, obtaining information on the actual microbial status of hides at the moment of dehiding is very relevant.

Levels of naturally occurring bacteria (i.e., total viable counts) on cattle hides and lamb pelts at abattoirs are commonly $>6\log$ CFU/cm^2 (Bacon et al., 2000; Duffy et al., 2000; Gracey and Collins, 1992). In the case of bovine carcasses, contamination with *E. coli* O157 is of particular concern. Recent studies have shown that the incidence of this pathogen on the brisket area of hides of cattle presented for slaughter can be as high as 11% (Elder et al., 2000) or 22% (Reid, Small, Avery, & Buncic, 2002). It is becoming clear that more and more attention needs to be paid to microbiological monitoring of cattle hides in the context of an integrated meat safety system.

For the microbiological sampling of carcasses, various evaluations of numerous methods have been published, with tissue excision methods and swabbing methods generally considered as the most accurate and the most practical, respectively (Gill and Jones, 2000; Stephan, 1997). On the other hand, the information on methods for microbiological sampling of animal coats is scarce.

* Corresponding author. Tel.: +44-117-928-9410; fax: +44-117-928-9324.
E-mail address: sava.buncic@bristol.ac.uk (S. Buncic).

0956-7135/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0956-7135(01)00093-7

In a few related studies (Bacon et al., 2000; Duffy et al., 2000), cattle hides or lamb pelts were sampled by use of the official swabbing technique developed for the sampling of carcasses (FSIS-USDA, 1996). The technique involves repeated swabbing (10 horizontal and 10 vertical passes) of a given area (100 cm^2) by the same wetted sponge. In other studies, cattle hide was sampled by a single-pass swabbing with wetted sponges (Reid et al., 2002) or by wetted gauze pads (Elder et al., 2000). However, detailed studies of actual recoveries of the microflora from cattle hides achieved by different methods, and their comparison, are lacking.

Therefore, the present study was conducted in order to: (a) assess bacterial recoveries achieved by consecutive passes during wet swabbing of cattle hide, and (b) compare bacterial recoveries from cattle hide achieved by single-pass wet swabbing, wet–dry swabbing and hair-clipping techniques.

2. Materials and methods

2.1. Inoculation of hide of experimental cattle

A broth culture of the marker organism *E. coli* K12 (resistant to 200 ppm nalidixic acid) was prepared by incubation in heart infusion broth (HI; Oxoid) at 37 °C for 24 h. Seven adult cattle (approximately 400–500 kg) in total were used in the study. Friesian cattle was used for hide surface-swabbing sampling, and Galloway for sampling of hair by clipping. Areas of approximately 2000 cm^2 were marked on hide (on sides) of these animals. Approximately half of these areas were topically inoculated with the culture of the marker organism using a paintbrush to work the organism onto the hide surface as uniformly as possible. Previous pilot experiments confirmed that this would result in surface contamination of the hide by the marker organism at the level of $>6 \log_{10}$ CFU/cm^2. The inoculated areas were left approximately 5 min to dry before any sampling was conducted. The remaining marked areas on hides of experimental cattle were not inoculated but were sampled to assess recovery of natural microflora by the same methods.

2.2. Sampling

The experimental/sampling protocol used in this study is shown in Table 1 (see below). In all cases, a square 100 cm^2 metal template was sterilised using alcohol, and held onto a given area of the cattle hide to be sampled.

2.2.1. Wet sponge swab sampling technique

General sponge cloths (20×18 cm^2) containing no antimicrobial additives were purchased from a local supermarket. Each sponge was cut into half widthwise, and each piece autoclaved at 121 °C for 15 min, dried, folded in half and placed in a sterile stomacher bag with the folded edge facing the bag opening. Each sponge swab was moistened, within the bag and immediately prior to use, with 10 ml of maximum recovery diluent (MRD; Oxoid). 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. The folded end of the swab was pressed onto the 100 cm^2 template area and passed from left to right in one motion, i.e., one-pass. The stomacher bag was then pulled back over the hand, encasing the swab inside, and kept chilled until sample processing. This area was swabbed further four times (i.e., by four consecutive passes) using the procedure detailed above and using a fresh swab each time.

2.2.2. Wet–dry sponge swab sampling technique

A sponge swab was prepared as described in Section 2.2.1, except that 15 ml MRD was added to moisten the sponge immediately prior to use. The 100 cm^2 template area was swabbed from left to right in one motion (i.e., single-pass) and placed in a sterile stomacher bag. The same area was then swabbed as above using another, dry (non-moistened) sponge swab, and this second swab added to the same bag as the wet swab.

2.2.3. Electrical hair-clipping sampling technique

The hair from the 100 cm^2 template area was clipped using Oster Professional clippers and a blade designed to cut to a depth of 1–1.5 mm from the skin surface, and collected into a sterile stomacher bag.

2.2.4. Manual hair-clipping sampling technique

The hair from the 100 cm^2 template area was cut manually using sterile scissors, and collected in a sterile stomacher bag.

2.2.5. Dehaired-skin swab sampling technique

The hair was first clipped from the 100 cm^2 template area to a depth of 1–1.5 mm from the skin surface by an electrical clipper (see Section 2.2.3), removed, and subsequently the same (dehaired) area was sampled by the one-pass wet sponge swab technique (see Section 2.2.1).

2.3. Microbiological analysis

Immediately after sampling, all samples were placed in a cool box on ice and transported to the laboratory within 2 h of sampling. In the laboratory, MRD was added to the samples in the stomacher bags; 90 ml to the swabs and 50 ml to the hair-clipping samples. Each sample was homogenised for 30 s using a Colworth Stomacher (type 400 for the swab samples, type 80 for the hair samples). Serial 10-fold dilutions were

Table 1
The experimental/sampling protocol

For assessment of recoveries of bacteria by consecutive passes		For comparative assessment of performance of swabbing and clipping techniques during wet-sponge technique							
Wet sponge swabbing of hide (multiple passes)		Wet–dry sponge swabs from hide		Wet sponge swabs (single-pass) from hide		Hair samples obtained by electrical clipper		Wet sponge swabs (single-pass) from dehaired-skin after hair-clipping	
A (from 10 sites)	B (from 10 sites)	A (from 15 sites)	B (from 15 sites)	A (from 10 sites)	B (from 10 sites)	A (from 15 sites)	B (from 15 sites)	A (from 15 sites)	B (from 15 sites)
Five consecutive passes separately taken from each site ($n = 50$)	Five consecutive passes separately taken from each site ($n = 50$)	Separate stomaching of each sample							
Final manual hair-clipping samples from each of the previously swabbed sites ($n = 10$)	Final manual hair-clipping samples from each of the previously swabbed sites ($n = 10$)	Determination of *E. coli* K12 counts (in samples A) or natural flora counts (in samples B)							
Separate stomaching of each of the samples		Comparison of sampling techniques in recovery of bacteria							
Determination of *E. coli* K12 counts	Determination of natural flora counts								
Comparison of recoveries between consecutive passes, for both inoculated and natural microfloras									

A – samples from left side of the animals (inoculated with *E. coli* K12); B – samples from right side of the animals (non-inoculated).

performed in MRD and from each dilution a 0.01 ml volume plated, using a modification of the calibrated loop method (Roberts et al., 1980) onto duplicate quarter sections of: (a) plate count agar (PCA, Oxoid) for enumeration of the natural microflora, and (b) MacConkey no 3 agar (Oxoid) containing 200 ppm of nalidixic acid for enumeration of the marker organism *E. coli* K12. Volume of 0.2 ml of each of the undiluted homogenates (i.e., neat samples) were also plated onto the same type of media, but at a level of 0.2 ml in order to detect low levels of the organisms. PCA and Mac Conkey agar plates were incubated at 30 °C/3 d and at 37 °C/24 h, respectively, and the colonies were counted. Calculation of the final counts was as described for decimal dilutions in BS EN ISO 6887 (if they were). The results are shown as mean $\log_{10}$ CFU/cm^2 determined at 10 or 15 different 100 cm^2 sampling sites (as indicated in Table 1).

3. Results and discussion

3.1. Recovery of bacteria from hide by consecutive passes during wet sponge swabbing

Results of the assessment of recoveries of bacteria by individual, consecutive passes during wet sponge swabbing of hide are shown in Fig. 1.

When considering recovery of a topically inoculated marker organism, it is clear that the average recovery achieved by the first swabbing pass (5.52 $\log_{10}$ CFU/cm^2) was significantly higher ($P < 0.05$) than average recoveries obtained by any of the four consecutive passes (ranging 3.87–5.02 $\log_{10}$ CFU/cm^2). To assess the level of the marker organism remaining on the hide after the five swabbing passes, the hair was clipped from the swabbed area and collected. The average count of the marker organism recovered from that hair was 5.15 $\log_{10}$ CFU/cm^2, i.e., relatively high and similar to the average recoveries from the 2nd to 5th passes, but yet significantly lower ($P < 0.05$) than the average count recovered by the first pass during swabbing.

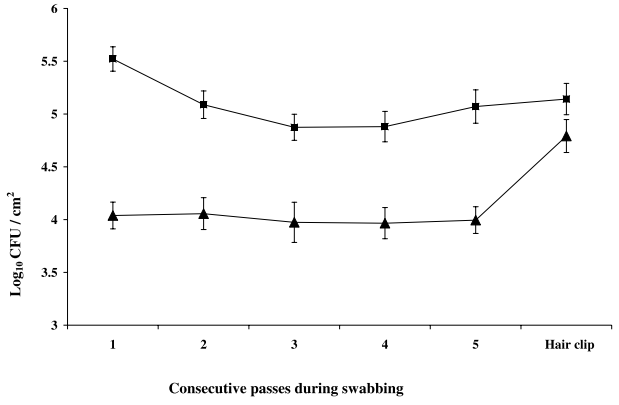

Fig. 1. Average recoveries of bacteria by consecutive passes during wet sponge swabbing of hide and by final hair-clipping (error bars represent SEM of the data): (■) – *E. coli* K12 topically inoculated on hide; (▲) – natural microflora (non-inoculated hide).

Conversely, when considering recovery of the naturally occurring bacteria (from non-inoculated hide), it was observed that the recoveries achieved by any of the five consecutive swabbing passes (ranging 3.96–4.04 $\log_{10}$ CFU/cm^2) were significantly lower ($P < 0.05$) than the recoveries of topically inoculated marker organism (ranging 4.87–5.52 $\log_{10}$ CFU/cm^2). Also, recoveries of natural flora by any of the five consecutive passes were significantly lower ($P < 0.05$) than the average level of the remaining bacteria found post-swabbing on the clipped hair (4.79 $\log_{10}$ CFU/cm^2).

When bacterial counts determined by all the five consecutive swabbing passes and by hair-clipping (at a given sampling site) were added, and the resulting sum expressed as 100%, then it could be calculated that the first pass yielded 35% and 10% of the marker organism and natural flora isolated, respectively.

Generally, it can be taken that swabbing techniques recover bacteria primarily present on the hide surface. When considering physical aspects of the collection of bacteria by sponge swab technique, one should be aware that two-way transfer of bacteria (from hide to sponge and from sponge to hide) can occur simultaneously during repeated swabbing of the same area. It could be speculated, therefore, that if the bacteria had the same "attachment affinity" for the hide and for the sponge, then the final distribution of the bacterial population after multiple swabbing passes would have been 50% on hide and 50% on sponge. In such a case, inherently, no bacterial recoveries >50% could have been expected from the swabbing technique. However, exact "attachment affinities" of various hide microorganisms for different swab materials are not known.

3.2. Comparison of bacterial recoveries achieved by swabbing and clipping techniques

Results of a comparative assessment of the performance of different hide sampling techniques are shown in Fig. 2.

When considering recovery of the topically inoculated marker organism from hide, it is clear that average recovery achieved by the wet–dry swabbing technique (5.95 $\log_{10}$ CFU/cm^2) was significantly higher ($P < 0.05$) than the recovery achieved by single-pass wet swabbing (5.52 $\log_{10}$ CFU/cm^2). The latter produced a higher recovery than the hair clipping technique (5.17 $\log_{10}$ CFU/cm^2).

In contrast, the highest recovery of natural microflora (from non-inoculated hide) was achieved by the hair-

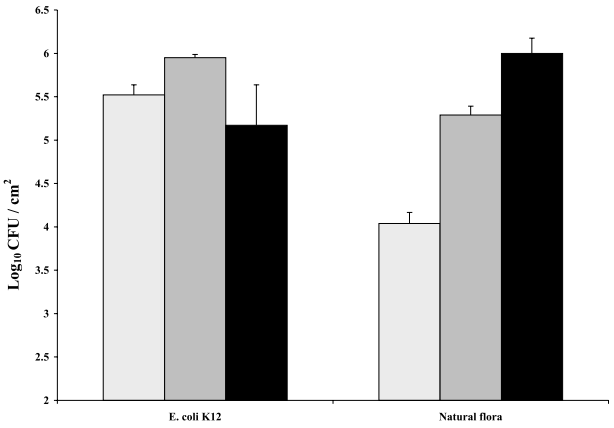

Fig. 2. Comparison of the recoveries of bacteria (topically inoculated *E. coli* O157 and natural flora) from hide by the three sampling techniques used (error bars represent SEM of the data): (□) – wet swabbing (single-pass); (▨) – wet/dry swabbing; (■) – hair-clipping.

clipping technique ($6.00\log_{10}$ CFU/cm^2) followed by wet–dry swabbing ($5.48\log_{10}$ CFU/cm^2) and single-pass wet swabbing ($4.04\log_{10}$ CFU/cm^2).

Nevertheless, even after hair-clipping, significant levels of the marker organism ($4.57\log_{10}$ CFU/cm^2) and natural flora ($6.48\log_{10}$ CFU/cm^2) remained on the surface of the "dehaired" skin (as determined by its wet swabbing).

Overall, the finding that the performance of the hair-clipping technique in the recovery of natural hide flora is superior to the performance of swabbing techniques is understandable and expected. However, lower recovery of topically inoculated marker organism by clipping hair technique, as compared with that achieved by wet–dry swabbing, is difficult to explain. The possible factors contributing to that phenomenon may include the fact that hair-clipping picks up a much heavier load of the natural flora, together with the marker organism, and the former could have suppressed the latter during processing and plating of the samples in the laboratory. In any case, further investigations are necessary to fully explain this aspect of the findings.

3.3. Practical consideration of the results

Generally, microbiological sampling of cattle hides can have two main applications: (a) to assess the level of actual, overall microbial loads on hide as they may not necessarily correlate with the visual assessment of animal cleanliness, and (b) to monitor the incidence/prevalence of foodborne pathogens introduced to slaughter line via hide. The latter is of particular interest, as the hide is frequently contaminated with *E. coli* O157 (Elder et al., 2000; Reid et al., 2002).

In theory, *E. coli* O157 present on the hide can be located either on top of the hair ("surface" contamination), or in a deeper layer of the hide (closer to the skin), or both. From the perspective of meat contamination during dehiding of slaughtered animals, "surface" hide location of *E. coli* O157 might represent a higher meat safety risk than the "deep layer of hide". Namely, transfer of the pathogen from hide onto meat seems to be the easiest, and most likely, via different types of direct surface-to-surface contacts: between hide and carcass, and/or between hide and hands/equipment. Nevertheless, it should be kept in mind that even if *E. coli* O157 is present only in deeper layers of the hide, it still can be transferred onto meat via knife used for opening cuts through the skin during dehiding.

The present study indicates that, if isolation of any *E. coli* O157 located within the hide layers close to the skin was required, then the sampling method of choice would have been hair-clipping. However, this technique is impractical and too laborious for use in routine testing under commercial abattoir conditions, mainly due to difficulties in the "aseptic" collection of clipped hair and/or sterilisation of the electrical clipper between sampling sites and different animals.

Surface hide swabbing techniques are much more practical, but their inherent weaknesses include the fact that they primarily recover bacteria located on tips of hair, and can easily miss those located on lower parts of the hair, i.e., closer to the skin. Therefore, performance of various versions of surface hide swabbing techniques, based on passing a wetted-only sponge over the surface of coats of bovines (Bacon et al., 2000; Duffy et al., 2000; Elder et al., 2000; Reid et al., 2002), could be taken as generally satisfactory as long as more qualitative microbiological data are required. The present study indicates that performance of such surface-swabbing techniques can be further improved if wet swab is immediately followed by a dry swab (i.e., combined wet–dry swabbing technique). Alternatively, where more quantitative data on overall microbial loads present throughout the hide layers are required, a hair-clipping-based sampling technique would have been preferred.

Presently, there are no sampling techniques specifically designed, and officially prescribed, for the microbiological testing of animal coats. Many variable factors can affect the performance of potentially most practical, hide-swabbing-based techniques including hair length, hardened dirt on hair, swab material, number of passes, etc. Obviously, further research is needed for their full optimisation and validation.

Acknowledgements

The study was funded by the UK Food Standards Agency (project reference M01009). Authors wish to thank to Mr. Bill Hudson for the technical help.

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.

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 qulity 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 prevalences in feces, hides, and carcasses of beef cattle during processing. *PNAS*, *97*, 2999–3003.

FSIS-USDA (1996). Pathogen reduction: hazard analysis and critical control points (HACCP) systems: final rule. *Federal Register*, *61*(1444), 38806–38989.

Gill, C. O., & Jones, T. (2000). Microbiological sampling of carcasses by excision or swabbing. *Journal of Food Protection*, *63*, 167–173.

Gracey, J. F., & Collins, D. S. (1992). Meat hygiene practice. In *Meat hygiene* (9th ed., pp. 178–204). London: Bailliere Tindall.

Reid, C.-A., Small, A., Avery, S.M., Buncic, S. (2002). Presence of food-borne pathogens on cattle hides. *Food Control*, Xref: S095671350100050-0.

Roberts, T. A., MacFie, H. J. H., & Hudson, W. R. (1980). The effect of incubation temperature and site of sampling on assessment of the numbers of bacteria on red meat carcasses at commercial abattoirs. *Journal of Hygiene, Cambridge*, *85*, 371–380.

Sheridan, J. J. (1998). Sources of contamination during slaughter and measures for control. *Journal of Food Safety*, *18*, 321–339.

Stephan, R. (1997). Sampling methods for microbial investigations in the red meat industry; An overview. In M. H. Hinton, & C. Rowlings (Eds.), *Factors affecting the microbial quality of meat: Microbial methods for the meat industry* (Vol. 4) (pp. 3–67). Bristol: University of Bristol Press.