Journal of Food Protection, Vol. 67, No. 11, 2004, Pages 2397–2402

Spread of Marker Bacteria from the Hides of Cattle in a Simulated Livestock Market and at an Abattoir

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MS 04-4: Received 14 January 2004/Accepted 7 May 2004

ABSTRACT

The spread of microbial contamination on the hides of beef was investigated at two stages in the meat chain: (i) in a simulated livestock market (''the market'') using 33 animals, and (ii) in the unloading-to-skinning area of a commercial abattoir using 18 animals. At both stages, harmless bacterial markers (nalidixic acid–resistant *Escherichia coli* K-12; rifampicin- and nalidixic acid–resistant *Pseudomonas fluorescens;* and a tetracycline-resistant *E. coli*) were inoculated on the hides of a small number of selected animals, and their transfer to other animals and the environment was examined. At the market, the initial prevalence of animals positive for the hide markers (9.1% in each phase) introduced in the presale pen, sale ring, and postsale pen changed to 39.4, 15.1, and 54.5%, respectively, by the end of the market process. In addition, widespread contamination of the market environment with the hide markers was observed. At the abattoir, the initial prevalence of animals positive for the hide marker (11.1%) inoculated at unloading increased to 100% (hide before skinning) and 88.8% (skinned carcass). In addition, another marker inoculated on environmental surfaces in lairage pens, races, and stunning box was detected on 83.3% (hide before skinning) and 88.8% (skinned carcass). These results, although obtained with a relatively small number of animals, demonstrate that both the livestock market process and the unloading-to-skinning process at abattoirs can facilitate the extensive spread of microbial contamination on hides not just within, but also between, batches of animals.

Cattle presented for slaughter with contaminated coats pose an increased risk for microbial contamination of carcass meat *(3–9, 11, 16).* The average prevalence of *Escherichia coli* O157 on cattle hides at abattoirs can range from 11 to >40% *(2, 4, 6, 16),* and even a prevalence of *Salmonella* of 89% on cattle hides at abattoirs has been reported *(4).* It is widely accepted that carcass tissues in healthy cattle are sterile before the first incision through the hide is made, but the cutting and removal procedures for hides can lead to the transfer of bacteria from the hide onto the carcass meat *(3, 5–9, 16).* Therefore, it can be assumed that any increase in hide contamination with pathogens during the preslaughter period will also increase meat safety risks.

Recent studies have indicated that a significant increase in the prevalence of foodborne pathogens on cattle hides can occur between the farm and slaughter, i.e., during transport and in abattoir lairages *(4),* and it can be presumed that similar occurrences take place in livestock markets. In addition to a stress-induced increased shedding of pathogens, the increase in the prevalence of pathogens on hides can be caused by the unavoidable physical contact between animals and with environmental surfaces during these events, due to a limited space. This can result in direct (animal to animal) or indirect (animal to environment to animal) cross-contamination of hides *(2, 11, 13).* While both modes of contamination can lead to hide contamination in previously pathogen-free animals, the latter is of particular concern because it can mediate cross-contamination within the same *(5, 6)* or between different batches of animals *(1, 2).* Such risks can be significant, because *E. coli* O157 prevalence on some environmental surfaces in the unloading-to-slaughter area in cattle abattoirs can be up to 50%, particularly on pen floors and in stunning boxes *(13).*

Previous studies have focused on the microbial contamination on hides that takes place during transport, in lairages, or in the slaughter hall *(1, 2, 4–9, 11, 13, 14, 16),* but they have not addressed the role of livestock markets—at least not directly. In addition, the assessment of microbial spread on hides has been based on either change in prevalence *(3, 4, 6, 8, 9)* or molecular characterization *(1, 2, 5, 16)* of bacteria. While these studies have provided very valuable information, the nature of their approach is to some extent indirect. Therefore, in the present study, specific bacterial markers (not existing in the animals naturally) were used to directly assess the main routes and the extent of hide cross-contamination that occur at livestock markets and in abattoir lairages.

MATERIALS AND METHODS

Marker organisms. *E. coli* K-12 that was resistant to nalidixic acid (200 μg ml^{-1}) was kindly supplied by Dr. J. Paiba (The Veterinary Laboratory Agency, Weybridge, UK) and used as marker organism 1 (M1; suspension, 4.8×10^6 CFU/ml) for the inoculation of selected animals in both the market and the lairage studies (see below). *E. coli* that was resistant to tetracycline (25

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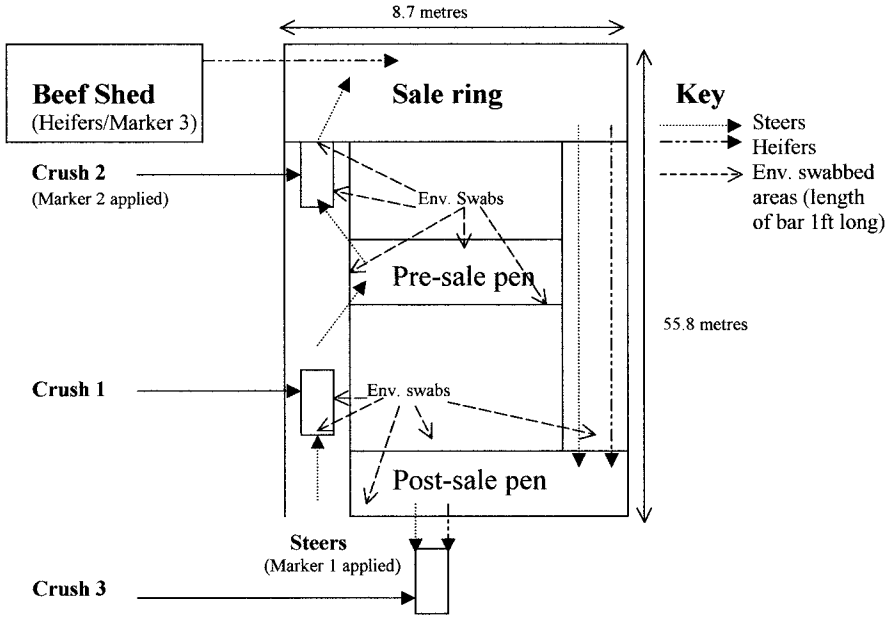
FIGURE 1. *General design of the market study.*



μg ml^{-1}) from the culture collection at The Direct Laboratories Ltd. (originally isolated from pig manure) was used as marker organism 2 (M2; suspension, 5.2×10^6 CFU/ml) for the inoculation of selected animals in the market study (see below). *Pseudomonas fluorescens* that was resistant to nalidixic acid (10 μg ml^{-1}) was kindly supplied by Dr. D. J. Daly (The National Food Centre, Dublin, Ireland); additionally, it was subsequently made resistant to rifampicin (100 μg ml^{-1}; through subculturing on media that contained this antibiotic) at The Direct Laboratories and was used as marker 3 (M3; suspension, 3.9×10^6 CFU/ml) for the inoculation of selected animals in the market study, as well as the environment in the lairage study (see below). Before use, an overnight broth culture of each of the markers was diluted in an appropriate amount of maximum recovery diluent (Oxoid, Basingstoke, UK), to achieve the final concentration.

Inoculation of hide and environments. Hides of selected animals in the market studies were inoculated using a sterile paintbrush as described previously *(10).* Using a sterile 100-cm^2 square metal template, 20 ml of suspension of M1, M2, or M3 organism was evenly spread on areas (100 cm^2 each) on the following regions of animal's body: shoulder, flank, hindquarter, and perianal (Figs. 1 and 2). In the abattoir study, in the same manner, 100-cm^2 areas of hide on the brisket, flank, and rump regions of selected animals were inoculated at unloading. Selected environmental surfaces in both market and abattoir studies were spray inoculated with M3 organism, using a pump sprayer, until visibly wetted.

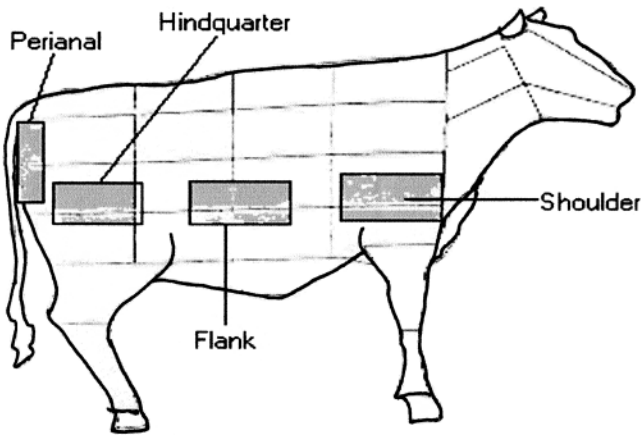


FIGURE 2. *Areas marked and swabbed on the cattle at market.*

Sampling of hides, carcasses, and environment. In the market studies, using sterile metal templates and wetted sponge swabs, samples from the hides of animals were taken from areas (100 cm^2 each) on the shoulder, flank, hindquarter and rump, and perianal regions (Fig. 2) by a one-pass swabbing technique as described previously *(10, 11).* In the abattoir study, surface areas (100 cm^2 each) from hides (postslaughter but preskinning) and the corresponding areas of the carcasses from the brisket, flank, and rump regions were sampled in the same manner. Therefore, in each of the market and abattoir studies, the inoculation and the sampling sites corresponded. In addition, environmental surfaces were swab sampled using the same technique. The swabs were placed in individual stomacher bags and transported on ice to the laboratory.

Microbiological examination of swab samples. Sponge swabs were stomached for 2 min in 90 ml of maximum recovery diluent, and aliquots (25 ml) were transferred to 225 ml of the enrichment medium for the respective marker. Because of technical issues, the market and the abattoir studies were conducted separately and by two different collaborating institutions. As a result, there were minor differences (as indicated below) in the marker organisms and the isolation methods used by the two laboratories. The *E. coli* marker M1 was enriched in brain heart infusion broth (Difco, Becton Dickinson, Sparks, Md.) for the abattoir study or in S/S brilliant green bile broth (Oxoid) for the market study, supplemented with nalidixic acid at 200 μg ml^{-1}, and incubated at 37°C for 24 h. The *E. coli* marker M2 was enriched in S/S brilliant green bile broth (Oxoid) supplemented with 25 μg of tetracycline per ml and incubated at 37°C for 16 to 20 h. The *P. fluorescens* marker (M3) was enriched in Tryptone Soya Broth (Oxoid) for the abattoir study or in *Pseudomonas* broth (Difco) for the market study, supplemented with 100 μg of rifamapicin per ml and 10 μg of nalidixic acid per ml, and incubated at 30°C for 24 h. After enrichment, the markers were plated: (i) marker M1 onto MacConkey agar (no. 3; Oxoid) for the abattoir study or Violet Red Bile Glucose (Oxoid) agar for the market

study—in each case, the sample was supplemented with 200 μg of nalidixic acid per ml; (ii) marker M2 onto Violet Red Bile Glucose agar supplemented with 25 μg of tetracycline per ml; and (iii) marker M3 onto *Pseudomonas* agar (Oxoid) containing glycerol (10 g liter^{-1}; Sigma Chemical Co., St. Louis, Mo.), preweighted CFC supplement (cetrimide, Fucidin, and cephalosporin; Oxoid), and 10 μg of nalidixic acid per ml. The agar plates were incubated at 37°C for 24 h (markers M1 and M2) or at 30°C for 24 to 48 h (marker M3).

Design of simulated livestock market study. The general layout of the simulated livestock market unit (hereafter called ''the market'') is shown in Figure 1. The market was set up using the cattle-handling facilities at the ADAS (Agricultural Development and Advisory Service) Rosemaund (Hereford, UK). The study was composed of three identical trials (11 animals each [33 total]) conducted on separate dates. Between dates, the market was thoroughly cleaned and disinfected, and the surfaces were checked to verify the absence of the marker organisms. In each trial, animals (six Welsh Black steers and five Charolais cross heifers; age, 12 to 14 months) were passed through the chain of events that are typical of commercial UK livestock markets. All animals were unfamiliar with the market environment, and the groups of steers and heifers were unfamiliar with each other. Finally, the results from all three trials were combined.

After being transported (0.5 h) to the market in a livestock transporter, the six steers were unloaded, moved into a race (race 1), and assessed for visual cleanliness using both the official Meat Hygiene Service Clean Livestock Policy five-category scoring system (i.e., categories 1 through 5) and the 35-point grid score method *(12).* Subsequently, the steers were moved individually into a cattle crush (crush 1), where they were held for approximately 1 min to allow time for reading of the ear tag and for weighing. Only one animal (the last one) was surface inoculated with a harmless marker organism M1 (see below). From crush 1, each animal was released into a ''presale'' pen, where the whole group of six steers (one inoculated and five noninoculated) remained for approximately 2 h. From the presale pen, the steers were moved through a race and individually through a crush (crush 2), where they were again visually assessed.

In crush 2, four of the noninoculated steers were surface hide sampled at three sites (shoulder, flank, and hindquarter) on the right side of the body. The fifth noninoculated steer was surface inoculated with a second marker organism M2 (see below) in the same manner as the animal inoculated with marker M1. Subsequently, the six ''familiar'' steers (one inoculated with M1, one inoculated with M2, and four noninoculated) were moved individually and consecutively into a ''sale ring,'' where they spent approximately 1 min, and then were moved to a ''postsale pen.'' During this period, a member of the study staff carried out the vocalization, and a group of people gathered around the pen to simulate the livestock sale process.

Five heifers were visually assessed for cleanliness. One of the heifers was surface inoculated with the third marker organism M3 (see below) in the same manner as the animals that were inoculated with markers M1 and M2. Subsequently, the group of heifers were moved directly through the sale ring to the postsale pen to join the six unfamiliar steers. After 2 h in the postsale pen, all of the animals were moved individually through crush 3, where they were sampled by hide swabbing. The eight noninoculated animals (four steers and four heifers) were sampled at three body sites (shoulder, flank, and hindquarter) on the left side, and the three inoculated animals (two steers and one heifer) were sampled at two sites (flank and perianal). In addition, various surfaces of the market environment (race 1, crushes 1 and 2, presale pen, postsale pen) were sampled within 1 h of animals passing each stage of the marketing process.

Design of abattoir preslaughter area study. From ''typical'' beef cattle arriving for normal slaughter at a commercial cattle abattoir, 18 animals were selected, assessed for cleanliness, and subsequently handled as indicated in Figure 3.

From the livestock transporter, 16 noninoculated animals were moved through the crush and along race 1 to three pens: six animals in pen 3, six animals in pen 2, and four animals in pen 1. In pens 2 and 1 (but not in pen 3), floor-surfaces-railings were previously inoculated with marker M3. After the 16 animals were penned, the two remaining animals were restrained in the crush, surface (hide) inoculated with marker M1, and moved to join the four noninoculated animals in pen 1. The animals remained in the pens for 2 h.

The walls and corners of the race leading to the stunning box, and the stunning box floor and roll-out ramp were inoculated with marker M3. Then, the animals were moved from the pens along race 2 for slaughter. After stunning and bleeding but before skinning, three sites on the hide of the animals were sampled (rump, flank, and brisket). After skinning, corresponding surface areas on the resultant carcasses were also sampled. In addition, the floors and walls of pens 1 and 2, the walls and corners of the race, the floor of the stunning box, and the roll-out ramp were sampled after all of the animals had been slaughtered.

RESULTS AND DISCUSSION

Spread of hide contamination in animals at livestock market. All animals were visually acceptably clean for slaughter, as they were scored between 1 and 2 using the Meat Hygiene Service five-category scoring system and between 9 and 18 using the 35-point grid scoring system. When considering all 33 animals that passed through the market (Table 1), the initial prevalence (9.1%) of animals carrying the markers on their hide increased. The prevalence of the M1 marker, introduced among animals after unloading but before the market process, increased by a factor of 4.3. The prevalence of the M2 marker, introduced at the middle stage of the market (i.e., at the entrance in the sale ring), increased by a factor of 1.7 overall, although it decreased when steers only were considered. The reasons for the latter observation are unclear; it may have been due to a multiple-contacts–mediated ''dilution'' of the marker on the hides, causing the final marker's counts to become too low to be detected. The prevalence of the M3 marker, introduced at the final stage (i.e., during postsale penning), increased by a factor of 6.

The transfer of bacterial hide contamination between inoculated and noninoculated animals was relatively rapid. For example, 33% of the noninoculated steers became surface contaminated with M1 after only 2 h of mixing with inoculated (M1) steers in the presale ring.

Bacterial transfer occurred regardless of whether the animals were familiar with each other before being penned together. For example, after 2 h in the postsale pen, the marker M1, originally introduced only among familiar steers, was also found on 40% of the unfamiliar (and M1 noninoculated) heifers from the same pen. Similarly, in the same pen, the M3 marker, originally introduced only among

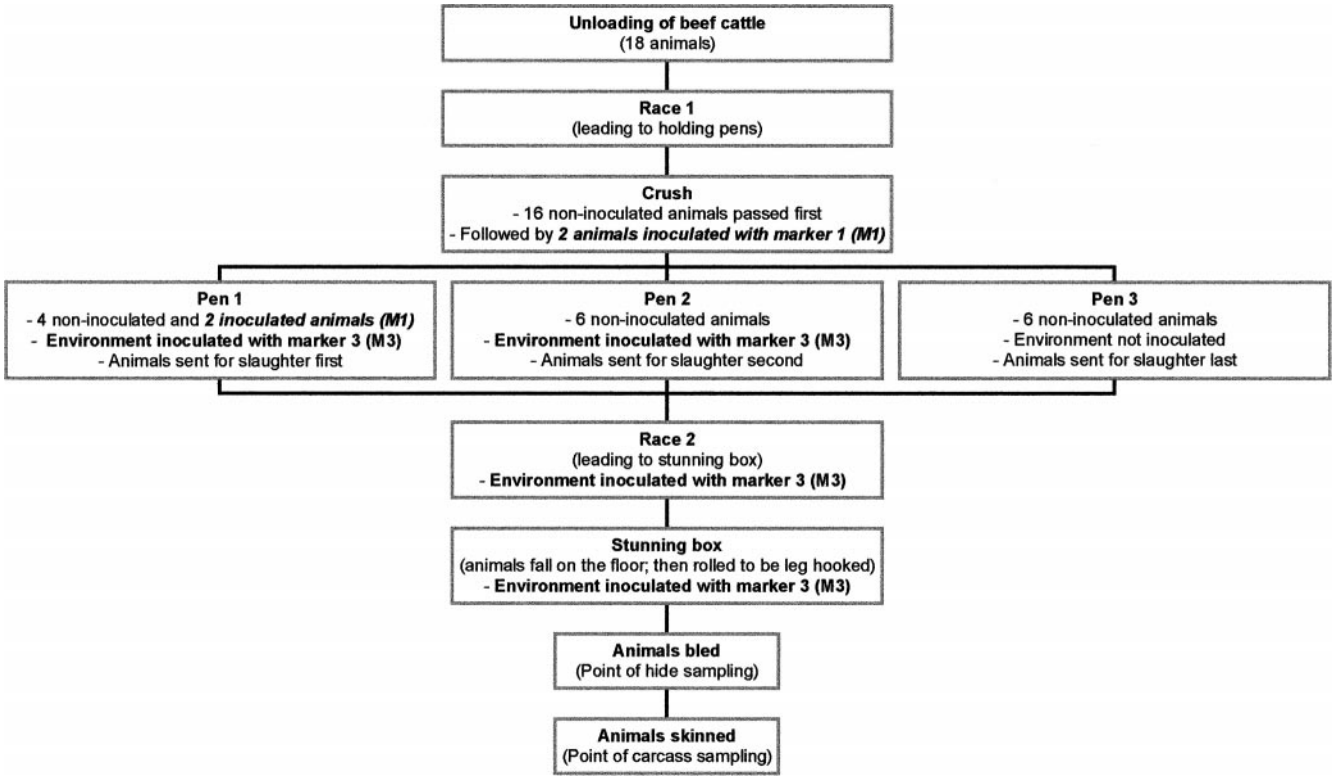


FIGURE 3. *General design of the abattoir study.*

familiar heifers, was also found on 50% of the unfamiliar, noninoculated steers.

It is likely that between-animal bacterial transfer within the holding pens occurred both directly (animal-to-animal contact) and indirectly (the animal-environment-animal route). The relevance of the environment is supported by the presence, for example, of markers M1 and M2 in 89 and 50% of environmental samples, respectively. Nevertheless, from the results of this study, it was not possible to quantitatively relate the contribution from each of the two main (direct and indirect) cross-contamination routes to the markers' spread.

Although a relatively small number of animals were used, this study demonstrated that the livestock market process can mediate the transfer of microbial contamination of hides not just within, but also between, batches of cattle. The implication is that livestock markets can facilitate the contamination of animals originating from ''noncontaminated'' farms. Ideally, skipping the markets, i.e., direct transport from farm to abattoir, would have been a desirable measure, reducing the meat safety risks. Nevertheless, further research is necessary to actually quantify the role of livestock markets in the epidemiology of meatborne diseases.

Spread of hide contamination in animals in abattoir lairage. All animals were acceptably clean for slaughter, since they were visually scored 1 or 2 using the official Meat Hygiene Service five-category scoring system. At the abattoir, both marker bacteria, which had been surface in-

TABLE 1. *Spread of bacterial contamination among cattle at simulated livestock market*

	Prevalence of the marker organisms on cattle at the end of market process[a]					
	Prevalence of the marker introduced among familiar animals at unloading (M1)		Prevalence of the marker introduced among familiar animals at entrance to sale ring (M2)		Prevalence of the marker introduced among unfamiliar animals in postsale ring (M3)[b]	
Animals	No. of inoculated animals (%)	No. of animals contaminated with M1 (%)[c]	No. of inoculated animals (%)	No. of animals contaminated with M2 (%)[c]	No. of inoculated animals (%)	No. of animals contaminated with M3 (%)[c]
Heifers (n = 15)	0 (0)	6 (40.0)	0 (0)	4 (26.6)	3 (20.0)	9 (60.0)
Steers (n = 18)	3 (16.6)	7 (38.9)	3 (16.6)	1 (5.5)	0 (0)	9 (50.0)
All (n = 33)	3 (9.1)	13 (39.4)	3 (9.1)	5 (15.1)	3 (9.1)	18 (54.5)

[a] From all three study repetitions together.
[b] These animals were subsequently mixed with familiar animals.
[c] Postmarket, including initially marked animal.

TABLE 2. *Distribution of bacterial contamination of preslaughter origin in slaughtered cattle*

Marker organisms	Slaughtered animals positive for the marker organisms on different body sites							
	No. positive on rump (%)		No. positive on flank (%)		No. positive on brisket (%)		No. positive on any site (%)	
	Hide before skinning	Skinned carcass	Hide before skinning	Skinned carcass	Hide before skinning	Skinned carcass	Hide before skinning	Skinned carcass
Marker (M1) inoculated on hides before lairaging[a]	9 (50.0)	3 (16.6)	11 (61.1)	0 (0)	11 (61.1)	15 (83.3)	18 (100.0)	16 (88.8)
Marker (M3) inoculated in lairage environment[b]	10 (55.5)	16 (88.8)	3 (16.6)	6 (33.3)	7 (38.8)	8 (44.4)	15 (83.3)	16 (88.8)

[a] Inoculated hides of 2 (11.1%) animals of 18.
[b] On surfaces in two (of three) pens, in race leading to stunning box, and in stunning box.

oculated onto a small number of animals or onto environmental surfaces in preslaughter areas, were recovered postslaughter not only from inoculated animals but also from a high proportion of noninoculated animals (Table 2).

Marker M1, inoculated on only 2 of 18 animals immediately after unloading, was found postslaughter on the hides of all the animals. Its frequency was slightly higher on the brisket and flank than on the rump (Table 2). Furthermore, the hide marker M1 was also found to have spread onto environmental surfaces, i.e., on floors and walls in pen 1 and the race leading to the stunning box. In a manner similar to that in the market situation, the spread of M1 probably occurred through both direct and indirect routes: (i) directly via body contacts between inoculated and noninoculated animals in the same pen, and (ii) indirectly via consecutive body contacts of inoculated and noninoculated animals with surfaces while moving along the race-stunning-bleeding route. Namely, animals from pen 1, containing the M1-inoculated animals, were sent for slaughter before M1-noninoculated animals from pens 2 and 3. Also, the environmental marker M3, which was inoculated on the surfaces of holding pens 1 and 2 (but not pen 3), the race leading to the stunning box, and the stunning box itself, was found postslaughter on the hides of 15 (of 18) animals (Table 2).

Overall, the spread of the hide marker at the abattoir was more extensive than at the market. A particular factor that probably caused this difference was the stunning box/roll-out area that was associated with the abattoir only. In this area, each one of the stunned animals fell and rolled on the same surfaces, which certainly potentiates the cross-contamination of hides.

Understandably, once markers M1 and M3 became, by whichever route, hide contaminants on the majority of the experimental animals, significant risks existed for their transfer, postslaughter, onto corresponding skinned carcasses. Indeed, the hide marker (M1) and the environmental marker (M3) were each found on 16 (of 18) corresponding skinned carcasses (Table 2). With respect to carcass sampling sites, the frequency of isolation was, in decreasing order, brisket-rump-flank for hide marker M1, while it was rump-brisket-flank for environmental marker M3.

From a meat hygiene perspective, the preslaughter holding of animals in separate lairage pens has two main goals: (i) to enable the penning of familiar, and prevent the mixing of unfamiliar, animals to reduce stress, and (ii) to prevent or reduce the transfer of microbial contamination between different batches of animals. Under the conditions of the present study, and confirming the findings from previous studies *(1, 2)*, the between-batches hide cross-contamination was not prevented by this procedure. Namely, the majority of the carcasses obtained from the animals from pen 3 were contaminated with both the hide marker M1 (83.3%) and the environmental marker M3 (100%), even though, in that particular pen, neither the animals nor the surfaces were inoculated. Also, all carcasses obtained from animals originating from pen 2 were contaminated with the hide marker M1, although none of these animals were inoculated with M1. In contrast, some other studies found intralot, but not extensive interlot, spread of carcass contamination *(5, 6)*. As previously indicated, the spread of microbial contamination between animals from different holding pens in the present study was likely mediated by postpen environmental surfaces, races, stunning boxes with roll-out ramps, or some combination thereof. It is not known whether, or how much, the skinning-associated factors (equipment, tools, and workers) contributed to such interpen cross-contamination.

Controls for hide contamination between farm and slaughter. This study, through the use of markers, and other studies, through the detection of pathogens *(1, 2, 4–6, 9, 11, 13, 14, 16)*, demonstrated that a significant hide-mediated spread of human health hazards, such as *Salmonella* and *E. coli* O157, can occur between the farm and slaughter. Bacterial transfer between animals within the same batch can occur *(5, 6)*, but possible interbatch (animal to environment to animal) transfer of bacterial contamination has even wider implications for the epidemiology of foodborne pathogens *(1, 2, 13, 16)*. Therefore, various approaches to the development of related control strategies must be considered.

By applying the principles of good hygienic practice, a reduction in the cross-contamination of hides during the farm-to-slaughter process appears achievable; however, its total prevention is unlikely. Additional difficulties include the fact that, presently, routine cleaning and sanitation of relevant surfaces (e.g., pens, stunning boxes) are either ir-

regular, ineffective, or both *(13, 14).* Further research is required to improve the efficacy of these control measures.

To eliminate microorganisms from hides completely, an effective decontamination treatment would be required. Hide decontamination would be less effective if conducted too early in the meat chain (i.e., on the farm), as recontamination of hides could occur during the transport and lairaging of animals *(4).* It is likely that hide decontamination applied immediately after slaughter but before skinning would be more beneficial. However, presently, hide decontamination treatments appear to eliminate only a proportion of hide microflora *(8, 15),* and some harsher hide treatments (e.g., heat) affecting the skin could be unacceptable to the leather industry. It seems that optimal hide decontamination treatments that are both effective and economical have yet to be developed.

Overall, this and other recent studies *(1, 2, 4, 8, 11, 12, 15, 16)* have demonstrated that the development of effective controls for hide cross-contamination that possibly combine preslaughter contamination-reducing measures and postslaughter decontamination of hides should be seen as one of the priorities for meat safety.

ACKNOWLEDGMENTS

The authors are grateful to a number of colleagues and technical staff for helping with the experimental work and to the Food Standards Agency (UK) for financial support. A. Small is supported by an RCVS John Lord Perry scholarship.

REFERENCES

1. Avery, S. M., E. Liebana, M. L. Hutchison, and S. Buncic. 2004. Pulsed field gel electrophoresis of related *Escherichia coli* O157 isolates associated with beef cattle and comparison with unrelated isolates from animals, meats and humans. *Int. J. Food Microbiol.* 92: 161–169.
2. Avery, S. M., A. Small, C.-A. Reid, and S. Buncic. 2002. Pulsed-field gel electrophoresis characterization of Shiga toxin-producing *Escherichia coli* O157 from hides of cattle at slaughter. *J. Food Prot.* 65:1172–1176.
3. Bacon, R. T., K. E. Belk, J. N. Sofos, R. P. Clayton, J. O. Reagan, and G. C. Smith. 2000. Microbial populations on animal hides and beef carcasses at different stages of slaughter in plants employing multiple-sequential interventions for decontamination. *J. Food Prot.* 63:1080–1086.
4. Barham, A. R., B. L. Barham, A. K. Johnson, D. M. Allen, J. R. Blanton, and M. F. Miller. 2002. Effects of transportation of beef cattle from the feedyard to the packing plant on prevalence levels of *Escherichia coli* O157 and *Salmonella* spp. *J. Food Prot.* 65:280–283.
5. Barkocy-Gallagher, G. A., T. M. Arthur, G. R. Siragusa, J. E. Keen, R. O. Elder, W. W. Laegreid, and M. Koohmaraie. 2001. Genotypic analysis of *Escherichia coli* O157:H7 and O157 nonmotile isolates recovered from beef cattle and carcasses at processing plants in the midwestern states of the United States. *Appl. Environ. Microbiol.* 67:3810–3818.
6. Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraire, and W. W. Laegreid. 2000. Correlation of enterohaemorrhagic *Escherichia coli* O157 prevalence in faeces, hides and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* 97:2999–3003.
7. Kain, M. L., S. L. Kochevar, J. N. Sofos, K. E. Belk, C. Rossiter, J. O. Reagan, and G. C. Smith. 2001. Relationship of live animal scores for ambulatory status, body condition, hide cleanliness, and fecal matter consistency to microbiological contamination of dairy cow carcasses. *Dairy Food Environ. Sanit.* 21:990–996.
8. McEvoy, J. M., A. M. Doherty, M. Finnerty, J. J. Sheridan, L. McGuire, L. S. Blair, D. A. McDowell, and D. Harrington. 2000. The relationship between hide cleanliness and bacterial numbers on beef carcasses at a commercial abattoir. *Lett. Appl. Microbiol.* 30: 390–395.
9. Midgley, J., and P. Desmarchelier. 2001. Pre-slaughter handling of cattle and shiga toxin-producing *Escherichia coli* (STEC). *Lett. Appl. Microbiol.* 32:307–311.
10. Reid, C.-A., S. M. Avery, M. L. Hutchison, and S. Buncic. 2002. Evaluation of sampling methods to assess the microbiological status of cattle hides. *Food Control* 13:405–410.
11. Reid, C.-A., A. Small, S. M. Avery, and S. Buncic. 2002. Presence of food-borne pathogens on cattle hides. *Food Control* 13:411–415.
12. Ridell, J., and H. Korkeala. 1993. Special treatment during slaughtering in Finland of cattle carrying an excessive load of dung: meat hygiene aspects. *Meat Sci.* 35:223–228.
13. Small, A., C.-A. Reid, S. M. Avery, N. Karabasil, C. Crowley, and S. Buncic. 2002. Potential for the spread of *Escherichia coli* O157, *Salmonella,* and *Campylobacter* in the lairage environment at abattoirs. *J. Food Prot.* 65:931–936.
14. Small, A., C.-A. Reid, and S. Buncic. 2003. Conditions in lairages at abattoirs for ruminants in Southwest England and in vitro survival of *Escherichia coli* O157, *Salmonella* Kedougou, and *Campylobacter jejuni* on lairage-related substrates. *J. Food Prot.* 66:1570–1575.
15. Small, A., B. Wells-Burr, and S. Buncic. An evaluation of selected methods for the decontamination of cattle hides prior to skinning. *Meat Sci*., in press.
16. Tutenel, A. V., D. Pierard, J. Van Hoof, and L. De Zutter. 2003. Molecular characterization of *Escherichia coli* O157 contamination routes in a cattle slaughterhouse. *J. Food Prot.* 66:1564–1569.