Journal of Food Protection, Vol. 66, No. 9, 2003, Pages 1570–1575

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

A. SMALL, C.-A. REID, AND S. BUNCIC*

Division of Farm Animal Science, School of Veterinary Science, University of Bristol, Langford, Bristol BS40 5DU, UK

MS 02-303: Received 28 August 2002/Accepted 27 March 2003

ABSTRACT

Information on lairages (regarding design, construction materials, and use of bedding and cleaning regimes) was collected for 21 commercial cattle and/or sheep abattoirs in southwest England. Overall, roughened or grooved concrete was the most common lairage flooring material. Straw bedding was used in the majority of lairages and was changed between animal batches, daily, weekly, and monthly in roughly 5, 60, 15, and 10%, respectively, of the surveyed lairages. Lairages were commonly washed with cold water with no detergents and/or disinfectants, and only about half the lairages were washed daily. Also, a three-pathogen cocktail inoculum comprising *Escherichia coli* O157 (NCTC 12900), *Salmonella* Kedougou (VLA S488/01), and *Campylobacter jejuni* (VLA C4) (at 8, 8, and 7 log CFU/ml or 8, 8, and 7 log CFU/g, respectively) was suspended in either broth (for nonfecal contamination) or bovine feces (for fecal contamination). Samples of the four most common substrates present in lairages (concrete, straw, metal, and hide) were contaminated in vitro with either fecal or nonfecal inocula and subsequently held in the laboratory at 10 or 25°C for 1 week. Bacterial counts for these samples were monitored daily and used to assess the number of days required for a 90% reduction of each pathogen population. In most cases, pathogens survived for >1 week, with survival rates being higher for straw or hide than for concrete or metal and higher for fecal contamination than for nonfecal contamination. Overall, if survival rates for the three pathogens under practical lairage conditions were similar to the in vitro survival rates found in this study, contamination of lairages with pathogens could be carried over from one batch of animals to another and/or from one day to the next.

Healthy cattle can be a reservoir for the major foodborne pathogens *Escherichia coli* O157, *Salmonella* spp., and *Campylobacter* spp. *(9, 11, 38),* and these organisms can be transferred from hides to meat during the slaughter and dressing of carcasses *(31, 45, 46, 49, 50).* The coats of animals destined for slaughter are a significant source of contamination of the resultant carcasses *(7, 45).* Consequently, an antemortem visual assessment of animal cleanliness via a scoring system is being used to prevent grossly contaminated animals from entering the slaughter line *(28, 42).* The lairaging of animals prior to slaughter can result in both animal-animal and animal-environment-animal cross-contamination *(3, 44, 52)* due to the potential persistence of foodborne pathogens in the environment in spite of routine cleaning procedures *(15, 44, 47).*

The excretion of foodborne pathogens by animals and contamination of animals with foodborne pathogens can increase as lairage time increases *(1, 36, 43).* Slaughterhouse lairages are designed primarily to facilitate animal handling and promote animal welfare and are constructed from materials that are durable and relatively easy to clean, thus reducing the risk of a buildup of pathogens *(13, 17–19, 24, 26).* Straw bedding may be provided, depending on the species held and the length of holding time, and such bedding may transfer contamination on the coats of animals *(26, 32).* However, there is little systematic information on actual lairage operation and cleaning regimes under commercial conditions and/or their effectiveness in preventing the accumulation of and cross-contamination with foodborne pathogens just before slaughter. Numerous published studies have shown that pathogenic bacteria can survive in farm-related environments for very long periods *(6, 22),* e.g., *Salmonella* Dublin was found to survive on fecally contaminated surfaces (nonwoven polyester, rubber, and concrete) for almost 6 years *(40),* and *E. coli* O157 was found to survive in bovine and ovine manure for several months *(30).* There is comparatively little information on the persistence of pathogens specifically in the immediate preslaughter environment and on the hides of slaughter cattle *(8, 27, 48).*

For these reasons, the main goals of the present study were (i) to obtain basic information on the routine operation of lairages at commercial cattle and sheep abattoirs and (ii) to assess the survival characteristics of *E. coli* O157, *Salmonella,* and *Campylobacter* under in vitro conditions simulating lairage conditions.

MATERIALS AND METHODS

Information on commercial lairage operations. From 21 commercial abattoirs (17 slaughtering both cattle and sheep, 2

* Author for correspondence. Tel: +44 117 928 9410; Fax: +44 117 928 9324; E-mail: sava.buncic@bristol.ac.uk.

slaughtering cattle only, and 2 slaughtering sheep only) in southwest England, basic information on the design of, the materials used for the construction of, and the use of bedding and cleaning regimes in lairages was gathered via a questionnaire. This information was validated through subsequent visits to most of the participating abattoirs. The abattoirs were grouped according to throughput status according to the abattoir license *(2)*. Fifteen abattoirs were full-throughput abattoirs (slaughtering more than 20 livestock units per week), and six were low-throughput abattoirs operating 1 or 2 days per week (slaughtering less than 20 livestock units). One livestock unit corresponds to 1 cow, 10 sheep, or 7 swine.

Preparation of bacterial inocula. A three-strain cocktail comprising 10 ml of a nonpathogenic *E. coli* O157 (NCTC 12900) culture grown overnight in brain heart infusion broth (Difco Laboratories, Sparks, Md.) at 37°C, 10 ml of *Salmonella* Kedougou (bovine strain VLA S488/01) culture grown overnight in brain heart infusion broth (Difco) at 37°C, and 20 ml of *Campylobacter jejuni* (bovine strain VLA C4) culture grown for 48 h in *Campylobacter* enrichment broth (Lab M, Lancashire, UK) at 42°C was prepared and used as a bacterial inoculum.

Fresh cattle feces were collected from a local farm, and the absence of *E. coli* O157, *Salmonella* spp., and *Campylobacter* spp. in these feces was verified by both direct plating and enrichment microbiological methods (see below). The bacterial inoculum described above was added to the feces, homogenized in a sterile pot with a sterile spatula, and used as the fecal inoculum to contaminate the substrates. For the nonfecal inoculum, the bacterial broth cocktail described above was used alone (i.e., without feces). Relatively high targeted levels of the three pathogens (8 log CFU/cm^2 or 8 log CFU/g of sample for *Salmonella* and *E. coli* O157 and 7 log CFU/ cm^2 or 7 log CFU/g for *C. jejuni*) were used in the inocula to enable the quantification of even large reductions in pathogen populations.

Inoculation of lairage-related substrates. Samples of four test substrates were used: smooth concrete (1.5-cm-diameter circular surfaces) handmade from a commercially available mixture, raw hide (washed three times in 50°C water and dried in air; square surfaces, 2 by 2 cm), unpainted galvanized steel (2-cm-diameter circular surfaces), and cut straw (1-g portions). Each of these samples was contaminated either fecally (with 1 g of the fecal inoculum) or nonfecally (with 0.3 ml of the nonfecal inoculum) by spreading the inoculum on the upper surface (for concrete, hide, and metal) or by mixing (for straw). The samples were placed (contaminated surfaces up) in the bases of sterile petri dishes and stored at either 10 or 25°C. For each sample at each temperature, two identical subsamples were used; one was stored at a high relative air humidity (90 to 96%) in a sealed plastic bag containing a wetted cellulose sponge, and the other was stored at a low humidity (40 to 49%) in a sealed plastic bag containing silica gel. Humidity was measured with a portable air humidity meter in preliminary experiments. The required number of samples was taken and examined immediately after preparation (time 0) and then daily over a 1-week period.

Sample analysis. For the direct plating method, three samples (see above) of each of the test substrates were placed in a sterile stomacher bag, and an amount of Sorensen's phosphate buffer (pH 7) that was ninefold larger than the original amount of the inoculum was added. The bags were manually squeezed for 2 min to release the bacteria from the sample. Subsequently, a series of decimal dilutions in maximum recovery diluent (Oxoid, Basingstoke, UK) were prepared. Each dilution was spread plated in triplicate onto xylose lysine desoxycholate (Oxoid), sorbitol MacConkey agar (CT-SMAC; Oxoid; containing 25 μl of cefixime per 500 ml and 25 μl of potassium tellurite [Dynal, Oslo, Norway] per 500 ml), and modified charcoal cefoperazone deoxycholate agar (mCCDA; Oxoid; containing selective supplement SR155E [Oxoid]) plates to determine counts of *Salmonella* Kedougou and *E. coli* O157 (after 24 h of incubation at 37°C) and *C. jejuni* (after 48 h at 42°C), respectively. For each pathogen, counts were determined at time 0 and then daily over a 1-week period. Counts were converted into terms of $\log_{10}$ CFU/ml or $\log_{10}$ CFU/g and used to calculate $D_{10°C}$- and $D_{25°C}$-values (times [in days] required for 90% reductions of a pathogen's population). For this study, for each sample, *D*-values obtained from two subsamples (high- and low-humidity samples) were used to calculate an arithmetic mean value. Whereas under practical lairage conditions in England air humidity varies widely between seasons and days (and even during the same day), in practice neither extremely high nor extremely low humidity would have been constant throughout a 1-week period.

For the enrichment method (i.e., to determine the presence or absence of a pathogen), the 10^{-1} dilution of the fecally contaminated sample was added to ninefold larger volumes of tryptone soya broth (containing 1.5 g of bile salts per liter, 1.5 g of K_2HPO_4 per liter, and 5 mg of novobiocin per ml; Oxoid), buffered peptone water (Oxoid), and *Campylobacter* enrichment broth (containing 2.5 ml of supplement CVTN, X132, and 5% defibrinated horse blood; Lab M). For the nonfecally contaminated samples, the 10^{-2} dilution was also used for enrichment in the same manner. Broths were incubated for 24 h at 37°C for *Salmonella* Kedougou and *E. coli* O157 and for 48 h at 42°C for *C. jejuni*. For the isolation of *Salmonella* Kedougou and *C. jejuni,* the broths were streaked onto xylose lysine desoxycholate and CCDA plates, respectively, and incubated as described for the direct plating method. *E. coli* O157 was isolated from the broth by the immunomagnetic separation technique (Dynal) with subsequent plating onto CT-SMAC agar *(12, 53)*.

Suspected *C. jejuni* colonies were confirmed with the *Campylobacter* test kit (Oxoid), suspected *Salmonella* Kedougou were confirmed with the use of Poly O and H antisera (Prolab Diagnostics, Liverpool, UK), and suspected *E. coli* O157 were confirmed with the latex agglutination kit (Oxoid).

RESULTS AND DISCUSSION

Lairages at commercial cattle and sheep abattoirs. The hygienic statuses of surfaces, floors, and walls in lairage holding pens can significantly affect the visible dirtiness of and pathogen loads on animal coats and feet *(16)*. Floors in both lairage pens and stunning boxes are particularly important because they are often contaminated with major foodborne pathogens *(44),* and many animals lie down during lairaging *(13, 25, 29)*.

Concrete was used as the flooring material in all surveyed abattoirs except one, which had brick flooring. In full-throughput abattoirs (Table 1), the most common types of concrete were roughened (46.6%) and grooved (40%) concrete, and the concrete was smooth in 26.6% of these abattoirs. Wire grid and concrete slat floorings were used in one lairage each. However, four of these lairages used two types of concrete flooring (in different areas). Generally, grooved and roughened flooring is used to give the animals' feet a better grip and prevent falls (thus improving the abattoir's animal welfare status), and this type of con-

TABLE 1. *Information on lairages at 21 commercial cattle and/or sheep abattoirs*[a]

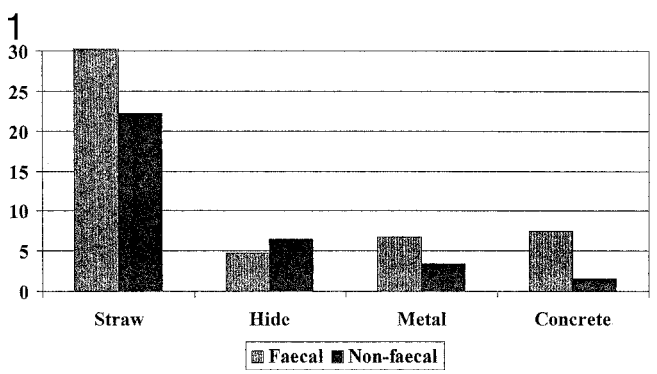
Lairage aspect	Full-throughput abattoirs (%)	Low-throughput abattoirs (%)	All abattoirs (%)
Flooring type (for lairages with concrete flooring)			
Smooth concrete	26.6	66.6	38.0
Roughened concrete	46.6	16.6	38.0
Grooved concrete	40.0	0	33.3
Concrete slats	6.6	0	4.8
Bedding			
Concrete, no bedding	20.0	16.6	19.0
Concrete with bedding	86.7	66.6	76.2
Brick with bedding	0	16.6	4.8
Wire grid, no bedding	6.6	0	4.8
Frequency of change of bedding (for lairages with bedding)			
After each batch of animals	0	16.6	4.8
Daily	60.0	50.0	57.1
Weekly	13.3	16.6	14.3
Monthly	6.6	16.6	9.5
Frequency of washing			
After each batch of animals	20.0	16.6	19.0
Daily	26.6	50.0	33.3
Weekly	6.6	16.6	9.5
Monthly	20.0	16.6	19.0
Bi-monthly	20.0	0	14.3

[a] Types of abattoirs are given in "Materials and Methods."

crete was present more often in newer abattoirs. On the other hand, in low-throughput abattoirs, 66.6% of lairages had smooth concrete flooring, while roughened concrete and brick floorings were used in one lairage each. Generally, good hygiene practice requires that construction materials used for abattoir lairages be both durable and easily cleanable. These requirements are satisfied by concrete and galvanized steel as used in the surveyed lairages. However, roughened or grooved floor surfaces, which are used particularly in newer lairages and in newer extensions of older lairages, may hinder cleaning and facilitate the persistence of microorganisms in the environment, as would crumbling edges of deteriorating or broken concrete *(47).*

Bedding is used in lairage holding pens both for animal welfare reasons and to speed up the drying of wet animals, particularly sheep *(34).* The majority of both high- and low-throughput abattoirs used bedding in holding pens (Table 1). For full-throughput abattoirs, straw bedding was used in 86.6% of lairages; in one lairage, straw bedding was used for lambs but no bedding was used for cattle; in another lairage, straw bedding was used overnight but not during the working day. In two lairages (the two with wire grid and concrete slat floorings), bedding was not used at all. For low-throughput abattoirs, straw bedding was used in 83.3% of lairages; no bedding was used in the other lairages. Sometimes, slatted flooring is recommended for sheep and cattle to prevent animal coat contamination if these animals lie down *(34).* The main problem encountered with slatted flooring is the need for a large manure collection pit below the floor and sufficient clearance for its regular emptying. Only one of the 21 abattoirs involved in this study used slatted flooring for cattle, and one used a wire grid for sheep.

With respect to the cleaning of lairages in full-throughput abattoirs, bedding was changed daily in 60% of the lairages, weekly in 13.3% of the lairages, and every 2 months in 6.7% of the lairages (Table 1). However, not all of these lairages were routinely washed at the time bedding was changed (Table 1). Only about one of five and one of four full-throughput lairages were washed after each animal batch and daily, respectively. For low-throughput abattoirs, bedding was changed daily in 50% of the lairages and more rarely on a weekly or monthly basis. None of the surveyed abattoirs used any detergent or disinfectant for lairage cleaning, although lairages were washed by hosing or with a pressure wash in all of the abattoirs. There is little doubt that lairage cleaning regimes are very important for meat hygiene and safety, since the removal of dirty and contaminated bedding and washing should reduce bacterial loads in the environment and contribute to an improvement in the visible cleanliness of animal coats *(16).* It is believed that holding animals in lairages with insufficient bedding or inadequate drainage could lead to fecal soiling of the coat



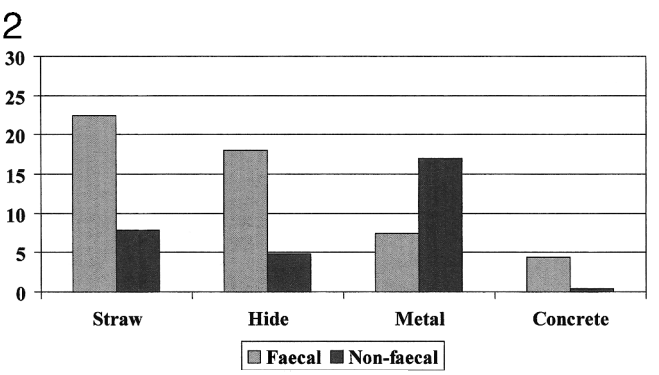
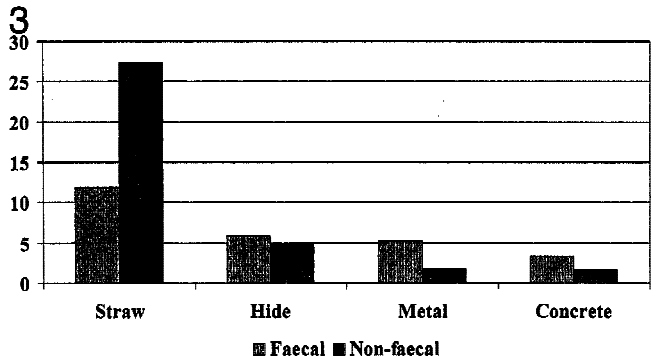


FIGURE 1. D-*values (days) for* Salmonella *Kedougou at 10°C on contaminated lairage-related substrates in vitro.*

FIGURE 2. D-*values (days) for* Salmonella *Kedougou at 25°C on contaminated lairage-related substrates in vitro.*



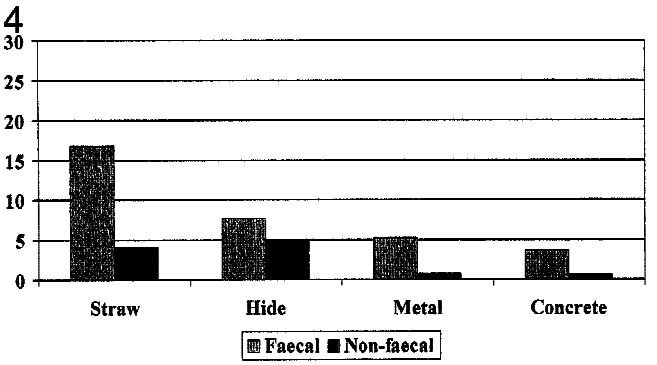


FIGURE 3. D-*values (days) for* E. coli *O157 at 10°C on contaminated lairage-related substrates in vitro.*

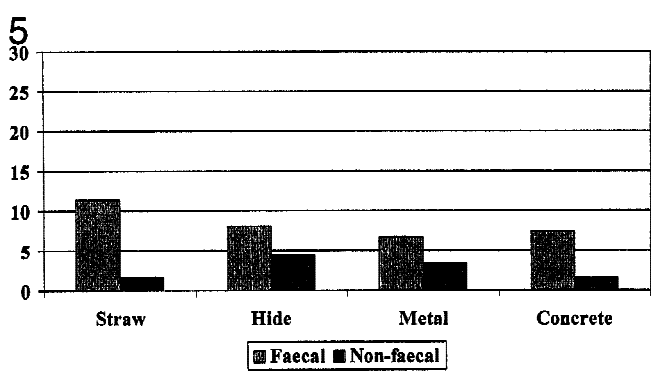
FIGURE 4. D-*values (days) for* E. coli *O157 at 25°C on contaminated lairage-related substrates in vitro.*

and skin *(20),* while improved coat cleanliness would have a positive effect on the microbiological status of the carcasses *(7, 21).* Nevertheless, the routine cleaning of cattle and sheep lairages does not necessarily eliminate *E. coli* O157 or *Salmonella* from the environment *(44),* and even the use of an alkaline chloride cleaning solution was found not to satisfactorily reduce rates of environmental *Salmonella* contamination in pig lairages *(47).* In conclusion, the lairage cleaning practices currently used in the surveyed abattoirs are unlikely to significantly reduce rates of environmental contamination with pathogens.

In vitro survival of pathogens on lairage-related substrates. In the majority of cases, initial pathogen counts decreased on lairage-related substrates under the in vitro conditions used in the present study. Generally, *Salmonella* Kedougou showed a marked ability to survive on straw (Figs. 1 and 2). At 10°C, the average *D*-values for this microorganism were as high as 31.2 and 22.2 days for fecally contaminated and nonfecally contaminated straw, respectively (Fig. 1). At the higher temperature (25°C), the average *D*-values were lower (22.5 and 7.9 days for fecally contaminated and nonfecally contaminated straw, respectively; Fig 2). Generally, *Salmonella* Kedougou survival rates for the other substrates examined (hide, metal, and concrete) were lower than the respective rates for straw at both 10 and 25°C (Figs. 1 and 2), with the sole exception of nonfecally contaminated metal at 25°C. No obvious explanation can be offered for *Salmonella* Kedougou's better survival on metal.

E. coli O157's behavior was similar to that of *Salmonella* Kedougou in that its overall survival rate was higher for straw than for the other substrates examined (Figs. 3 and 4). At 10°C, *E. coli* O157's *D*-values were 11.9 and 27.4 days for fecally contaminated and nonfecally contaminated straw, respectively (Fig. 3). At the higher temperature (25°C; Fig. 4), *E. coli* O157's *D*-values were 16.7 and 4.1 days for fecally contaminated and nonfecally contaminated straw, respectively. The presence of feces had a protective effect on *E. coli* O157 on straw at 25°C (similar to the effect seen for *Salmonella* Kedougou) but not at 10°C, a finding that is difficult to explain. This result may be due to the effects of some other variable factors, such as different counts and/or different of competing background flora activities in the feces at these two temperatures, but this possibility was not investigated. On the whole, *E. coli* O157 survival rates for the other substrates examined (hide, metal, and concrete) tended to be lower than the respective rates for straw (particularly for fecally contaminated substrates) at both 10 and 25°C (Figs. 3 and 4).

At 10°C, *C. jejuni* most often survived for days on the lairage-related substrates examined (Fig. 5) and also had a tendency to survive longer in the presence of feces. At 25°C (Fig. 6), populations of the pathogen on nonfecally contaminated substrates decreased to below the level of detection



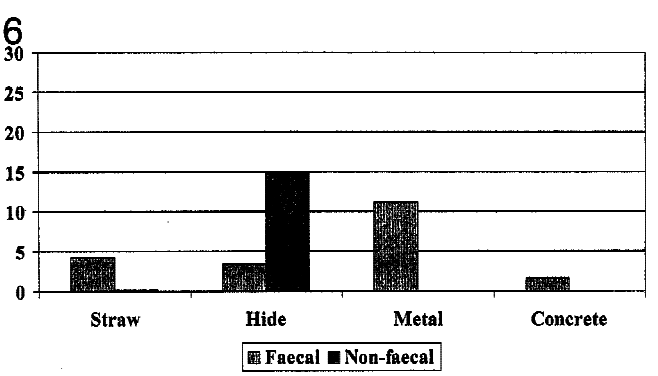


FIGURE 5. D-*values (days) for* C. jejuni *at 10°C on contaminated lairage-related substrates in vitro.*

FIGURE 6. *D-values (days) for* C. jejuni *at 25°C on contaminated lairage-related substrates in vitro.*

within 1 day, with the sole exception of the populations on hide. Interestingly, the overall better survival rates for straw than for the other substrates clearly observed for *Salmonella* Kedougou and for *E. coli* O157 were observed only at 10°C, and not at 25°C, for *C. jejuni*.

When these in vitro results were considered overall, in spite of some unexplained inconsistencies probably caused by certain variable factors such as unknown behavior of background flora in feces under different conditions, some general trends were apparent. First, in most cases the pathogens survived better on straw than on other lairage-related substrates examined. Second, all three pathogens had a tendency to survive better on fecally contaminated substrates than on nonfecally contaminated substrates. Third, *C. jejuni* populations decreased to undetectable levels more often than *Salmonella* Kedougou and *E. coli* O157 populations.

Practical implications of the results. Heavy microbial loads are normally associated with animal hides and hooves *(4).* Total microbial counts for cattle hides have been found to range from 3.53 to 12.50 $\log_{10}$ CFU/cm^2 *(6), E. coli* levels have been found to range from 2.08 to 7.50 $\log_{10}$ CFU/cm^2 *(5),* and a *Salmonella* Dublin level of about 4×10^6 cells per g has been found *(39).* The average microbial count for sheep fleece was found to be 5.38 $\log_{10}$ CFU/cm^2 *(37).* Recent studies have demonstrated that animal coats at slaughter frequently carry foodborne pathogens, e.g., the prevalence of *E. coli* O157 on cattle hides ranged from several to tens of percentage points *(3, 14, 35, 41, 44).* Since the microbial status of cattle hides and sheep fleeces can influence the microbial contamination of carcasses *(14, 23),* it is a relevant meat safety issue.

Various approaches to reducing the risk of the transfer of pathogens from the hide to the carcass, including the shaving of tags off cattle hides, the slowing of slaughter lines *(51),* the postmortem chemical dehairing of hides *(10),* and hide decontamination *(33),* have been considered, but the reduction rates achieved have been relatively limited.

It was very difficult to simulate and fully control all the possible lairage-related variables under in vitro conditions, but large-scale experiments involving the contamination of commercial abattoirs with fully pathogenic strains were not permissible. Because of the inherent experimental variability, as well as uncertainties associated with the possible behavioral diversity of wild strains of the three pathogens, the in vitro survival rates determined in this study cannot be directly extrapolated to a range of practical conditions encountered in abattoir lairages. However, these rates do support the possibility that a marked proportion of pathogen populations could survive in lairage environments during the routine animal lairaging period of 24 h (i.e., overnight), enabling their carryover from one animal batch to another and from one day to another. More in-depth research is necessary to fully evaluate how and to what extent common lairage construction, operation, and cleaning regimes contribute to cross-contamination with such pathogens and to develop adequate control strategies.

ACKNOWLEDGMENTS

This study was funded by the Food Standards Agency, United Kingdom (project M01009). The authors thank Howard Carpenter and Chris Beek for technical assistance and Dr. Diane Newell (Veterinary Laboratory Agency, Weybridge, UK) for bacterial strains.

REFERENCES

1. Anderson, E. S., N. S., Galbraith, and C. E. D. Taylor. 1961. An outbreak of human infection due to *Salmonella typhimurium* phage type 20a associated with infection in calves. *Lancet* i:854–858.
2. Anonymous. 1995. Fresh meat (hygiene and inspection) regulations. 1995. Her Majesty's Stationery Office, London.
3. 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.
4. Ayres, J. 1955. Microbiological implications in the handling, slaughtering and dressing of meat animals. *Adv. Food Res.* 6:110–162.
5. 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.
6. Bale, M. J., and M. Hinton. 1992. Bacteria and agriculture animals: survival and gene transfer, p. 14–23. *In* J. C. Fry and M. J. Day (ed.), Plant and microbial biotechnology research series 2. Cambridge University Press, Cambridge.
7. Bell, R. G. 1997. Distribution and sources of microbial contamination on beef carcasses. *J. Appl. Microbiol.* 82:292–300.
8. Burrows, M. R., and J. Deans-Rankin. 1970. A further examination of the survival of pathogenic bacteria in cattle slurry. *Br. Vet. J.* 126: 32–34.
9. Buxton, A. 1957. Public health aspects of salmonellosis in animals. *Vet. Rec.* 69:105–109.
10. Castillo, A., J. S. Dickson, R. P. Clayton, L. M. Lucia, and G. R. Acuff. 1998. Chemical dehairing of bovine skin to reduce pathogenic bacteria and bacteria of faecal origin. *J. Food Prot.* 61:623–625.
11. Chapman, P. A., C. A. Siddons, D. J. Wright, P. Norman, and E. Crick. 1993. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. *Epidemiol. Infect.* 111: 439–447.
12. Chapman, P. A., D. J. Wright, and C. A. Siddons. 1994. A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from bovine faeces. *J. Med. Microbiol.* 40:424–427.
13. Cockram, M. S., and K. T. T. Corley. 1991. Effect of preslaughter handling on the behaviour and blood composition of beef cattle. *Br. Vet. J.* 147:444–454.
14. Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalences in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* 97:2999–3003.
15. Gibson, E. A. 1961. Salmonellosis in calves. *Vet. Rec.* 73:1284.
16. Gracey, J. E., and D. S. Collins. 1999. Meat hygiene. W. B. Saunders, London.
17. Grandin, T. 1983. Welfare requirements of handling facilities, p. 137–149. *In* S. H. Baxter, M. R. Baxter, and J. A. D. MacCormack (ed.), Farm animal housing and welfare. Martinus Nijhoff, The Hague, The Netherlands.
18. Grandin, T. 1990. Design of loading facilities and holding pens. *Appl. Anim. Behav. Sci.* 28:187–201.
19. Grandin, T. 1997. Survey of stunning and handling in federally inspected beef, veal, pork and sheep slaughter plants. Available at: http://www.grandin.com/usdarpt.html.
20. Gregory, N. G. 1994. Pre-slaughter handling, stunning and slaughter. *Meat Sci.* 36:45–56.
21. Hadley, P. J., J. S. Holder, and M. H. Hinton. 1997. Effects of fleece soiling and skinning method on the microbiology of sheep carcasses. *Vet. Rec.* 140:570–574.
22. Heard, T. W. 1969. Housing and *Salmonella* infections. *Vet. Res.* 85: 482–483.

23. Hudson, W. R., G. C. Mead, and M. Hinton. 1998. Assessing of abattoir hygiene with a marker organism. *Vet. Rec.* 139:587–589.
24. Jarvis, A. M., and M. S. Cockram. 1994. Effect of handling and transport on bruising of sheep sent directly from farms to slaughter. *Vet. Rec.* 135:523–527.
25. Jarvis, A. M., and M. S. Cockram. 1995. Some factors affecting the resting behaviour of sheep in slaughterhouse lairages after transport from farms. *Anim. Welfare* 4:53–60.
26. Jarvis, A. M., C. D. A. Messer, and M. S. Cockram. 1996. Handling, bruising and dehydration of cattle at the time of slaughter. *Anim. Welfare* 5:259–270.
27. Jones, P. W. 1980. Health hazards associated with the handling of animal wastes. *Vet. Rec.* 106:4–7.
28. Jordan, D., S. A. McEwan, B. Wilson, W. B. McNab, and A. M. Lammerding. 1999. Reliability of an ordinal rating system for assessing the amount of mud and faeces (tag) on cattle hides at slaughter. *J. Food Prot.* 62:520–525.
29. Kim, F. B., R. E. Jackson, G. D. H. Gordon, and M. S. Cockram. 1994. Resting behaviour of sheep in a slaughterhouse lairage. *Appl. Anim. Behav. Sci.* 40:45–54.
30. Kudva, I. T., K. Blanch, and C. J. Hovde. 1998. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. *Appl. Environ. Microbiol.* 64:3166–3174.
31. Lammerding, A. M., M. M. Garcia, E. D. Mann, Y. Robinson, J. Dorward, R. B. Truscott, and F. Tittiger. 1988. Prevalence of *Salmonella* and thermophilic *Campylobacter* in fresh pork, beef, veal and poultry in Canada. *J. Food Prot.* 51:47–52.
32. McClain, J., J. E. Wohlt, K. H. McKeever, and P. H. Wart. 1997. Horse hair coat cleanliness is affected by bedding material: a comparison of clean and used wheat straw, wood shavings and pelleted newspaper. *J. Eq. Vet. Sci.* 17:156–160.
33. McEvoy, J. M., A. M. Doherty, J. J. Sheridan, I. S. Blair, and D. A. McDowell. 2001. Use of steam condensing at subatmospheric pressures to reduce *Escherichia coli* O157 numbers on bovine hide. *J. Food Prot.* 64:1655–1660.
34. McGrath, J. F., and J. T. Patterson. 1969. Meat hygiene—the preslaughter treatment of fatstock. *Vet. Rec.* 85:521–524.
35. Midgley, J., and P. Desmarchelier. 2001. Preslaughter handling of cattle and Shiga toxin-producing *Escherichia coli* (STEC). *Lett. Appl. Microbiol.* 32:307–311.
36. Morgan, I. R., F. L. Krautil, and J. A. Craven. 1987. Effect of time in lairage on caecal and carcass *Salmonella* contamination of slaughter pigs. *Epidemiol. Infect.* 98:323–330.
37. Newton, K. G., J. C. L. Harrison, and A. M. Wauters. 1978. Sources of psychrotrophic bacteria on meat at the abattoir. *J. Appl. Bacteriol.* 45:75–82.
38. Paiba, G. A., and J. C. Gibbens. 2000. Prevalence of faecal carriage of VTEC O157 and other foodborne pathogens by cattle and sheep at slaughter in Great Britain, VLA/MHS Collaborative Project. VLA, Weybridge, UK
39. Petersen, G. V. 1983. The effect of swimming lambs and subsequent resting periods on the ultimate pH of meat. *Meat Sci.* 9:237–246.
40. Plym-Forshell, L., and I. Ekesbo. 1996. Survival of salmonellas in urine and dry faeces from cattle—an experimental study. *Acta Vet. Scand.* 37:127–131.
41. 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.
42. 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.
43. Samuel, J. L., D. A. O'Boyle, W. J. Mathers, and A. J. Frost. 1979. Isolation of *Salmonella* from mesenteric lymph nodes of healthy cattle at slaughter. *Res. Vet. Sci.* 28:238–241.
44. 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* spp., and *Campylobacter* spp. in the lairage environment at abattoirs. *J. Food Prot.* 65:931–936.
45. Sofos, J. N., S. L. Kochevar, G. R. Bellinger et al. 1999. Sources and extent of microbiological contamination of beef carcasses in seven United States slaughtering plants. *J. Food Prot.* 62:140–145.
46. Sofos, J. N., S. L. Kochevar, J. O. Reagan, and G. C. Smith. 1999. Incidence of *Salmonella* on beef carcasses relating to the US meat and poultry inspection regulations. *J. Food Prot.* 62:467–473.
47. Swanenburg, M., H. A. P. Urlings, D. A. Keuzenkamp, and J. M. A. Snijders. 2001. *Salmonella* in the lairage of pig slaughterhouses. *J. Food Prot.* 64:12–16.
48. Thunegard, E. 1975. On the persistence of bacteria in manure. *Acta Vet. Scand.* 56(Suppl.):1–86.
49. Turnbull, P. C. B., and P. Rose. 1982. *Campylobacter jejuni* and *Salmonella* in raw red meats—a Public Health Laboratory Service survey. *J. Hyg. Camb.* 88:29–37.
50. Vanderlinde, P. B., B. Shay, and J. Murray. 1998. Microbiological quality of Australian beef carcass meat and frozen bulk packed beef. *J. Food Prot.* 61:437–443.
51. van Donkersgoed, J., K. W. F. Jericho, H. Grogan, and B. Thorlakson. 1997. Preslaughter hide status of cattle and the microbiology of carcasses. *J. Food Prot.* 60:1502–1508.
52. Watson, W. A. 1975. Salmonellosis and meat hygiene: red meat. *Vet. Rec.* 96:374–376.
53. Wright, D. J., P. A. Chapman, and C. A. Siddons. 1994. Immunomagnetic separation as a sensitive method for isolating *Escherichia coli* O157 from food samples. *Epidemiol. Infect.* 113:31–39.