

Potential for the Spread of *Escherichia coli* O157, *Salmonella*, and *Campylobacter* in the Lairage Environment at Abattoirs

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ABSTRACT

Prevalences of *Escherichia coli* O157, *Salmonella* spp., and *Campylobacter* spp. were examined in 270 swabs taken from selected sites along the unloading-to-slaughter routes of animal movement in lairages of six commercial abattoirs, three for cattle and three for sheep. The overall prevalences of the pathogens in the respective lairage environments were compared with those for 270 swabs from the pelts of 90 lambs examined in the present study and 270 swabs from the hides of 90 cattle examined in a previous study that were slaughtered at the same abattoirs on the same days. Also, the results obtained were analyzed with the aim of identifying critical points at which animal-environment-animal transfer of the pathogens in lairages occurs. The results showed that (i) the overall prevalences of *E. coli* O157, *Salmonella* spp., and *Campylobacter* spp. were 27.2, 6.1, and 1.1%, respectively, in cattle lairages and 2.2, 1.1, and 5.6%, respectively, in sheep lairages; (ii) the overall prevalences of the three pathogens on cow hides (28.8, 17.7, and 0%, respectively) and sheep pelts (5.5, 7.8, and 0%, respectively) were higher than the overall prevalences in the respective lairage environments; (iii) the most frequently contaminated sites in cattle lairages were holding pen floors (50% of swabs positive for one or more pathogens), entrance gates of stun boxes (27.8% of swabs positive for one or more pathogens), and stun box floors (22.2% of swabs positive for one or more pathogens); (iv) the most frequently contaminated sites in sheep lairages were unloading ramp floors, holding pen floors, and water troughs (33.3, 22.2, and 22.2%, respectively); and (v) overall, cattle lairages and cow hides were more frequently contaminated with the pathogens than were lamb lairages and lamb pelts. Further research is needed to develop strategies for the incorporation of pathogen control in lairages into integrated microbial meat safety systems.

The major foodborne bacterial pathogens associated with the contamination of meat, such as *Escherichia coli* O157, *Salmonella* spp., and *Campylobacter* spp., are asymptotically carried in the gastrointestinal tracts of ruminants and other food animals and are shed in feces. This fecal material, and hence bacterial pathogens, can be spread within animal-related environments (1, 6, 9, 10). Understandably, animal coats and hooves are easily contaminated.

In addition to being contaminated on the farm, animal coats may be “freshly” contaminated during farm-to-abattoir transport, during unloading-to-pen movement in the lairage, while the animal is resting in the pen, and along the pen-to-slaughter route. Numerous routes of transfer of pathogens within the lairage exist, including animal-to-animal, animal-to-environment, and environment-to-animal routes. Recent studies have shown that the prevalence of *E. coli* O157 on the hides of slaughtered cattle can be as high as 10.7% (7) or 28.8% (14). From a global meat safety assurance perspective, the possibility that animals from pathogen-free farms can become contaminated with pathogens introduced into the lairage environment by animals originating from “contaminated” farms is of particular concern. If such spreading of pathogens were to occur within lairages to a significant extent, it could largely diminish or negate positive effects achieved with on-farm control; it is

well recognized that any contamination of the animal coat can result in meat contamination during the skinning and processing of the carcass (2, 8).

Recent studies have shown that extended lairaging of pigs at abattoirs increases the contamination of slaughtered pigs with *Salmonella* spp. because of the high prevalence (20 to 90%) of this pathogen in the lairage environment (e.g., on floors and walls), which consequently serves as a pig contamination source (11, 13, 15, 16). However, information on prevalences of other major pathogens in lairages of abattoirs for other food animals, such as cattle and sheep, is scarce. Therefore, the present study was conducted to determine prevalences of pathogens contaminating the lairage environment and animal coats and to identify critical points in the lairage that contribute to the animal-environment-animal spread of pathogens.

MATERIALS AND METHODS

Lairage environment samples: cattle lairages. Samples from the lairage environments of three beef cattle abattoirs in southwest England were collected twice: during work (i.e., the production process) and before work (i.e., just before the start of processing in the morning). In each of the three cattle abattoirs, both before and during work, swabs were taken from each of the following 10 sites within the lairage:

1. floor of ramp at unloading area (three swabs from horizontal surface)

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2. corner between unloading area and holding pen area (three swabs from vertical surface)
3. walls of holding pens (three swabs from vertical surface)
4. floor of holding pens (three swabs from horizontal surface)
5. water troughs in pens (three swabs from trough edge and water)
6. corner between holding pen and race (three swabs from vertical surface)
7. walls of funnel leading into race (three swabs from vertical surface)
8. cattle crush (two swabs from vertical surface and one swab from horizontal surface)
9. gates between race and stunning box (three swabs from vertical surface)
10. stunning box floor (three swabs from horizontal surface)

Therefore, 18 samples per site (180 samples in total) were taken from cattle lairage environments. All vertical surfaces were sampled at a height roughly corresponding to the horizontal middle of the side of the body of an average beef bovine.

Lairage environment samples: sheep lairages. Samples from each of three sheep abattoirs in southwest England were collected only during work. In each of the three sheep abattoirs, three swabs were taken separately from each of 10 sites that generally corresponded to those listed above for cattle lairages (see above). The exceptions were that the crush was not sampled at sheep abattoirs; passageway floor sites were sampled instead. Overall, 9 samples per site (90 in total) were taken from sheep lairages. All vertical surfaces were sampled at a height roughly corresponding to the horizontal middle of the side of the body of an average lamb.

Animal coat samples. At each of the three sheep abattoirs, at the same time the lairage environment sampling was being conducted during work, three sites (the rump, the flank, and the brisket) on the pelt of each of 30 randomly selected lambs were sampled immediately after bleeding out but before depelting. Thus, a total of 90 lambs were sampled (i.e., 270 pelt samples were taken). Sampling of the hides of 90 cattle was conducted at three cattle abattoirs in an identical manner and has previously been reported (14).

Sampling procedure. Plain cellulose sponge cloths (20 by 18 cm) containing no antimicrobial additives were halved widthwise, and each half was folded in half and placed in a sterile stomacher bag with the folded edge facing the bag opening. Each of these swabs was moistened immediately prior to use with 10 ml of maximum recovery diluent. The base of each sponge was grasped through the stomacher bag, and the stomacher bag was pulled over the hand to reveal the folded edge of the swab. A metal template providing a window of 10 by 10 cm (i.e., 100 cm²) was disinfected with alcohol and placed on the chosen sampling site (i.e., the lairage environment surface or the lamb pelt). The folded end of the swab was pressed onto the template area and passed from left to right in one motion. On rounded surfaces on which metal template could not be placed flat in lairages—such as water trough edges or corners—swabs were taken from an area determined by visual assessment to be ca. 100 cm². The stomacher bag was then pulled back over the hand, encasing the swab inside. The resulting samples were held on ice and processed in the laboratory within 2 h.

Microbiological analysis. In the laboratory, 90 ml of maximum recovery diluent was added to each stomacher bag, and the sponge was repeatedly squeezed manually from the outside of the

bag for 1 min to release any microorganisms present on the sponge surface. The resulting liquid was divided into three portions for three enrichment procedures, which were handled as follows. For the enrichment of *E. coli* O157, 25 ml was added to 225 ml of modified tryptone soya broth base (Oxoid, Hampshire, England, UK) containing bile salts (1.5 g/liter; Oxoid), K₂HPO₄ (1.5 g/liter; Sigma), and 5 mg of novobiocin (Sigma) per ml. For the enrichment of *Campylobacter* spp., 25 ml of the liquid obtained from the stomacher bag was added to 225 ml of Park Sanders broth (brucella broth [28 g/liter; Difco Laboratories, Detroit, Mich.], sodium citrate [1 g/liter; Sigma], and sodium pyruvate [0.25 g/liter; Sigma]) containing 1 ml of reconstituted supplement A (Mast Diagnostics, Merseyside, England, UK) and 11.8 ml of lysed horse blood (Oxoid). For the enrichment of *Salmonella* spp., 10 ml of the liquid obtained from the stomacher bag was added into 240 ml of buffered peptone water (Oxoid).

After enrichment of the modified tryptone soya broth for 24 h at 41.5°C, the isolation of *E. coli* O157 was carried out with immunomagnetic separation (4, 17) followed by plating onto sorbitol MacConkey agar containing cefixime (25 µl/500 ml; Dynal, Oslo, Norway) and potassium tellurite (25 µl/500 ml; Dynal) and onto sorbitol MacConkey agar containing cefixime (25 µl/500 ml; Dynal) and rhamnose (2.5 g/500 ml; Sigma). Plates were incubated for 24 h at 37°C, and colorless colonies and colonies with dark pink centers were picked and plated onto plate count agar for further examination. Suspect colonies were confirmed by latex agglutination with an *E. coli* O157 test kit (Oxoid) with no H-antigen characterization.

After enrichment of the buffered peptone water for 16 to 24 h at 37°C, 0.1 ml was transferred to 10 ml of Rappaport Vassiliadis broth (Oxoid) and 10 ml was transferred to 100 ml of selenite cysteine broth (E&O Laboratories, Bonnybridge, Scotland, UK). The broths were incubated at 42 and 37°C, respectively, for 24 h. Aliquots from each broth were spread in duplicate onto xylose lysine desoxycholate (Oxoid) and brilliant green agar (Oxoid) plates and incubated at 37°C for 24 h. After plating, the selenite cysteine broths were returned to the incubator for a further 24 h and plated out as described above. Suspect colonies on either xylose lysine desoxycholate or brilliant green agar were confirmed with Gram staining; catalase, oxidase, and urease tests; poly O and H antisera (Prolab Diagnostics, Cheshire, England, UK); and API 20E kits (BioMerieux, Marcy l'Etoile, France).

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

RESULTS AND DISCUSSION

Overall prevalences of pathogens in lairages and on animal coats. Overall prevalences of *E. coli* O157 and *Salmonella* spp. in cattle lairages were very similar, 7.2 and 6.1%, respectively (Table 1). No major difference in the

TABLE 1. Prevalences (%) of foodborne pathogens in the lairage environment and on animal coats in abattoirs.

Foodborne pathogen	Lairage environment swabs from cattle abattoirs		Hides of slaughtered bovines before dehidng (90 animals) ^{a,b}	Lairage environment swabs in sheep abattoirs during work (n = 90)	Pelts of slaughtered lambs before depelting (90 animals) ^b
	Before/during work (n = 90 each)	Overall (n = 180)			
<i>Escherichia coli</i> O157	6.7/7.8	7.2	28.8	2.2	5.5
<i>Salmonella</i> spp.	1.1/11.1	6.1	17.7	1.1	7.8
<i>Campylobacter</i> spp.	2.2/ND ^c	1.1	ND	5.6	ND

^a These results have previously been reported (14) but were obtained at the same abattoirs at the same time the lairage sampling was conducted.

^b Percentage of animals testing positive for pathogens on any of the three sampled sites (brisket, flank, and rump).

^c ND, not detected.

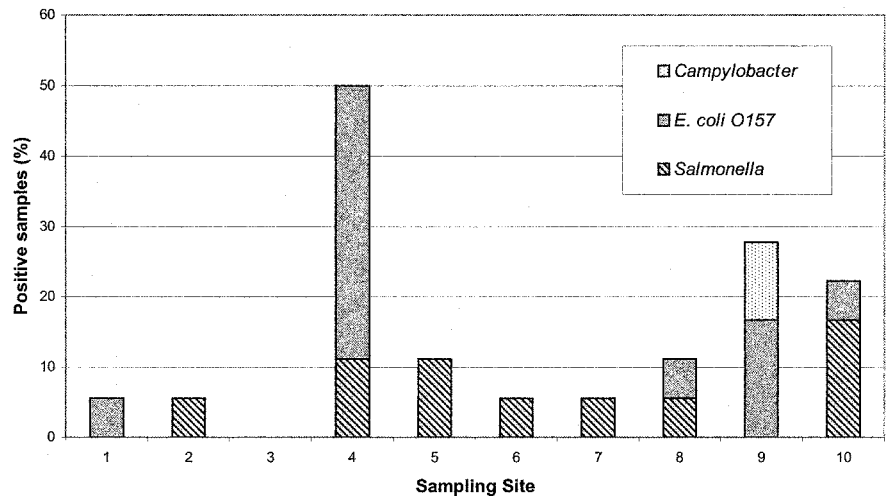
overall prevalence of *E. coli* O157 in samples collected before work started and that in samples taken during the production process in the lairage environment was observed. This finding indicates that *E. coli* O157 contamination within the lairage was, in practice, fully carried over from one day to the next, in spite of routine cleaning conducted between days. In contrast, the overall prevalence of *Salmonella* spp. in lairages before work was low but increased 10-fold during working hours.

Generally, the presence of pathogens within the lairage environment makes their transfer onto the hides of at least some cattle during lairaging inevitable. The overall prevalences of the pathogens on the hides of slaughtered bovines were published elsewhere (14) but are shown again here (Table 1) as they relate to the animals slaughtered at the same abattoirs on the same days. It is noticeable that the prevalences of both *E. coli* O157 and *Salmonella* spp. on hides were higher than their respective overall prevalences in the lairage environment (Table 1). This finding could mean that a certain proportion of the hide contamination probably took place within the lairage environment, but part of it could also have originated from the prelairage chain of events (i.e., on the farm, during transport). However, to distinguish the exact extents of prelairage and within-lairage hide contamination, the prevalence of pathogens on the hides of animals before lairaging would have to have been known but was not investigated. Also, a proportion of each pathogen population could have been transferred from hide to hide (i.e., via direct animal-to-animal physical contact within the lairage but without the involvement of the lairage environment itself). With respect to distribution of the pathogens on different hide areas (previously reported (14)), the mean frequencies of contamination with *E. coli* O157 and *Salmonella* spp. were 22.2 and 10.0%, respectively, for the brisket; 4.4 and 8.8%, respectively, for the flank; and 3.3 and 2.2%, respectively, for the rump. On the other hand, *Campylobacter* spp. were found only rarely in the cattle lairages (i.e., six- to sevenfold less frequently than *E. coli* O157 or *Salmonella* spp., and not at all on the hides of slaughtered bovines; Table 1). There may be many reasons for this phenomenon, including the possibility of less fecal shedding by the cattle and survival rates of *Campylobacter* spp. on dry surfaces and/or hides that were correspondingly lower than those of the other two pathogens.

Microbiological sampling in lairages of abattoirs for sheep was conducted only during the production process. Relatively low overall prevalences of *E. coli* O157 and *Salmonella* spp. were found in these environments (2.2 and 1.1%, respectively; Table 1). However, these pathogens were found on the pelts of slaughtered lambs with prevalences roughly twofold (*E. coli* O157) and sevenfold (*Salmonella* spp.) higher than the respective average prevalences found in the lairage environments. These results indicate that some of the pelt contamination could have occurred during lairaging, but some could also have originated from the prelairage period. Moreover, a certain proportion of each pathogen population on the pelt could be acquired through direct animal-to-animal physical contact without the involvement of the lairage environment itself. With respect to the distribution of the pathogens in different pelt areas, the average frequencies of contamination with *Salmonella* spp. and *E. coli* O157 were 3.3 and 2.2%, respectively, for the brisket; 3.3 and 2.2%, respectively, for the flank; and 1.1% for both pathogens for the rump. It is unclear why *Campylobacter* spp. were not found on the pelt of any lamb examined, although this pathogen was present in the sheep lairage environments more frequently than the other two pathogens were. Further research is necessary to determine whether *Campylobacter* survival rates differ for the fleece and the floor material.

Prevalences of the pathogens in lairages, as well as on the coats of slaughtered animals, varied between individual abattoirs, even those slaughtering the same animal species (results not shown). This finding can probably be attributed to numerous factors that differed between abattoirs (e.g., animal origin, lairage design, and animal handling, cleaning, and disinfection practices). However, the focus of the present study was primarily on general trends attributable to cattle or sheep abattoirs, and the abattoir-specific factors were not explored for the abattoirs tested. Generally, when comparing cattle and sheep abattoirs, it is important to note that overall prevalences of *E. coli* O157 in the lairage and on hides in the cattle abattoirs were markedly higher than the respective prevalences of this pathogen in lairages and on pelts in the sheep abattoirs (Table 1). Similar but less marked trends were observed for *Salmonella* spp. It could be speculated that the higher prevalence of *E. coli* O157 in cattle abattoirs was the consequence of more extensive fecal

FIGURE 1. Incidences of foodborne pathogens at selected sites in cattle lairages: (1) unloading ramp (horizontal surface); (2) corner between unloading area and lairage pen (vertical surface); (3) pen walls (vertical surface); (4) pen floor (horizontal surface); (5) water trough (interior and water); (6) corner between lairage pen and race (vertical surface); (7) funnel walls (vertical surface); (8) crush (one swab from horizontal surface and two swabs from vertical surface); (9) gates between race and stunning box (vertical surface); and (10) stunning box floor (horizontal surface).



shedding for cattle than for sheep. In one study (3), the overall shedding of *E. coli* O157 for cattle was shown to be 15.7% and that for sheep was shown to be 2.2%.

Critical points mediating the transfer of pathogens within lairages. Generally, the spread of pathogens in lairages can occur through direct animal-to-animal contact and/or via animal-environment-animal routes. To assess the possibilities for the contamination of animal coats from the lairage environment itself, the presence of pathogens at 10 different sites (i.e., surfaces along the route from unloading to slaughter) was investigated. In spite of some differences in lairage design between abattoirs, 10 corresponding sites could be identified in each of the three cattle lairages and the three sheep lairages.

The most frequently contaminated point in the cattle lairages (Fig. 1) was the floor of the holding pen, for which 50% of the swabs tested positive for one or more of the pathogens under study. The next most frequently contaminated points were the gate between the race and the stun box at the entrance (with 27.8% of swabs testing positive) and the floor of the stun box (with 22.2% of swabs testing positive). The crush and the water troughs each produced 11.1% positive swabs. All other lairage sites (except the pen walls) were also contaminated, but at lower levels (5.5%). The high prevalences of the pathogens on floors, particularly in the holding pen and in the stun box, are clearly related to the highest prevalences of the same pathogens on the brisket area of the hides of slaughtered bovines (see above). It can be expected that the brisket area of the hide can easily become contaminated if the animal lies in the holding pen. Furthermore, the stun box floor is a particularly critical contamination point, since every single stunned animal falls on the same spot and pathogens from the brisket of one animal could consequently be transferred via the floor onto the briskets of consecutively stunned animals. On the other hand, some vertical surfaces within narrow spaces such as the entrance to the stun box or the crush, against which every animal is forced to rub its side, also serve as critical contamination points, primarily for the contamination of the flank area of the hide.

As with cattle lairages, the most frequently contami-

nated sites in sheep lairages included floors (Fig. 2), particularly the unloading ramp floor (with 33.3% of swabs testing positive for one or more pathogens) and the holding pen floor (with 22.2% of swabs testing positive), followed by floors in lairage passageways (with 11.1% swabs testing positive). Contamination of lower areas of the pelts (including the brisket area) from lairage floors seems particularly likely for lambs, even if the animals do not lie down, because of their closer proximity to floors and, as a consequence, more extensive exposure to contaminated floor-generated dust and/or aerosols. On the other hand, water troughs in holding pens were also frequently contaminated (with 22.2% of swabs testing positive). Other studies have also indicated that water troughs can serve as sources of *E. coli* O157 infections for farm animals (10). However, at other sampling sites in sheep lairages, including vertical surfaces, no pathogens were detected. The absence of pathogens in swabs from vertical surfaces was somewhat surprising, since the pathogens were found on related areas (the flanks) of the pelts of some of the slaughtered sheep (see above). It could be speculated that because of the flocking behavior of sheep, extensive direct flank-to-flank pathogen transfer between lambs may have contributed to that discrepancy.

General relevance of lairage contamination to integrated meat safety assurance. It is clear that the problem of control of major foodborne pathogens along the meat production chain is very complex. The total eradication of pathogens by preharvest interventions or their total elimination during the slaughter, processing, and retailing phases does not seem achievable. Success therefore might be limited to pathogen reductions at all points of the meat production chain from preharvest to consumer levels (10). While significant research and information on intervention measures to reduce the prevalence of pathogens at farm and slaughter line levels has accumulated, the epidemiology and control of pathogens during the period between farm and slaughter, i.e., during transport and lairaging of animals, has attracted relatively much less attention. This is not a desirable situation, for two main interrelated reasons: (i) the microbial safety of carcasses is largely determined by the

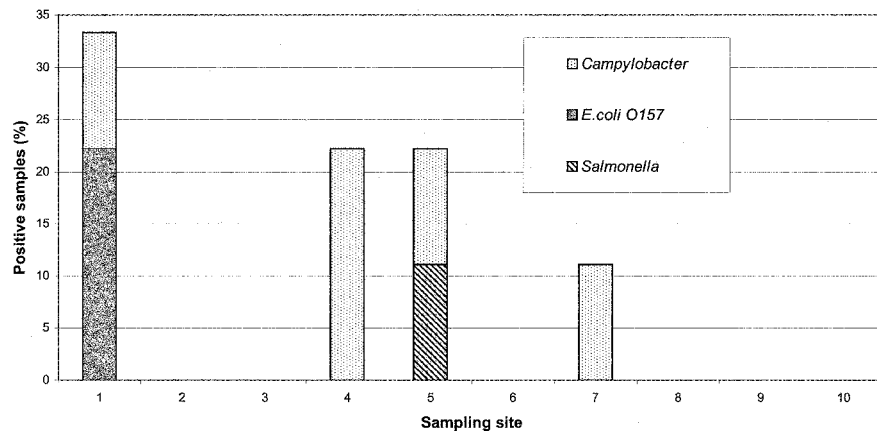


FIGURE 2. Incidences of foodborne pathogens at selected sites in sheep lairages: (1) unloading ramp (horizontal surface); (2) corner between unloading area and lairage pen (vertical surface); (3) pen walls (vertical surface); (4) pen floor (horizontal surface); (5) water trough (interior and water); (6) corner between lairage pen and race (vertical surface); (7) passageway floor (horizontal surface); (8) funnel walls (vertical surface); (9) gates between race and stunning box (vertical surface); and (10) stunning box floor (horizontal surface).

prevalence of pathogens on animal coats, and (ii) if significant pathogen exchange via coat-coat and/or coat-environment-coat routes took place just before slaughter, it could diminish or completely negate pathogen reductions achieved on farms.

One should keep in mind that abattoir lairages, like livestock markets, are places where, directly or indirectly, mixing of animals from different farms takes place, with potential negative consequences from the perspective of the epidemiology of zoonotic agents. Within a given pen, animals in the same group obviously exchange pathogens: cattle do lie down in lairages; 26% of them are lying after 3 h (12), and lairaging sometimes lasts for up to 27.5 h (5). However, some critical abattoir lairage contamination points identified in the present study (Figs. 1 and 2) can come in intimate contact with every single animal and consequently produce an “indirect mixing” of animals from different groups or farms that consecutively pass through the same premises on any given day. From a microbiological perspective, therefore, “mixing” of different groups may occur via critical contamination points even if different groups are kept physically separate for the entire unloading-to-slaughter period as required by good manufacturing practice/good hygiene practice and animal welfare principles. In addition, the results of this study show that lairage-mediated exchange of pathogens is possible even between groups of animals slaughtered on different days, because the contamination can be carried over from one processing day to the next (Table 1), despite the routine washing down of the lairage overnight. Although the results of the present study do not provide a direct explanation for the sources and/or routes of hide and pelt contamination, they indicate that the risks of contamination of the coats of previously pathogen-free animals with pathogens during the unloading-to-slaughter period can be high.

The results of this study and those of other recent studies (1, 6, 7, 11, 13–15) confirm at least qualitatively that the contamination of the preslaughter environment and the contamination of animal coats are very relevant meat safety issues, since the contamination rates of animal coats can determine the contamination rates of the carcasses. Further research is required, however, for a better understanding of the epidemiology of, and potential control strategies for, zoonotic agents during the unloading-to-slaughter phase. The main risk-assessment-based directions for future re-

search would include (i) the quantification of the relationship between lairage design and the prevalence of pathogens on animal coats, (ii) the quantification of the effects of animal behavior and activities during lairaging on the spread of pathogens, and (iii) the development of new critical control point(s) along unloading-to-slaughter route at abattoirs.

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