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Presence of *Salmonella* in the Red Meat Abattoir Lairage after Routine Cleansing and Disinfection and on Carcasses

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ABSTRACT

Foodborne pathogens, such as *Salmonella,* may remain in abattoir lairages after cleansing and pose a risk of transfer and contamination from one processing day to the next. These organisms may be transferred to the outer surface of animals held in lairage facilities, and the skin or hide may be a significant source of microbial contamination on the red meat carcasses subsequently produced. Sponge samples were taken from various sites in the lairage ($n = 556$), and single-pass sponge samples were taken from one side of red meat carcasses ($n = 1{,}050$) at five commercial abattoirs in Southwest England and tested for the presence of *Salmonella.* Of these, 6.5% of lairage samples were positive, containing estimated numbers of up to 10^4 *Salmonella* organisms per sampled area (50 by 50 cm). *Salmonella* was found on 9.6% of 240 lamb carcasses, 12.7% of 330 beef carcasses, 31% of 70 pig carcasses, 20% of 80 calf carcasses younger than 14 days of age, and none of 330 cull cow and bull carcasses. Subtyping divided the 137 isolates into seven serogroups and three pulsed-field gel electrophoresis clusters, and sensitivity testing against a bank of 16 antimicrobials indicated that 47 isolates had resistance to one or more antimicrobial agents. These results indicate that *Salmonella* contamination can persist in the lairage environment from one processing day to the next and that *Salmonella* is present on red meat carcasses, although the implications of residual lairage contamination on carcass meat microbiology are not clear from this study. Abattoir owners should take steps to reduce the level of contamination in their premises to prevent contamination from being carried over from one processing day to the next.

Zoonotic agents, such as *Salmonella,* can be carried asymptomatically in the intestines of healthy animals and are shed into the environment when feces are voided *(7, 13).* Animals sent for slaughter may contaminate the lairage holding and stunning areas by shedding human pathogens in their feces; contamination may also occur through the mechanical transfer of organisms carried on the animals' hides *(5, 23). Salmonella* may persist for several days in the environment *(24)* and can be transferred onto the hides and skins of animals in subsequent batches handled within the same facility *(5, 23).* In pigs, lairage contamination with *Salmonella* has been shown to be a source of intestinal colonization *(12).* Legislation and good manufacturing practices demand that abattoir operators take steps to limit contamination and that cleaning regimes be put into place in both the food processing area and the lairage. These cleaning regimes vary widely and may or may not entail the use of chemical cleaning products *(24).* ''Normal'' cleaning programs in United Kingdom lairages are ineffective in eliminating foodborne pathogens *(22),* and indeed, an improved cleaning regime implemented in a pig lairage in The Netherlands similarly did not eliminate *Salmonella* contamination *(27).*

Under new European Union legislation (EC 2073/2005) *(1),* red meat abattoir operators are required to test carcasses produced on their premises against criteria for the presence of *Salmonella.* There are few available data on the current isolation rate of *Salmonella* on red meat carcasses in the United Kingdom, because recent studies of red meat species have concentrated on fecal carriage or hide contamination. This study was carried out to establish current performance against the criteria for the isolation rate of *Salmonella* on red meat carcasses in United Kingdom abattoirs and to investigate the prevalence of *Salmonella* contamination in the preharvest areas, after routine cleaning operations, in various commercial abattoirs in the United Kingdom.

MATERIALS AND METHODS

Origin of samples. Five abattoirs participated in the study, and each was visited on two or more occasions to collect samples. Abattoir A was a medium-sized sheep and cattle facility, processing approximately 700 steers and heifers, 150 calves younger than 14 days of age, and 1,000 sheep each week. In this abattoir, routine cleaning of the stun boxes and roll-out area involved the use of pressure washing and quaternary ammonium cleaning products, while cleaning of the holding pens entailed the removal of soiled bedding with a pitchfork and scraper and then the addition of fresh straw bedding. At the end of each week, all bedding was removed, and the pens were steam cleaned and allowed to dry before fresh bedding was laid. Abattoir B was a small multispecies plant, processing 6 cattle, 10 pigs, and 10 sheep each week, and all areas were cleaned at the end of the processing day by using a pressure wash with a quaternary ammonium–based cleaning product. Ab-

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attoir C was a medium-sized multispecies plant, processing 1,000 pigs, 2,000 sheep, and 500 calves each week. The stun box was cleaned daily by pressure washing with a hypochlorite solution, while the cleaning regime for the holding pens involved the removal of soiled bedding and daily brushing, and the pens were pressure washed with a broad-spectrum virucidal disinfectant solution once weekly, on a rotational basis. Abattoir D was a large sheep and cattle plant, processing 1,500 steers and heifers and 5,000 sheep each week, and abattoir E was a medium-sized cull cow and bull plant, processing 800 cows and bulls each week. Although geographically distant, the same company owned abattoirs D and E, and the cleaning regimes were identical. Stun boxes were cleaned at the end of each working day with a pressure wash and then detergent foam. The box was then rinsed, and a terminal quaternary ammonium sanitizer was applied. The holding pens were pressure washed after each batch of animals, but no chemicals were used.

The stunning facilities used for cattle in abattoirs A, B, D, and E consisted of a race leading to an individual stunning box, from which the animal, once stunned, would roll out to be shackled and hoisted. In abattoir D, sheep were processed by a restrainer-conveyor system, with the stunned sheep rolling onto a bleeding table. In abattoirs A, B, and C, all the small species (sheep, pigs, and calves) were processed through a group stunning pen, where the stunned animal would fall to the floor of the group pen to be shackled and hoisted.

Collection of samples: environmental samples. A total of 556 samples were taken at these red meat abattoir lairages from the holding pens and stunning areas early in the morning before animals were delivered and processing commenced. The lairages had undergone routine cleansing operations at the end of the previous day's processing. Within the holding pens, samples were taken from the floors, walls, edges (two-dimensional corner between floor and wall), and corners (three-dimensional corner between the floor and two walls). In the stunning areas, samples were taken from the stun box walls, floors, and corners (three-dimensional corner between the floor and two walls) and from the roll-out ramp for cattle stun boxes or sheep restrainer-conveyor systems. Sample sites were chosen randomly. For edge or wall samples, the researchers took three paces along the wall of the pen and then sampled the area adjacent to their hands. For floors, the researchers took three paces into a pen and gently tossed the template onto the floor to choose the site. In large pens, up to three samples were similarly taken three paces apart.

Samples were collected with gauze swabs (Readiwipes Super, Robinson Healthcare 5345, Chesterfield, UK) presoaked in 100 ml of buffered peptone water (BPW; CM0509, Oxoid Ltd., Basingstoke, UK). Excess BPW was squeezed from the swab into the transport container, and the swab was rubbed vigorously over an area measuring 50 by 50 cm before being returned to the transport container. These were then stored on ice and returned to the laboratory within 2 h of collection.

Collection of samples: carcass samples. Carcasses of the red meat species commonly processed for human consumption in the United Kingdom were sampled after meat inspection, but before chilling, by a single-pass sponge swab technique. Proprietary cellulose sponges (20 by 20 cm; Spongyl 87, MapaSpontex Ltd., Worcester, UK) were cut into four pieces (10 by 10 cm), which were autoclaved at 121°C for 15 min. Each piece was then aseptically placed into a medium stomacher bag (BA6041, Seward, Worthing, UK) to form a single sponge swab. Immediately before use, the sponge was moistened with 10 ml of peptone salt broth (maximum recovery diluent, CM0733, Oxoid). The sponge was grasped through the bag, and the bag was inverted over the hand to expose the moistened sponge. The sponge was then passed with firm manual pressure over the entire carcass surface in a single sweep from hindquarter to shoulder, and the bag was pulled back over the hand to enclose the sponge. Samples were taken randomly from the ventral, dorsal, and lateral surfaces to ensure that all parts of the carcass were represented in the study. The exact area sampled was not determined, as microbial counts were not required in this part of the study.

TABLE 1. *Estimation of numbers of* Salmonella *organisms in the original sample*

Lowest dilution giving positive results	Interpretation
Neat	1–10 organisms in the sampled surface area
−1	10–100 organisms in the sampled surface area
−2	100–1,000 organisms in the sampled surface area
−3	1,000–10,000 organisms in the sampled surface area
−4	10,000–100,000 organisms in the sampled surface area

In total, 240 lamb carcasses, 330 steer and heifer carcasses, 330 cull cow and bull carcasses, 70 pig carcasses, and 80 calf carcasses were sampled. The samples were taken over several months of multiple visits to each of the five abattoirs and were taken at different times during the processing day.

Sample processing: environmental samples. On return to the laboratory, the transport containers containing the swab and BPW were vigorously shaken, and 10 ml was decanted into a universal container (UNI1). The original samples were then refrigerated at 4°C. From UNI1, decimal dilutions made in BPW were incubated for 24 h at 37°C. After this enrichment phase, 0.1 ml was taken from UNI1 and from the refrigerated original sample, inoculated into DIASALM selective enrichment medium (1.09803, Merck, Hoddesdon, UK), and incubated at 41.5°C for 24 h. The original sample and the dilution series were refrigerated at 4°C. On day 3, a 10-μl loopful of growth from each of the DIASALM plates was streaked onto Rambach chromogenic agar (1.07500, Merck) and incubated at 37°C for 24 h for identification of *Salmonella.*

When cultures yielded presumptive *Salmonella* colonies on Rambach agar (cerise-colored colonies), 0.1 ml of the associated refrigerated dilution series was enriched and plated with DIASALM and Rambach agar as outlined above. Numbers of *Salmonella* in the original sample could then be estimated on the basis of the lowest dilution at which *Salmonella* isolates were identified on Rambach agar (Table 1). Presumptive *Salmonella* isolates were confirmed with API 20E strips (no. 20100, bioMérieux, Basingstoke, UK). The proportions of samples containing *Salmonella* were calculated and compared by the χ^2 test by means of MINITAB software (Minitab Inc., State College, Pa.).

Sample processing: carcass samples. The samples were stored on ice packs, returned to the laboratory within 2 h of collection, and processed in the following manner. Ninety milliliters of BPW was added to the bag containing the sponge, which was then massaged vigorously by hand for 1 min. The BPW was next transferred to a sterile jar and incubated at 37°C for 24 h. Thereafter, 0.1 ml of the incubated BPW was transferred into 10 ml of

Rappaport-Vassiliadis Soya Peptone broth (CM0866, Oxoid) and incubated for 24 h at 41.5°C, after which time 1 ml was transferred into Müller-Kauffmann tetrathionate-novobiocin broth (CM1048, Oxoid) and incubated at 37°C for 24 h. Thereafter, 10 μl of this enrichment broth was plated onto brilliant green agar (CM0263, Oxoid) and xylose-lysine-deoxycholate agar (CM0469, Oxoid). After 24 h of incubation at 37°C, presumptive *Salmonella* colonies identified on these agars were confirmed with API 20E kits (20100, bioMérieux).

Typing of isolates. All *Salmonella* isolates were serogrouped and tested by disk diffusion for resistance to a panel of 16 antimicrobial agents: amikacin, ampicillin, amoxicillin-clavulanic acid, apramycin, chloramphenicol, ceftazidime, ciprofloxacin, cefotaxime, furazolidone, gentamicin, neomycin, nalidixic acid, streptomycin, compound sulfonamides, sulfamethoxazole-trimethoprim, and tetracycline. Selected isolates from within the broad groupings thus determined were then fully serotyped, and a further subset underwent pulsed-field gel electrophoresis (PFGE) analysis and plasmid profiling according to the following procedures. (i) PFGE was performed according to the ''One-Day (24–28 h) Standardized Laboratory Protocol for Molecular Subtyping of Nontyphoidal *Salmonella* by PFGE'' described by PulseNet (Centers for Disease Control and Prevention, Atlanta, Ga.) *(4).* A single colony of each isolate was streaked on tryptic soy agar (TSA) and incubated overnight at 37°C. With a cotton swab, a portion of the growth on the agar plate was transferred to 2 ml of cell suspension buffer (100 mM Tris–100 mM EDTA [pH 8.0]), and the cell concentration was adjusted to 0.48 to 0.52 optical density in a Dade Microscan Turbidity Meter (Dade Behring, Deerfield, Ill.). Thereafter, 400 μl of this cell suspension was transferred to a 1.5-ml microcentrifuge tube containing 20 μl of proteinase K (20 mg/ml), mixed with 400 μl of melted 1% SeaKem Gold (Cambrex, East Rutherford, N.J.)–1% sodium dodecyl sulfate agarose prepared with TE buffer (10 mM Tris–1 mM EDTA [pH 8.0]), and pipetted into disposable plug molds. Three plugs were transferred to 50-ml polypropylene screw-cap tubes containing 5 ml of cell lysis buffer (50 mM Tris–50 mM EDTA [pH 8.0] plus 1% sarcosyl) and 25 μl of proteinase K (20 mg/ml) and incubated at 54°C in a shaking water bath for 2 h. Thereafter, the plugs were washed twice with 15 ml of sterile water and three times with TE buffer at 50°C for 15 min. Chromosomal DNA was digested with 50 U of *Xba*I (Promega, Southampton, UK). PFGE was performed on a CHEF DRIII system (Bio-Rad Laboratories, Hercules, Calif.) in 0.5× Tris-borate-EDTA (TBE) extended-range buffer (Bio-Rad) with recirculation at 14°C. DNA macrorestriction fragments were resolved on 1% SeaKem Gold Agarose (Cambrex) in 0.5× TBE buffer. DNA from *Salmonella* Braenderup H9812 restricted with *Xba*I was used as a size marker. Pulse times were ramped from 2.2 to 63.8 s during an 18-h run at 6.0 V/cm. Macrorestriction patterns were compared by BioNumerics software (Applied Maths, Sint Martens-Latem, Belgium). Different profiles were designated with the letter X (*Xba*I types) in accordance with the restriction patterns. (ii) Plasmid DNA was isolated by the alkaline lysis method as described previously *(14).* Samples were analyzed by electrophoresis in 1× TBE buffer at 150 V for 4.5 h on 0.8% agarose gels with recirculation at 20°C. Plasmid-containing *Escherichia coli* strain 39R861 and a supercoiled DNA ladder (GIBCO BRL, Paisley, UK) were used as size markers. Plasmids were compared by BioNumerics software. The molecular weights of the plasmids were calculated by comparison with the external markers, and images were normalized accordingly.

RESULTS

Environmental samples. Overall, 36 (6.5%) of the 556 samples were positive for *Salmonella,* with populations ranging from <10 to 10,000 (Table 2). No *Salmonella* organisms were found on the stun box walls or roll-out ramps. High levels of *Salmonella* were not associated with any particular sampling site, but positive samples originated from sites where the swab collected visual contamination or where the integrity of the surface sampled had been broken because of the corrosion of metal or the shattering of concrete. These areas were prone to insufficient cleaning, where either physical removal of bedding from pens was the cleaning method employed or damage to the corners and edges of the lairage and preslaughter areas allowed contamination to collect and be bypassed during cleaning.

Holding pens from abattoirs B and E yielded no *Salmonella*-positive samples, whereas those from abattoirs A, C, and D had isolation rates of 7.8% (5 of 64), 11.1% (7 of 99), and 13.2% (10 of 76), respectively. The overall isolation rate of *Salmonella* contamination in the holding areas was 10%, and the walls were the least often contaminated.

In cattle stun boxes, 7.8% of 102 samples were positive for *Salmonella,* and the corners (16.7% [4 of 24]) and floors (10.3% [4 of 39]) were more likely to harbor contamination than the walls (0 of 30). In abattoir A, 4 of 10 samples taken from the stun box floor and corner were contaminated. In small animal species, 2 (4.8%) of 42 samples taken from the stun box floor and corner yielded *Salmonella.*

The overall isolation rate for *Salmonella* from the lairages of abattoirs B (0%) and E (2.9%) was significantly lower ($P < 0.05$) than that for abattoirs A (9.3%), C (10.1%), and D (9.5%), and the isolation rate for *Salmonella* from holding pen walls (1%), stun box walls (0), and roll-out ramps (0) was significantly lower ($P < 0.01$) than from the other sites sampled. There were no statistically significant differences between the isolation rates from holding pen floors (10%), holding pen corners (9.2%), holding pen edges (13.8%), stun box floors (cattle, 10.3%; small species, 3.8%), and stun box corners (cattle, 10%; small species, 6.3%). In abattoir A, no significant difference was seen between the isolation rate for *Salmonella* from holding pen floors (15%) and small species stun box floors (16.7%), while those from the cattle stun box floor (33.3%) and cattle stun box corner (50%) were significantly higher ($P < 0.05$). In abattoir E, no significant difference was seen between isolation rates from cattle stun box corners (10%) and cattle stun box floors (13.3%). While all holding pen samples were negative, in abattoir D, the isolation rates from the corners of the holding pens (30.8%) and the cattle stun box (25%) and the holding pen edge (23.1%) were each significantly greater than those from the floors at each site (8% on stun box floors and 0% on holding pen floors) ($P < 0.01$). In abattoir C, no significant difference in isolation rate was observed between the holding pen floors and edges (20 and 23%, respectively), while the small species stun box corner was more highly contaminated than the floor (20 versus 0%) ($P < 0.01$).

TABLE 2. *Environmental samples taken at each abattoir, showing numbers of positive samples with estimated numbers of* Salmonella *isolates in each positive sample indicated in parentheses and percentages indicated in braces*[a]

	Abattoir A		Abattoir B		Abattoir C		Abattoir D		Abattoir E		Total		
Sample site	Samples taken	Positive samples	Samples taken	Positive samples	Samples taken	Positive samples	Samples taken	Positive samples	Samples taken	Positive samples	Samples taken	Positive samples	% positive
Holding pen wall	20	0	15	0	25	0	25	1 (1–10) {4%}	15	0	100	1 (1–10)	1
Holding pen floor	20	3 (10–100) {15%}	15	0	25	3 (10–100) 2 (1–10) {20%}	25	1 (1–10) 1 (10^2–10^3) {8%}	15	0	100	1 (10^2–10^3) 6 (10–100) 3 (1–10)	10
Holding pen corner	12	1 (10–100) 1 (10^2–10^3) {16.7%}	5	0	23	0	13	4 (1–10) {30.8%}	12	0	65	4 (1–10) 1 (10–100) 1 (10^2–10^3)	9.2
Holding pen edge	12	0	4	0	26	5 (1–10) 1 (10^3–10^4) {23.1%}	13	1 (1–10) 1 (10–100) 1 (10^2–10^3) {23.1%}	10	0	65	6 (1–10) 1 (10–100) 1 (10^2–10^3) 1 (10^3–10^4)	13.8
Stun box wall	6 beef 6 sheep	0 0	12 beef 11 smalls	0 0	6 smalls	0	6 beef 12 sheep	0 0	15	0	39 beef 17 smalls 18 sheep	0	0
Stun box floor	6 beef 6 sheep	2 (10–100) {33.3%} 1 (1–10) {16.7%}	12 beef 11 smalls	0 0	9 smalls	0	6 beef	0	15	1 (10^2–10^3) 1 (1–10) {13.3%}	39 beef 20 smalls 6 sheep	1 (1–10) 2 (10–100) 1 (10^2–10^3) 1 (1–10)	10.3 (beef) 3.8 (smalls)
Stun box corner	4 beef 4 sheep	1 (1–10) 1 (10–100) {50%}	6 beef 7 smalls	0 0	5 smalls	1 (10^2–10^3) {20%}	4 beef	1 (10^2–10^3) {25%}	10	1 (10–100) {10%}	24 beef 12 smalls 4 sheep	1 (1–10) 2 (10–100) 1 (10^2–10^3) 1 (10^2–10^3)	10 (beef) 6.3 (smalls)
Roll-out ramp	12 beef	0	11 beef	0			8 beef 24 sheep	0 0	12	0	43 beef 4 sheep	0	0
Total											556		

[a] Smalls, small species (pigs and sheep in abattoir B; pigs, sheep, and calves in abattoir C).

TABLE 3. *Positive carcass samples/samples taken at each abattoir*

	Abattoir:					
	A	B	C	D	E	Total
Sheep	13/40	0	10/80	0/120	0	23/240 (9.58%)
Pig	0	11/20	10/50	0	0	21/70 (31%)
Steer and heifer	0/10	0/20	0	42/300	0	42/330 (12.73%)
Cow and bull	0	0	0	0	0/330	0/330 (0%)
Calves, <14 days old	8/50	0	8/30	0	0	16/80 (20%)
Total	21/100 (21%)	11/40 (27.5%)	28/160 (17.5%)	42/420 (10%)	0/330 (0%)	

Carcass samples. A total of 23 (9.6%) of 240 lamb carcasses were positive for *Salmonella,* as were 42 (12.7%) of 330 steer and heifer carcasses, 0 (0%) of 330 cull cow and bull carcasses, 21 (31%) of 70 pig carcasses, and 16 (20%) of 80 calf carcasses younger than 14 days of age (Table 3). *Salmonella* was isolated from carcasses at each abattoir. The rate of *Salmonella* contamination on carcasses varied markedly between visits, with several visits (13 of 27) yielding no *Salmonella* isolates and other visits showing 71% of carcasses to be positive (data not shown). The overall isolation rate for *Salmonella* on carcasses from abattoirs A, B, and C was not statistically different, being between 17.5 and 28%, while the isolation rate of *Salmonella* on carcasses from abattoir D, the largest and most highly mechanized facility, was less (10%) than in the smaller, more traditional abattoirs ($P < 0.05$). No positive samples were found in abattoir E.

Typing of isolates. Seven serogroups of *Salmonella* were found among the isolates (Table 4). Serogroup C1 was the most predominant, consisting of 40 isolates—12 from the lairage of abattoir A, 10 from the lairage of abattoir D, and 18 from beef carcasses in abattoir D. All of these isolates were sensitive to all 16 antimicrobials tested. Serogroup *C1 (initially ROUGH serogroup) was also recovered from six beef carcasses in abattoir D, and these isolates also demonstrated sensitivity to the range of antimicrobials tested. Thirteen isolates of serogroup C1 and *C1 from abattoir D were subtyped as *Salmonella* Mbandaka and belonged to the same PFGE cluster (i.e., cluster 4), with plasmid profile clusters (4a, 4b, and 4c) differing by one band only (Fig. 1).

Thirty-two isolates belonged to the ROUGH serogroup. Six of these were subtyped as Orough:i:l,w, all of which were nalidixic acid resistant and devoid of plasmids; all belonged to PFGE cluster 3. ROUGH isolates were found in abattoirs A, C, and D but on the carcasses only, not in the environment. All carcass species were represented. Seven ROUGH isolates from beef carcasses in abattoir D showed no resistance to any of the antimicrobials tested, and two of these were subtyped as Orough:z10:e,n,z_{15}. These belonged to PFGE cluster 4 and plasmid profile cluster 4b, suggesting that they were genetically similar to the *Salmonella* Mbandaka found on beef carcasses at the same abattoir, during the same visit.

Serogroup B was found in abattoirs A (one calf carcass—*Salmonella* Kimuenza, sensitive to all 16 antimicrobials), B (11 pig carcasses—*Salmonella* Typhimurium DT208, tetracycline resistant), and C (nine pig carcasses, one sheep carcass, and seven environmental samples). Isolates from abattoir C were identified as *Salmonella* Derby and belonged to PFGE cluster 2 and plasmid profile clusters 2a and 2b. Some were sensitive to all antimicrobials tested, but others, including five from pig carcasses, one from a sheep carcass, and one from the environment, were resistant to sulfamethoxazole-trimethoprim and compound sulfonamides.

Abattoir C yielded *Salmonella* Dublin (serogroup D) on both calf and sheep carcasses on the same processing day. Sixteen isolates of serogroup D were found—eight each on calf and sheep carcasses. All were sensitive to the 16 antimicrobials tested and belonged to PFGE clusters (1A, 1B, and 1C) and plasmid profile clusters (1a and 1b) that differed by one band only.

Nine isolates of serogroup E1 were identified, which were from the lairage environment of abattoirs C and E and from the calf carcasses in abattoir A. These were subtyped as *Salmonella* Anatum, and all lairage isolates were sensitive to all 16 antimicrobials tested, while the six carcass isolates were resistant to chloramphenicol.

There was one isolate of serogroup C3—a sulfamethoxazole-trimethoprim and compound sulfonamide-resistant strain of 8,20:−:z_6 from the holding pen floor of abattoir C—and one isolate of serogroup O61—a fully sensitive strain of IIIb O61:−:1,5,7, normally associated with sheep, also from the holding pen in abattoir C but found in the two-dimensional corner between the floor and wall.

Initially we had hoped to investigate the relationships between strains isolated from carcasses and strains isolated from the lairage, but ultimately, too few isolates were found that coincided between the two groups. On only two occasions were similar isolates recovered from the lairage environment prior to the animals arriving and the carcasses produced during subsequent processing, namely (i) isolates of *Salmonella* Mbandaka found in the lairage of abattoir D in the early morning of 12 August 2004 and on beef carcasses that same day and (ii) isolates of the same serovar at the same abattoir found in the lairage on the evening of 5 December 2005 and on beef carcasses produced on the morning of 13 May 2005 (Table 4).

Of the 137 isolates, 90 (65.7%) were sensitive to all

TABLE 4. Salmonella *isolates: results of subtyping*[a]

Abattoir	Date of sampling (mo/day/yr)	Sample site	Serogroup	Antimicrobial resistance	*Salmonella* subtypes	PFGE cluster	Plasmid profile cluster
A	11/10/04	Sheep carcass (13)	ROUGH	NAL	1× ROUGH	—	—
					1× Orough:i:l,w	3	None
	11/18/04	Holding pen corner (2)	C1	A/S	1× Mbandaka	—	—
		Holding pen floor (5)	C1	A/S	—	—	—
		Stun box corner (2)	C1	A/S	—	—	—
		Stun box floor (3)	C1	A/S	1× Infantis	—	—
		Calf carcass (9)	ROUGH (2)	NAL	1× Orough:i:l,w	3	None
			B (1)	A/S	1× Kimuenza	—	—
			E1 (6)	A/S (2)	1× Anatum	—	—
				C10 (4)	2× Anatum	—	—
B	02/07/05	Pig carcass (11)	B	TET	2× Typhimurium DT208	—	—
C	02/05/05	Holding pen edge (1)	O61	A/S	IIIb O61:−:1,5,7	—	—
	02/17/05	Pig carcass (10)	B (9)	SXT/SU (5)	2× Derby	2	2a
						2	2b
				A/S (4)	2× Derby	2	2a
						2	2b
			ROUGH (1)	A/S	—	—	—
	03/10/05	Calf carcass (8)	D	A/S	2× Dublin	1A	1a
		Sheep carcass (10)	D (8)	A/S	2× Dublin	1C	1a
						1B	1b
			B (1)	SXT/SU	Derby	2	2a
					One isolate, no data shown		
	03/12/05	Holding pen edge (5)	B	A/S (4)	2× Derby	2	2b
				SXT/SU (1)	Derby	2	2a
		Holding pen floor (3)	B (2)	A/S	—	—	—
			C3 (1)	SXT/SU/S13	8,20:−:z_6	—	—
		Stun box corner (1)	E1	A/S	Anatum	—	—
D	12/01/04	Holding pen corner (1)	C1	A/S	Mbandaka	4	4a
		Holding pen edge (2)	C1	A/S	—	—	—
	12/08/04	Holding pen corner (3)	C1	A/S	1× Mbandaka	4	4b
		Holding pen edge (1)	C1	A/S	—	—	—
		Beef carcass (30)	C1 (8)	A/S	2× Mbandaka	4	4b
					1× 6,7:−:nonmotile	—	—
			*C1 (6)	A/S	3× Mbandaka	4	4b
			ROUGH (14)	NAL (8)	3× Orough:i:l,w	3	None
				A/S (6)	2× Orough:z10:e,n,z_{15}	4	4b
					Two isolates, no data shown		
	02/03/05	Beef carcass (2)	ROUGH	A/S (1)	—	—	—
				NAL (1)	Orough:i:l,w	3	None
	04/05/05	Stun box corner (1)	C1	A/S	—	—	—
	05/12/05	Holding pen floor (2)	C1	A/S	2× Mbandaka	4	4a
						4	4c
	05/13/05	Beef carcass (10)	C1	A/S	4× Mbandaka	4	4b
E	04/05/05	Stun box floor (1)	C1	A/S	4× Mbandaka	4	4b
	05/12/05	Stun box floor (1)	E1	A/S	Anatum	—	—
			E1	A/S	Anatum	—	—

[a] Numbers in parentheses are number of isolates; 1×, 2×, 3×, number of isolates tested and found to be of that subtype. NAL, nalidixic acid; A/S, ampicillin-sulbactam; Cl0, chloramphenicol, 10 μg; TET, tetracycline; SXT, sulfamethoxazole-trimethoprim; SU, compound sulfonamides; S13, streptomycin (13-mm inhibition zone, borderline sensitivity); —, analysis not carried out on this isolate.

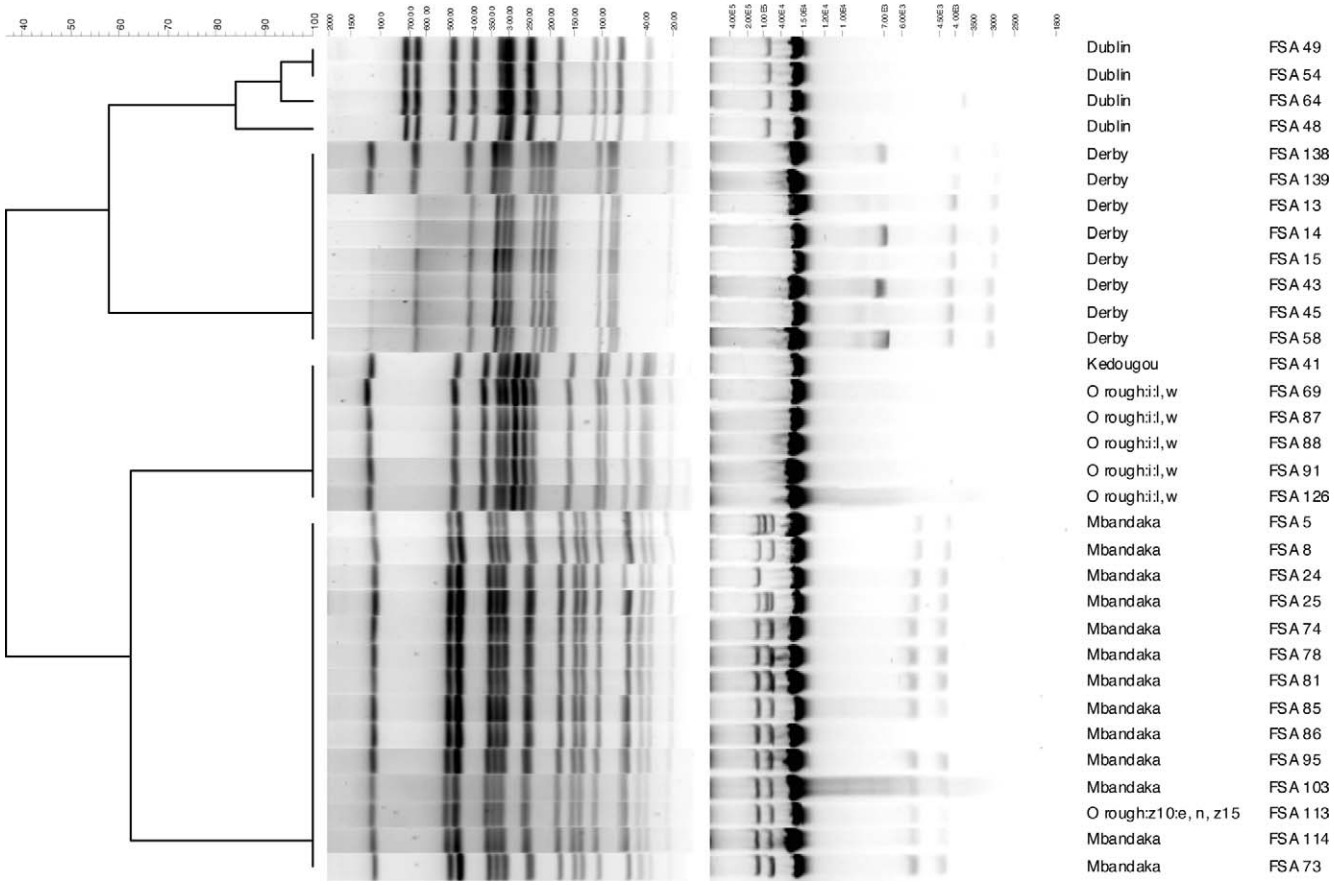

FIGURE 1. *Dendrogram of PFGE and plasmid profiling results.*

16 antimicrobials tested, 24 (17.5%) were resistant to nalidixic acid, 11 (8%) were resistant to tetracycline, 8 (5.8%) were resistant to compound sulfonamides and sulfamethoxazole-trimethoprim, and 4 (3%) were resistant to chloramphenicol.

DISCUSSION

In this study, *Salmonella* was readily isolated from red meat abattoir lairages and carcasses of red meat species in the abattoirs sampled. These findings show that routine cleansing operations in the lairage do not remove *Salmonella* from the environment, a conclusion in broad agreement with that of previous studies *(22, 27).* The organism can remain in holding pens in significant numbers of up to 10^4 organisms per sample. Abattoirs B and E yielded no *Salmonella*-positive samples in the holding pens, suggesting that the cleaning regime in these lairages was sufficient to remove contamination, but in abattoir D, where the cleaning regime was identical to that in abattoir E, the isolation rate for *Salmonella* from holding pens (13.2%) was similar to that in abattoirs A and C (7.8 and 11.1%, respectively) ($P > 0.1$). Within individual abattoirs, the contamination of a particular sampling site in the holding pens was as high as 30.8% (holding pen corners, abattoir D). At this particular abattoir (abattoir D), the pens drained from the corners, and it is possible that contamination gathered around the drains. Contamination of the floor and corners in the cattle stun box in abattoir A was high (40% [4 of 10]), but this facility in particular showed heavy corrosion in the corners and wear to the floor, where contamination may have been harbored. The *Salmonella* isolation rate in the stunning pens was lower for small species (4.8%) than for cattle (16.7%), which was not expected, as pigs and calves are more likely than cattle to excrete *Salmonella.* However, the number of samples taken in these areas was relatively low; this may be an artifact of sample size rather than an indication that cleaning was more effective in small species stunning facilities than in those for cattle. The holding pen walls sampled in this study yielded no isolates of *Salmonella.* This may have been a result of the reduced accumulation of feces or the desiccation of material seen adhering to the walls in many instances, although *Salmonella* can survive for up to 6 years in dried feces *(19).* Several walls were constructed of galvanized steel, and an antimicrobial effect of free zinc (Zn^{2+}) ions may have contributed to the decreased persistence of these organisms. Other authors have reported the decreased survival of *Salmonella* and other foodborne pathogens on galvanized metal and under dry conditions *(24, 32).* Nevertheless, the persistence of *Salmonella* in lairage holding pens is a cause for concern, as organisms present in the environment could be transferred to animals processed through the lairage and stunning facility and then to subsequent carcasses *(11, 17, 25).*

Each abattoir yielded a particular population of *Salmonella* strains. *Salmonella* Derby and *Salmonella* Dublin were isolated only in abattoir C, which processes sheep, calves, and pigs. *Salmonella* Derby was the second most

commonly isolated *Salmonella* serovar in pigs in Great Britain in 2004 and the fourth most common serovar from sheep *(15).* Carcasses of both species yielded *Salmonella* Derby in abattoir C. *Salmonella* Dublin, which ranks third in incidence in sheep, was isolated from sheep carcasses, but in this case, cross-contamination from calves, which were processed immediately before the sheep and also yielded similar isolates, was a more likely source than the sheep themselves, with *Salmonella* Dublin being the most common serovar found in British cattle. Tetracycline-resistant *Salmonella* Typhimurium DT208 was isolated from pig carcasses during one visit to abattoir B, suggesting carriage in the group of pigs being processed *(3). Salmonella* Kimuenza and *Salmonella* Infantis were found only in abattoir A, which processes sheep, cattle, and calves. *Salmonella* Anatum was found in abattoirs A (sheep, cattle, and calves), C (sheep, calves, and pigs), and E (cattle only). *Salmonella* Mbandaka was found in abattoirs A and D, both of which process ruminants only. In abattoir D, strains of *Salmonella* Mbandaka PFGE/plasmid cluster 4/4a were isolated from the holding pens both in December 2004 and May 2005, while cluster 4/4b isolates were recovered from the holding pens and beef carcasses during one visit, 1 week after the December isolation of cluster 4/4a. These clusters differed by one band alone, suggesting that this small genetic difference is partly responsible for the enhanced persistence of this isolate in the environment. Environmentally persistent strains would likely contaminate subsequent animals during processing, while other strains are likely to enter the abattoir regularly on incoming animals. Similarly, in May 2005, plasmid clusters 4/4a and 4/4c were found in the holding pens in abattoir D on the evening before cluster 4/4b was isolated from the beef carcasses processed there, providing stronger evidence that *Salmonella* Mbandaka was being transferred from the environment to the resultant carcasses. Rostagno et al. *(20)* found similar evidence of environmental contamination matching carcass contamination in a pig slaughterhouse.

Nalidixic acid–resistant isolates of Orough:i:l,w belonging to PFGE/plasmid cluster 3/0 were found on carcasses in abattoirs A and D but not in the environment. This strain may be less persistent than others found in the lairage, such as *Salmonella* Mbandaka, or it may be present in the slaughter environment rather than in the lairage. In abattoir A, this strain was found on sheep carcasses on one sampling occasion in November 2004 and then on beef carcasses 1 week later, while in abattoir D, it appeared on beef carcasses in December 2004 and again in February 2005. As the strain was not found in the lairage environment, it is possible that the organism arrived at the abattoir associated with a particular producer's animals and that this producer was supplying animals to both affected abattoirs. Rough strains, however, are more often associated with environmental contamination than with animal carriage; hence, the reservoir may be within the slaughterhouse rather than the lairage. Isolates of *Salmonella* Derby PFGE/plasmid cluster 2/2a that were resistant to both compound sulfonamide and sulfamethoxazole-trimethoprim were found in abattoir C on three separate occasions, on pig carcasses in February 2005, on sheep carcasses in March 2005, and in the holding pens 2 days later in March 2005. The strain may be regularly arriving with animals from a particular farm, but environmental isolation suggests that the organism also persists in the lairage and presents a potential source of contamination to subsequent groups of animals.

A chloramphenicol-resistant strain of *Salmonella* Anatum was isolated from calf carcasses on one occasion. Chloramphenicol has often been used in calf-rearing establishments in the United Kingdom to treat respiratory illness *(8)* but has been banned in the European Union for many years. Chloramphenicol resistance in *Salmonella,* however, appears to persist independently of usage, and it is not thought that the use of an analog drug, florfenicol, is responsible for selection of chloramphenicol resistance *(28).*

The isolation rate for *Salmonella* from carcasses in this study was relatively high (9.6% of lamb carcasses, 12.7% of steer and heifer carcasses, 31% of pig carcasses, and 20% of calf carcasses) compared with other reports, including 1.3% *(2)* to 7.6% *(18)* for cattle, 1.5% *(6)* to 10% *(21)* for sheep carcasses, 17.5% for pork *(16),* and 4.1% for veal *(16).* It may be that the United Kingdom has a higher isolation rate for *Salmonella* than other countries or that the sampling technique used allows far greater recovery, but it is likely that the results obtained in the present study are an artifact of the limited size of the study. Importantly, carcasses produced in the United Kingdom undergo no decontamination treatment, in accordance with European Union requirements, with our carcasses sampled before any final potable water wash and before chilling, whereas a number of published studies refer to carcasses that have been sampled after chilling. Therefore, the bacterial load of these carcasses is likely to be somewhat greater than that found on carcasses sampled after decontamination. Various serovars were found on carcasses and in the environment, of which *Salmonella* Infantis was the only member of the top 10 *Salmonella* organisms associated with human illness in Great Britain in 2004 *(15).* Although *Salmonella* Typhimurium ranks second, DT208—the strain isolated in this study—is not among the top 10 *Salmonella* Typhimurium DT strains causing human illness.

On individual sampling visits, the isolation rate for *Salmonella* from carcasses varied from 0 to 71%. Nearly half the visits yielded negative results for *Salmonella,* whereas 15% of the visits yielded isolation rates of 33% or more, with positive carcasses often clustered temporally (data not shown). This clustering suggests that the batch of animals slaughtered at that time were all contaminated, but given that sampling was relatively random, not necessarily taking consecutive carcasses, it is likely that the carcasses represented different producer groups. Thus, the clustering of positive carcasses is more likely a result of cross-contamination during dressing. Cross-contamination during processing is well documented and can occur via the workers and their implements *(10, 26, 31).* That almost 50% of sampling visits yielded no *Salmonella* when up to 60 carcasses were sampled has implications for the development of sampling programs and performance criteria for *Salmonella* surveillance in red meat carcasses. Although poor hygienic

practices were found in abattoir E during seven sampling visits, as evidenced by the visible fecal contamination on the carcasses at the end of the slaughter line, no positive carcasses were found in this abattoir, which suggests that positive carcasses were most likely missed. A higher *Salmonella* isolation rate would be expected from cull cow and bull carcasses than from prime beef carcasses, as older animals, particularly from the dairy industry, often have high fecal carriage rates *(9, 29, 30).* Also, at the time of the study, in the United Kingdom, no cull cattle over the age of 30 months were permitted to enter the human food chain, and although they were slaughtered, dressed, and inspected under normal conditions, the carcasses were destroyed, with little attention given to hygienic practices in the cull slaughter facilities.

A larger, random survey would correct for clustering by abattoir and slaughter day or batch and would probably also give a lower overall prevalence. If isolation rate criteria were set on the basis of the isolation rate found in this study in abattoir E, i.e., zero or very low, there could be serious consequences for an abattoir sampling when a much higher isolation rate is found.

This study shows that the cleaning regimes used in lairages at United Kingdom red meat abattoirs are often insufficient to remove *Salmonella* contamination from the holding pens and stun boxes. As a result, there is a risk of *Salmonella* persisting in the environment and potentially contaminating animals and carcasses processed on the same and subsequent days. Abattoir managers should ensure that the state of repair of the facility is such that cleaning can be carried out effectively. In addition, systems for monitoring the effectiveness of cleaning and decontamination need to be established.

A significant proportion of red meat carcasses coming from United Kingdom abattoirs may be contaminated with *Salmonella* on a particular processing day, but the incidence of strains commonly associated with human illness appears to be low in this small study. *Salmonella* contamination rates on carcasses from individual abattoirs are highly variable and are probably influenced initially by the *Salmonella* carriage rate for the incoming animals on any particular day and, subsequently, by the standard of hygiene during processing. Identification of groups of animals that carry *Salmonella* in their gastrointestinal tracts and on their hides would allow these animals to be slaughtered separately, thus reducing the introduction of the organism into the abattoir environment. The use of strict hygienic measures to reduce cross-contamination is also recommended to decrease the *Salmonella* isolation rate from carcasses.

The results of this study clearly show that care is needed when implementing surveillance programs and setting criteria for the isolation rate of *Salmonella* on red meat carcasses, as the isolation rate can vary widely between abattoirs and between processing days. Day-to-day variability in test results has important implications regarding enforcement of the new European Union legislation *(1)* for *Salmonella* testing in red meat plants, with study required to understand the role of persistent colonization of the lairage in contamination of carcasses during processing.

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