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Prevalence of Salmonellae, Listeriae, and Yersiniae in the Slaughterhouse Environment and on Work Surfaces, Equipment, and Workers

MICHELA LUCIA SAMMARCO,[1] GIANCARLO RIPABELLI,[1] ADDOLORATO RUBERTO,[2] GIORGIO IANNITTO,[2] and GUIDO MARIA GRASSO[1]*

[1]*Chair of Hygiene, Department of Animal, Plant and Environmental Science, Faculty of Agriculture, University of Molise, 86100 Campobasso, Italy; and* [2]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale," Diagnostic Unit of Campobasso, 86100 Campobasso, Italy*

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ABSTRACT

In 1995 and 1996 a nine-month study was carried out in 11 pig abattoirs located in the Molise region (Italy) to evaluate the degree of contamination of the slaughterhouse environment, work surfaces, equipment, and personnel by *Salmonella* spp., *Listeria* spp., and *Yersinia* spp. A total of 219 samples were taken over three replications including slaughtering floor and wall, hooks, worktables, chopping blocks, knives, cleavers, dehairing devices, hands of personnel, clothing, hand-wash basins, and cold room handles, floor, wall, and hooks. Overall, six abattoirs (54.5%) had one or more positive sites, while only 14 of the 219 sites (6.4%) tested were positive for any of considered microorganisms. *Salmonella* spp. were isolated from 1 of 9 cleavers (11.1%), 1 of 16 worktables (6.25%), and 1 of 18 slaughtering floors (5.6%). *Yersinia enterocolitica* was found on 3 slaughtering floors (16.7%) and on 2 worktables (12.5%). *Yersinia kristensenii* was detected on 2 slaughtering floor swabs (11.1%). *Listeria monocytogenes* was isolated from 2 of 20 cold room floor swabs (13.3%) and from 1 of 14 hand-wash basins (7.1%). Other species of *Listeria* were detected on slaughtering wall and floor swabs and on chopping blocks. Our study indicates that slaughtering floors, cold room floors, and worktables are important sites in abattoirs that may possibly harbor pathogens like *Salmonella* spp., *Yersinia enterocolitica,* and *Listeria monocytogenes,* and that cleaning and sanitizing of the slaughterhouse environment and equipment need a greater emphasis.

Key words: *Salmonella* spp., *Listeria* spp., *Yersinia* spp., slaughterhouse, environmental sampling

The muscle tissues of healthy living animals are usually free from microorganisms, and their contamination during slaughtering is undesirable but cannot be avoided in the transformation of live animals into meat. Contamination occurs mostly by means of the animal exterior surface, the gastrointestinal tract, and the introduction of pathogens onto the meat surfaces during slaughtering, handling, cutting, processing and storage *(4, 14, 19, 20).* During slaughtering, transfer of microorganisms continues from carcasses to hands of workers and equipment surfaces, and from them to other carcasses. Thus, it is very important that slaughter follow sanitary guidelines and that hazard analysis critical control point (HACCP) programs guarantee the raw products against further contamination. Nevertheless, few studies have been carried out to evaluate the degree of contamination of abattoirs *(8, 11, 15).*

Historically, poultry, red meat, and meat products have been considered the primary source of foodborne salmonellae *(21, 22). Yersinia* has a wide distribution in nature, and numerous authors have determined swine to be a natural reservoir for pathogenic serotypes of *Yersinia enterocolitica (1)*; meat and meat products could be involved in the transmission of this pathogen. *Listeria monocytogenes* is presently one of the bacteria of most concern in the meat industry. Until the last decade or so, few individuals in the food industry were familiar with this organism. Then, a series of listeriosis outbreaks caused by contaminated foods brought *L. monocytogenes* to the forefront as an important foodborne pathogen with increasing evidence that contamination of foods is more likely to originate from environment *(5, 9).*

The objectives of the present study were to determine the prevalence of *Salmonella* spp., *Listeria* spp., and *Yersinia* spp. in the environment and on work surfaces, equipment, and workers of abattoirs and to identify areas within slaughterhouses which are likely to harbor pathogens.

MATERIALS AND METHODS

Collection of samples

A total of 219 samples were obtained in 11 different hog abattoirs located in an inland territory of Central Italy (Molise region) between May 1995 and January 1996. The visits took place at noon to catch an in-work situation. Environmental sampling sites included slaughterhouse floors and walls; hand-wash basins; and cold room handles, floors, and walls. Equipment sites and work surfaces included hooks, worktables, chopping blocks, knives, cleavers, dehairing devices, and cold room hooks. Abattoir workers were monitored by sampling hands and clothing.

* Author for correspondence. Tel: 39 874 481259; Fax: 39 874 481025.

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Each sample consisted of three sterile cotton swabs taken from adjacent areas from the same location. Swabs were wetted in sterile saline solution and were uniformly stroked 10 to 15 times across the surface of an area approximately 5.9 by 5.9 in. (15 by 15 cm) of floors, walls, worktables, chopping blocks, dehairing devices, hand-wash basins, and clothing. The swabs were then rotated and stroked another 10 to 15 times perpendicular to the first swabbing direction. Handle, hook, knife, and cleaver samples were obtained by swabbing a part of the equipment being tested. The samples taken from the hands of the personnel were collected by swabbing the palm of the right hand. Care was taken to obtain a sample which was representative of the whole equipment and which was from an area as close as possible to the total surface area tested in environmental samples.

After the swabbing procedure each swab was placed aseptically in one of three sets of test tubes containing 10 ml of buffered peptone water (BPW; Oxoid, Basingstoke, U.K.), *Listeria* primary selective enrichment medium (UVM1) (Oxoid, Basingstoke, U.K.), and peptone sorbitol bile broth (PSBB) *(24),* respectively. BPW and UVM1 tubes were transported at ambient temperature; PSBB tubes were carried in a refrigerated container.

Isolation and identification of Salmonella *spp.*

After 18 to 24 h of incubation at 37°C, 1 ml of preenrichment culture (BPW) was subcultured to 9 ml of selenite cystine (SC) broth (Biolife, Milan, Italy) and incubated at 37°C for 18 to 24 h. The SC broth cultures were streaked onto Hektoen agar (Oxoid, Basingstoke, U.K.) and Rambach agar (Merck, Darmstadt, Germany). Plates were incubated for 37°C overnight, and suspect colonies were picked onto slants of triple sugar iron agar (Oxoid, Basingstoke, U.K.) and lysine iron agar (Oxoid, Basingstoke, U.K.) media. Biochemical characterization of isolates showing typical characteristics was performed with the API 20E system (Biomerieux, Marcy l'Etoile, France). Serological confirmation was carried out with *Salmonella* O and *Salmonella* H antisera (Wellcome Diagnostic, Dartford, U.K.).

Isolation and identification of Yersinia *spp.*

The PSBB tubes were refrigerated at 4°C up to six weeks. At two, four, and six weeks, samples were inoculated on MacConkey agar no. 3 (Oxoid, Basingstoke, U.K.). Plate were incubated at room temperature for 48 h. Typical colonies were inoculated on Kligler iron agar (Oxoid, Basingstoke, U.K.). Biochemical characterization of isolates showing typical characteristics was performed with the API 20E system (Biomerieux, Marcy l'Etoile, France). *Y. enterocolitica* biotyping was performed by the method of Wauters *(23, 24)*; serotyping was performed with O:3, O:8 and O:9 antisera (Biogenetic, Padua, Italy).

Isolation and identification of Listeria *spp.*

After 18 to 24 h of incubation at 30°C, 0.1 ml of primary enrichment broth (UVM1) was used to inoculate 10 ml of *Listeria* secondary selective enrichment medium (UVM2) (Oxoid, Basingstoke, U.K.). UVM2 tubes were then incubated at 30°C for 18 to 24 h and streaked to *Listeria* selective agar (Oxford) (Oxoid, Basingstoke, U.K.) plates. Oxford agar plates were incubated at 37°C for 18 to 24 h. Up to five colonies showing characteristic blackening with dimpled centers were picked and restreaked to fresh Oxford agar. Following incubation at 37°C for 18 to 24 h, each isolate was Gram stained and evaluated for catalase reaction using hydrogen peroxide. Biochemical characterization of Gram-positive rods exhibiting catalase production was performed with the API *Listeria* system (Biomerieux, Marcy L'Etoile, France).

Statistical analysis

The presence/absence of *Salmonella* spp., *Yersinia* spp., and *Listeria* spp. was recorded for each of the environmental and work surface/equipment sites. Because neither *Salmonella* spp. nor *Yersinia* spp. nor *Listeria* spp. were isolated from abattoir workers, a statistical analysis was not performed for hand of personnel and clothing. The difference between environmental and work surface/equipment sites was then tested using Pearson's chi-square test; a Fisher's exact test was calculated if any expected cell value in a two-by-two table was less than 5. A two-sided significance level of <0.05 was used.

RESULTS

Six abattoirs (54.5%) had one or more positive sites (Table 1), while only 14 of the 219 (6.4%) sites tested were positive for *Salmonella* spp., *Listeria* spp., or *Yersinia* spp.; 2 of these sites were positive for both *Salmonella* spp. and *Yersinia* spp. Five of 14 (35.7%) positive sites were on slaughtering floors and 2 (14.3%) were on slaughtering walls, cold room floors, and worktables. Seventeen isolates were obtained from these sites; one site yielded both *Y. enterocolitica* and *Y. kristensenii* (Table 2). The species most often identified were *Y. enterocolitica* and *L. monocytogenes,* which represented 29.4% (5/17) and 17.6% (3/17) of isolates, respectively.

Salmonella spp. were isolated from 2 of 11 abattoirs (18.2%) (Table 1) and from 3 of 219 sites (1.4%) (Table 2). Frequency of isolations from positive abattoirs was 3.8% (1 of 26 samples) and 15.4% (2 of 13 samples). One *Salmonella* serotype was identified: *S. derby* was isolated from 1 of 9 cleavers (11.1%), 1 of 16 worktables (6.25%), and 1 of 18 slaughtering floor swabs (5.6%).

Three abattoirs (27.3%) (Table 1) and six sites (2.7%) (Table 2) yielded *Yersinia* spp. Prevalence of isolations in positive slaughterhouses ranged from 7.7% (2 of 26 samples) to 12.5% (2 of 16 samples) to 15.4% (2 of 13 samples). Two species of *Yersinia, Y. enterocolitica* and *Y. kristensenii,* were identified. *Y. enterocolitica* was isolated from 2 of 16 worktables (12.5%) and from 3 of 18 slaughtering floor swabs (16.7%). *Y. kristensenii* was detected on 2 of 18

TABLE 1. *Distribution of* Salmonella *spp.,* Yersinia *spp., and* Listeria *spp. in abattoirs*

		Isolates					
		Salmonella		*Yersinia*		*Listeria*	
Abattoir	No. of samples	No.	(%)	No.	(%)	No.	(%)
A	26	0	—	0	—	0	—
B	25	0	—	0	—	3	(12.0)
C	27	0	—	0	—	1	(3.7)
D	19	0	—	0	—	0	—
E	24	0	—	0	—	0	—
F	19	0	—	0	—	3	(15.8)
G	13	2	(15.4)	2	(15.4)	0	—
H	16	0	—	0	—	0	—
I	8	0	—	0	—	0	—
L	16	0	—	2	(12.5)	0	—
M	26	1	(3.8)	2	(7.7)	0	—

TABLE 2. *Distribution of* Salmonella *spp.,* Listeria *spp., and* Yersinia *spp. in specimens*

Sample type	No. of samples	Isolates: *Salmonella* No.	(%)	*Yersinia* No.	(%)	*Listeria* No.	(%)
Environmental sites							
Slaughtering floor	18[a]	1[b]	(5.6)	4[c]	(22.2)	1[d]	(5.6)
Slaughtering wall	19	0	—	0	—	2[e]	(10.5)
Hand-wash basin	14	0	—	0	—	1[f]	(7.1)
Cold room handles	9	0	—	0	—	0	—
Cold room floor	15	0	—	0	—	2[f]	(13.3)
Cold room wall	14	0	—	0	—	0	—
Equipment sites							
Hooks	16	0	—	0	—	0	—
Worktables	16[a]	1[b]	(6.2)	2[g]	(12.5)	0	—
Chopping blocks	8	0	—	0	—	1[d]	(12.5)
Knives	16	0	—	0	—	0	—
Cleavers	9	1[b]	(11.1)	0	—	0	—
Dehairing devices	7	0	—	0	—	0	—
Cold room hooks	14	0	—	0	—	0	—
Abattoir workers							
Hands	22	0	—	0	—	0	—
Clothing	22	0	—	0	—	0	—
Total	219	3	(1.4)	6	(2.7)	7	(3.2)

[a] One site was positive for both *Salmonella* and *Yersinia.*
[b] *S. derby.*
[c] *Y. enterocolitica* (three samples) and *Y. kristensenii* (two samples).
[d] *L. innocua.*
[e] *L. welshimeri* (one sample) and *L. innocua* (one sample).
[f] *L. monocytogenes.*
[g] *Y. enterocolitica.*

slaughtering floor swabs (11.1%); one of these yelded both *Y. enterocolitica* and *Y. kristensenii.* Of the five *Y. enterocolitica* isolates, four belonged to biotype 6 and one to biotype 1A. None of the *Y. enterocolitica* isolates belonged to serotypes O:3, O:8, and O:9. The presence of *Salmonella* was always related to presence of *Yersinia* in the same abattoir.

Listeria spp. were detected in 3 abattoirs (27.3%) (Table 1) and in 7 sites (3.2%) (Table 2). Frequency of isolations from positive abattoirs ranged from 3.7% (1 of 27 samples) to 12.0% (3 of 25 samples) and to 15.8% (3 of 19 samples). Three species of *Listeria, L. monocytogenes, L. welshimeri,* and *L. innocua,* were identified. *L. monocytogenes* was isolated from 1 of 14 hand-wash basins (7.1%) and 2 of 20 cold room floor swabs (13.3%). *L. welshimeri* was identified from 1 of 19 slaughtering wall swabs (5.3%) and *L. innocua* came from 1 of 8 chopping blocks (12.5%), 1 of 18 slaughtering floors (5.6%), and 1 of 19 slaughtering wall swabs (5.3%).

No *Salmonella* spp., *Yersinia* spp., or *Listeria* spp. were isolated from hooks, knives, dehairing devices, hands of personnel, clothing, and cold room walls and handles.

The distribution of the positive samples in the slaughterhouse environment and on work surfaces, equipment, and personnel is shown in Table 3. *Salmonella* spp. were isolated from 1.1% of environmental sites and from 2.3% of work surface/equipment sites. *Yersinia* spp. were isolated from four environmental sites (4.5%) and from two work surface/equipment sites (2.3%), but the difference was not significant ($P = 0.7$). *Listeria* spp. were isolated from 6 environmental samples (6.7%) and from one work surface (1.2%), with no significant difference between environmental and work surface/equipment sites ($P = 0.1$). No *Salmonella* spp., *Yersinia* spp., or *Listeria* spp. were isolated from hands or clothing of any abattoir workers.

TABLE 3. *Distribution of positive samples in the slaughterhouse environment, work surfaces/equipment, and workers*

Site	No. of samples	Isolates: *Salmonella* No.	(%)	*Yersinia* No.	(%)	*Listeria* No.	(%)
Environment	89	1	(1.1)	4	(4.5)	6	(6.7)
Work surfaces/ equipment	86	2	(2.3)	2	(2.3)	1	(1.2)
Workers	44	0	—	0	—	0	—
Total	219	3	(1.4)	6	(2.7)	7	(3.2)

DISCUSSION

Researchers investigating the environment as a source of microbiological contamination have revealed a wide variation in the extent of slaughterhouse contamination. Kampelmacher et al. *(11)* in the Netherlands found 17.5% of specimens obtained from hand scrapers or scraping machines of six hog slaughterhouses containing *Salmonella;* the percentage decreased to 9.2% after cleaning of scraping machines and hand scrapers. Lowry and Tiong *(13)* in New Zealand found that 30 to 65% of work surfaces and knives were positive for *L. monocytogenes.* Mafu et al. *(15)* in Canada evaluated the degree of contamination of the slaughterhouse environment by *Salmonella* and *Y. enterocolitica* and reported a considerable *Salmonella* contamination (25% of floor abattoir swabs and 12.5% of cold room swabs); they also found 6.2% cold room swabs positive for *Y. enterocolitica.* The differences in results are likely due to the structural characteristics of abattoirs, the species of the slaughtered animals, the slaughtering practices, the sanitation practices, and the sampling procedure. Moreover, results vary considerably in relation to the culture methods adopted (preenrichment, enrichment, incubation temperature and time, plating media, picks of suspect colonies, confirmation, etc.). Indeed, the choice of isolation methods and media could influence the prevalence of microorganisms in environmental samples, and the use of a single isolation procedure could result in an underreporting of the true prevalence. Pritchard et al. *(17)* determined that the use of three primary enrichment media increased the number of *Listeria*-positive sites in dairy processing plants.

More important is to identify within abattoirs the areas and instruments that, harboring pathogens, may be considered possible sources of meat contamination. To identify the

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possible routes of contamination, it is also necessary to consider the specific features of the pathogens. Both *Salmonella* and *Yersinia* belong to the family Enterobacteriaceae, are found in the intestinal tract of animals, and are associated with fecal contamination. *Y. enterocolitica* has a wide distribution in nature (especially water) with swine as a probable reservoir for pathogenic serotypes *(1)*. Results of biotyping and serotyping showed that none of the *Y. enterocolitica* isolates was pathogenic. Indeed, the important human pathogenic strains are included mainly among biotypes 2, 3, 4, and 5 and serotypes O:3, O:8, and O:9 *(1, 12)*. For the purpose of our study this was not as important as the fact that conditions exist at the sites which allow certain pathogens to develop. The habitat of *Listeria monocytogenes* is quite different: this microorganism has been isolated from a variety of sources, and it is now recognized to be widely distributed in nature (soil and vegetation) *(10)*. *Listeria* can adapt from its external niche to become a gastrointestinal commensal *(14)*.

The frequent isolation of *L. monocytogenes* from cooler and freezers reflects the psychrotrophic nature of these microorganisms *(2, 16, 17)*. This characteristic has an impact on many food processing environments. Indeed, *L. monocytogenes* and *Y. enterocolitica* can grow at refrigeration temperatures in meat, and could reach infectious levels at the times of product consumption *(18)*.

In contrast to our findings, *Salmonella* and *Listeria* have been isolated from the hands or gloves of workers of both meat and poultry slaughterhouses *(3, 6, 7)*. Genigeorgis et al. *(7)* found at the slaughterhouse level 30.0% (27/90) of turkey meat handlers harboring *Listeria* spp. on their hands and gloves, 12.2% harboring *L. monocytogenes,* and 17.7% harboring *L. welshimeri.* They also found a higher prevalence of *Listeria* in retail products than in slaughterhouse samples, and assumed that handling of turkey carcasses might play a major role in spreading the contamination and increasing the *Listeria* prevalence at the end of the processing line. Whelehan et al. *(25),* found that automation of a beef slaughterline did not significantly alter the bacterial populations on the carcasses and bacterial count did not differ significantly between the original manual line and the automated line. Slaughterhouse environment, work surfaces, and equipment in contact with meat are important sources of *Listeria (6, 8)*. Once the abattoir environment is contaminated, *Listeria* may establish itself in the plant; therefore the environment plays a major role in spreading the contamination to carcasses. Cross-contamination among carcasses and hands of personnel can contribute to the spreading of pathogenic microorganisms. In any case, the persistence of these pathogenic microorganisms on raw meats presents a potential health risk and requires proper cooking and handling prior to human consumption.

In conclusion, our results indicate that slaughtering floors, cold room floors, and worktables are important sites in abattoirs that may possibly harbor pathogens like *Salmonella* spp., *Y. enterocolitica,* and *L. monocytogenes,* and demonstrate the need of stringent cleaning and sanitizing regimes in the slaughterhouse environment. Moreover, our findings indicate a slightly higher prevalence of *Yersinia* spp. and *Listeria* spp. in environmental sites than in work surface/equipment sites. The practice of allowing sanitizers and cleaners to flow off of equipment and walls onto the floor is not effective as a means to eliminate pathogenic microorganisms from the environment. It is necessary to apply more potent sanitizers to the environment, particularly walls and floors, and develop proper sanitation schemes for refrigerated areas. The use of good manufacturing practices and the decontamination of carcasses should also be recommended to decrease the contamination of pathogens which may be present on meat products, and to avoid postslaughter environmental contamination *(4)*. The use of such practices and the implementation of specific guidelines for slaughter procedures will help reduce environmental contamination and control the spreading of pathogens. Further investigations are needed to develop a simple and economical microbiological method useful in testing the effectiveness of sanitizing procedures and in suggesting a likely presence of pathogens in the slaughterhouse environment.

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