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Prevalence of *Listeria* spp. in Poultry Meat at the Supermarket and Slaughterhouse Level

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ABSTRACT

The prevalence of *Listeria* spp. in the skins of poultry legs (drumsticks), wings, and whole livers, representing 160 packages, three national brands, and purchased from three supermarkets in Davis, California was investigated first. Overall, *Listeria* spp., *L. monocytogenes*, *L. innocua*, and *L. welshimeri* were present in 40.6, 13.1, 26.3, and 1.3% of the chicken parts. For *L. monocytogenes* spp. the overall prevalence in the skins of wings, drumsticks, and livers was 10, 15, and 14% and varied extensively with sampling day. Next, we evaluated the presence of *Listeria* spp. in 12 locations and products collected from a slaughterhouse during four visits. A total of 188 samples were analyzed. No *Listeria* spp. were isolated from feathers (5 g composite sample from 10 birds), scalding tank water overflow (25 ml), chiller incoming water (25 ml), neck skin (5-9 g), whole liver after chilling, and cecum and large intestine content (1 g) samples. *Listeria* spp. were present in 18.8% of feather picker drip water (25 ml), 12.5% of chiller water overflow (25 ml), 37.5% of recycling water for cleaning gutters (25 ml), 25% of hearts, and 31.3% of mechanically deboned meat samples (25 g). The prevalence of *L. monocytogenes* in packaged livers and skins of drumsticks and wings at the end of the processing line was 33.3, 36.7, and 70.0%, respectively. After 4 d storage of the same packages at 4°C, *L. monocytogenes* was recovered from 40, 52, and 72% of the respective products. The prevalence of *L. monocytogenes* on the hands and gloves of the persons hanging birds after chilling, cutting carcasses, and packaging parts was 20, 45.5, and 59%, respectively. Overall, the study demonstrated the high prevalence of *Listeria* spp. and *L. monocytogenes* in poultry products and, that through certain improvements and innovations at the slaughterhouse level, contamination with *Listeria* can be minimized.

In recent years, *L. monocytogenes* has been established as a foodborne pathogen and has become a major concern to the food industry.

Globally, several outbreaks of *L. monocytogenes* have been associated with the consumption of raw or recontaminated foods such as coleslaw (*38*), pasteurized milk (*15*), and soft cheeses (*1,3,30*).

Although dairy products have been the principal foods involved in outbreaks of listeriosis, other raw or recontaminated foods of animal (*4,18,39*) or vegetable (*21*) origin may serve as vehicles of transmission of this pathogen. *L. monocytogenes* has been frequently isolated from poultry and red meat and meat products (*2,7,10,12,13,17,22,26, 28,36,42*) but these foods have not been associated with documented outbreaks of human listeriosis. Nevertheless, the high prevalence of *L. monocytogenes* in these products presents a potential risk which may lead to outbreaks of listeriosis, especially if they are eaten raw or undercooked.

A case-control study involving 82 sporadic cases of listeriosis and 233 controls was conducted recently (*39*) by the Centers of Disease Control to identify risk factors for the disease. One third of the cases were perinatal and the remaining occurred in elderly and immunosuppressed individuals. Cases were significantly more likely than controls to have eaten undercooked chicken or uncooked hot dogs with 20% of the overall risk of the disease attributable to consumption of these foods.

The present studies was undertaken in order to determine the prevalence of *Listeria* spp. in fresh chicken meat and organs at the supermarket level. Products originating from three suppliers, collected from three local supermarkets, were evaluated. Furthermore, the study evaluated the potential contribution of slaughterhouse practices to the prevalence of *Listeria* spp. in the finished products.

MATERIALS AND METHODS

Sample collection from supermarket

Fresh and semi-frozen packaged chicken wings, drumsticks (legs), and livers were collected from three local supermarkets. The samples representing three national brands (A, B, and C) were brought to the laboratory in an ice chest and analyzed within 24 h. Semi-frozen samples which arrived at the supermarket as fresh and exposed to <0°C to extend shelf life by few days were analyzed at the end of the working day.

Sample collection in slaughterhouse

A federally inspected California chicken processing plant cooperated in this study. Conventional slaughtering and process-

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ing techniques are used in this plant which is part of a fully integrated operation with its own source of chickens. The birds were scalded at 130-132 F (54.4-55.5°C) for 2 min 40 s. Next, they had a wing dip (half of the body) at 137 F (58.3°C) for 10 s and a neck dip at 170 F (76.7°C) for 5 s between the 1st and 2nd feather picker. Water of drinking quality was used in the scalding tank and feather pickers. Final rinsing was done with water containing 25 ppm total chlorine, while chiller tank water contained 20 ppm chlorine. Equipment and environment were washed every 2 h with water containing 25 ppm chlorine. Every 4 h all equipment was brushed and cleaned with caustic detergent and then rinsed. Every 8 h they were brushed, cleaned with detergent, then rinsed and finally sanitized with a 200 ppm guaternary ammonium preparation.

Slaughterhouse sampling sites and tissue samples included: feathers from live, hanging birds, scalding tank water overflow (SWO), feather picker drip water (FPDW), recycled water for cleaning gutters (RWCG) in the receiving room, chiller incoming (CIW) and overflow water (CWO), ceca (CC) and the last part of the large intestine (LI) from the evisceration line, neck skins, drumsticks, and wings cut aseptically from whole birds after they came out of the chilling tank, hearts (HT) and livers (LV) taken immediately after coming out of the giblet chiller, and mechanically deboned meat (MDM) taken directly from the machines outlet.

Ten consecutive feather samples were taken from every fifth bird and placed in a sterile "Whirl-Pak" bag to form a composite feather sample. Feathers were plucked from the breast region. Individual heart, liver, neck skin, leg and wing samples, and mechanically deboned meat samples were collected aseptically and placed in "Whirl-Pak" bags. Several crystals of sodium thiosulfate were added to each water sample to neutralize any residual chlorine. A total of 16 samples per each site (12 samples of neck skin) were taken during four sampling trips to the slaughterhouse.

In addition to individual part samples, 75 packaged products were obtained from the packaging station at the end of the processing line and all were analyzed the same day and 65 again after storage at 4°C for four d.

Swabbing workers hands and gloves

This was done by wetting sterile cotton swabs in Food and Drug Administration *Listeria* enrichment broth (FDA-EB) (*31,32*), swabbing an area of 2x2 cm^2 and placing the swabs back into tubes containing 9 ml of FDA-EB. All slaughterhouse samples and final product packages were placed in an ice chest, brought to the laboratory and analyzed within 5 h after collection.

Sample processing

All water samples in volumes of 25 ml were placed in 100 ml screw cup bottles containing 25 ml of double strength FDA-EB. One gram of ceca or large intestine contents were placed in sterile "Whirl Pak" bags containing 9 ml of EB and homogenized by hand for 1 min. Each composite feather sample (approximately 5 g) and each whole liver and heart were placed in 50 ml of EB and massaged by hand. Market livers (25-30 g) were placed in 225 ml FDA-EB. Neck skins of approximately 9x6 cm to 12x8 cm long in the "Whirl-Pak" bags were mixed with 34-60 ml of FDA-EB at a ratio of 1 ml broth to 1.6 cm^2 skin surface and then massaged by hand for 1 min. Legs (drumsticks) and wings originating from supermarkets and whole carcasses brought to the laboratory from the slaughterhouse were handled as follows: the whole skin from each drumstick of approximately 7 to 10 g weight and between 9x5 cm to 13x8 cm surface was removed aseptically and placed in a "Whirl-Pak" bag. Enrichment broth was added to each bag at a ratio of 1 ml to 1.6 cm^2 skin. The tip of each wing 20 cm^2 to 32 cm^2, representing metacarpi-falangean bones, was cut and placed in a bag. In addition 15 cm^2 to 22.0 cm^2 wing skin was removed and placed in the bag also. The total skin placed in each bag was 35.0 cm^2 to 54.0 cm^2. FDA-EB was added to each bag at ratio of 1 ml broth to 1.6 cm^2 skin and massaged for 1 min. Twenty-five grams of MDM was placed in a bag containing 225 ml EB and homogenized.

All tubes, bottles, and bags containing the samples (primary enrichment) were incubated aerobically at 30°C for 24 h. Next, the contents were mixed very well and 0.1 ml was transferred to 9 ml FDA-EB containing tubes (secondary enrichment). The tubes were incubated for 24 h at 30°C and then 0.01 ml of the subculture was streaked onto Lithium chloride-phenylethanol-moxalactam (LPM) plating agar (*27,32*) and modified Mc.Bride *Listeria* agar (MMA) (*34*). A number of secondary enrichment broths after the 24 h incubation at 30°C were placed at 4°C for 15 d and streaked again on LPM and MMA agar. Both agars were incubated at 37°C for 24 to 48 h. After 24 and 48 h incubation, the plates were checked with a dissecting microscope under Henry's 45°C transillumination (*27,32*) for typical *Listeria* colonies. Three to four suspect colonies were subcultured for purity on brain heart infusion (BHI) agar (Difco), and then transferred to BHI agar slants for further biochemical characterization, which was done within a week using selected criteria proposed by Seelinger and Jones (*41*) and Lovett (*31*). They included tests for: Gram stain, production of beta-hemolysis on sheep blood agar, catalase, motility and utilization of esculin, glucose, rhamnose, mannitol, xylose, and alpha-methyl-D-mannopyranoside.

Production of hemolysin was determined by stabbing thick sheep blood agar plates with BHI agar cultures and incubating the plates for 48 h at 37°C. Positive and negative controls were used as reference (*31*).

To evaluate motility we used a semisolid agar made of proteose (Difco) 5 g tryptose (Difco) 5 g, Lab Lemco powder (Oxide) 4 g, sodium chloride 1 g, glucose 1 g, disodium phosphate 5 g, and agar 2.5 g per liter. After inoculation the agar tubes were incubated for up to 48 h at 25°C and observed for mobility and umbrella formation.

Carbohydrate utilization tests were based on the use of purple broth base (BBL) supplemented with 1% of filter sterilized glucose, mannitol, L (+) rhamnose and D(+) xylose (Sigma), and 0.5% of alpha-methyl-D-mannopyranoside (Aldrich). The tubes were inoculated with cultures from BHI slants and incubated for up to 3 d at 37°C. Esculin utilization was evaluated by streaking BHI agar cultures on a plate agar made of proteose 10 g, ferric ammonium citrate 1 g, esculin (Sigma) 1 g and agar, 15 g per liter. The plates were incubated for 24 h at 37°C. Formation of black colonies was considered a positive reaction.

RESULTS

Twice during the period of June to November 1988 we visited three local supermarkets and bought a total of 160 packages of fresh and semi-frozen chicken wings, drumsticks, and livers in order to determine the prevalence of *Listeria* spp.

TABLE 1. *Prevalence of* Listeria *spp. on raw chicken products representing three companies and purchased from local supermarkets in Davis, California during the period of June through November 1988.*

Product	Species	Trip	Companies A	B	C	ABC Total	ABC %
			Percentages				
Wings	*L. monocytogenes*	1	40	0	10[b]	5/30	17
		2	0	0	0	0/20	0
	L. innocua	1	70	0	60	13/30	43
		2	0	20[a]	10[c]	2/20	10
	L. welshimeri	1	0	0	0	0/30	0
		2	0	20[a]	0[c]	1/20	5
Total	*Listeria* spp.	1,2	66	6.7	35	18/50	36%
Drumstick (legs)	*L. monocytogenes*	1	0	50	10	7/40	8
		2	0	20	10[c]	7/20	10
	L. innocua	1	30	50	20	12/40[d]	30
		2	80	60	0	7/20	35
Total	*Listeria* spp.	1,2	46	93	23	28/60	46%
Livers	*L. monocytogenes*	1	10	30	10	5/30	17
		2	0	20	10[c]	2/20	10
	L. innocua	1	50[b]	0	20[b]	7/30	23
		2	20	0	0[c]	1/20	5
	L. welshimeri	1	10[c]	0	0	1/30	3
		2	0	0	0[c]	0/20	0
Total	*Listeria* spp.	1,2	46.7	26	15	14/50	28%

[a] = One sample harbored both *L. innocua* and *L. welshimeri*.
[b] = One sample harbored both *L. monocytogenes* and *L. innocua*.
[c] = Semi-frozen samples.
[d] = Number of positive samples/total samples.

Table 1 summarizes the findings with respect to the sources (companies), type of product (fresh or semi-frozen), and type of *Listeria* spp. Of the 50 samples of wings, 60 samples of legs (drumsticks), and 50 samples of livers, 18 (36%), 28 (46.6%), and 14 (28%) harbored *Listeria* spp. respectively.

L. monocytogenes was detected in 10% of the wings, 15% of the legs, and 14% of the livers. *L. innocua* was detected in 30% of the wings, 31.7% of legs, and 16% of livers. *L. welshimeri* was detected in 2% of the wings and livers and 0% of legs. The prevalence differed with the sampling time (trip 1 vs trip 2) significantly for all products, companies, and *Listeria* species. Company A had a *Listeria* spp. prevalence of 63.3%, company B had 43.3%, and company C had 35% for all three types of meats during the first trip. The corresponding figures for the 2nd trip were 33.3, 40, and 10%. The latter figure represents semi-frozen samples (Table 2).

Table 2 presents the observed variations in the overall prevalence of *L. monocytogenes, L. innocua,* and *L. welshimeri* in the products of each company. *L. innocua* was the most prevalent species (26.3%) followed by *L. monocytogenes* (13.1%), and *L. welshimeri* which was present only in 1.3% (2/160) samples. For company A, 53.3% of its products harbored *Listeria* spp., including 11.1% *L. monocytogenes*, 44.4% *L. innocua*, and 2.2% *L. welshimeri*. For company B, 42.2% of its products harbored *Listeria* spp., including 22.2% *L. monocytogenes* 20.2% *L. innocua* and 2.2% *L. welshimeri*. Finally, for company C, 40.0% of its products harbored *Listeria* spp., including 10.0% *L. monocytogenes*, and 30.0% *L. innocua* in the fresh samples, and 6.6% *L. monocytogenes*, and 3.3% *L. innocua* in the semi-frozen samples. The prevalence of *Listeria* spp. was significantly ($p<0.01$) higher in fresh parts than semi-frozen parts (43.8% versus 10.0%, respectively). The prevalence of *Listeria* spp. in fresh parts did not differ significantly among companies ($p>0.1$). All individual *Listeria* species were also more prevalent in the fresh parts than the semi-

TABLE 2. *Grand total numbers and percentages of fresh and frozen chicken parts representing companies and harboring various* Listeria *spp.*

Microorganisms	Companies A Total	A %	B Total	B %	C Fresh Total	C Fresh %	C Frozen Total	C Frozen %	ABC Total %
L. monocytogenes	5/45	11.1	10/45	22.2	4/40	10.0	2/30	6.6	13.1
L. innocua	20/45	44.4	9/45	20.0	12/40	30.0	1/30	3.3	26.3
L. welshimeri	1/45[a]	2.2	1.45[a]	2.2	0/40[a]	0.0	0/30	0.0	1.3
Listeria spp.	24/45	53.3	19/45	42.2	14/40	35.0	3/30	10.0	40.6

[a]= Number of positive samples / Total samples.

frozen ones. Five samples harbored more than one *Listeria* spp. All *Listeria* spp. recoveries mentioned above were based on the isolation of the agent in selective agars inoculated with 24 h secondary enrichment broth cultures. When 30 negative for *Listeria* spp. secondary broths, (representing ten of each wings, legs, and livers) were stored at 4°C for 15 d and then plated on agars, two more samples (one leg and one liver) were detected as harboring *L. innocua* and one (liver) as harboring both *L. innocua* and *L. welshimeri*.

During four trips to company A slaughterhouse a total of 188 samples were obtained from 12 stations. The prevalence of *Listeria* species by trip, sampling sites, and summary data are presented in Table 3. No *Listeria* spp. was isolated form feather, scalding tank water overflow, chiller incoming water, neck skin, liver, cecum, and large intestine content samples. *Listeria* spp. were isolated from 18.8% of FPDW, 12.5% of CWO, 37.5% of RWCG, 25.0% of hearts, and 31.3% of mechanically deboned meat. *L. monocytogenes* was present only in CWO (12.5%), RWCG (6.3%), and mechanically deboned meat (18.8%). Overall, from 188 samples, 20 (10.6%) were positive for *Listeria* spp., 6 (3.2%) for *L. monocytogenes*, and 14 (7.4%) for *L. innocua*.

The prevalence of *Listeria* spp. in chicken parts packages of company A was 53.3% at the supermarket level and 0, 0 and 25% in the individual neck skin, liver and heart samples, respectively, at the slaughterhouse level. Because of this big difference in the frequency of *Listeria* present in retail products and slaughterhouse samples, we initiated a study in order to identify the contributing factors. First, we determined the prevalence of *Listeria* spp. in the skins of legs and wings from birds coming out of the chilling tank and individual livers coming out of the giblet chiller. None of the 20 wing and 20 leg samples collected during four trips to the slaughterhouse harbored detectable *Listeria* spp. (Table 4). Only 2/31 (6.5%) liver samples collected during six trips, harbored *Listeria* (*L. innocua*). During the same trips we also collected packaged livers, wings, and legs from the packaging station. Individual parts were taken from each package and analyzed the same day while the rest were placed at 4°C and analyzed again after 4 d. In parts from packages analyzed the day of production we found *Listeria* spp. in 46.7% (7/15) of livers, 73.3% (22/30) of wings, and 80% (24/30) of legs. The corresponding percentages for *L. monocytogenes* were 33.3, 70.0, and 36.3%. For *L. innocua* the corresponding percentages were 26.7, 20 and 63.3%, respectively (Table 4). After 4 d storage at 4°C the prevalence of *L. monocytogenes* increased for livers, wings, and legs to 40.0, 72.0 and 52.0%, respectively. The corresponding percentages for *L. innocua* were 26.7, 16.0, and 68.0%, and for total *Listeria* spp. 60.0, 76.0, and 76.0%, respectively.

We assumed that the added handling for hanging the carcasses after the chilling process, the cutting into legs (drumsticks) and wings, and the handling during packaging was responsible for the dramatic increase in prevalence at the end of the processing line. Storage of packages for 4 products at 4°C slightly increased the prevalence of *L. monocytogenes* for all products and also slightly for *L. innocua* in packaged legs. Overall, storage increased the prevalence for livers and wings by 13.3% and 2.7%, respectively and decreased the prevalence in legs by 4%.

The potential contribution of chicken meat handlers in the slaughterhouse to an increasing *Listeria* prevalence through cross-contamination was evaluated next. The presence of *Listeria* in the hands and gloves of handlers in three stations was determined during three trips to the slaughterhouse; Table 5 presents the findings. Of the persons handling chicken carcasses immediately after chilling 10% (2/20) harbored *L. monocytogenes*, 10% harbored *L. innocua*, and 20% (4/20) harbored *Listeria* spp. The prevalence of *L. monocytogenes* and *Listeria* spp. on the hands of persons cutting the carcasses into legs and wings was increased to 36.4% (4/11) and 45.5% (5/11), respectively. Finally, 45.5% (20/44) and 59% (26/44) of the hands and

TABLE 3. *Prevalence of* Listeria monocytogenes *and* Listeria innocua *in product and environmental samples collected from different sites during four trips to a slaughterhouse.*

		Chicken sampling sites or products												
Trip	Micr	Ftr	SWO	FPDW	CIW	CWO	RWCG	NK	LV	HT	CC	LI	MDM	Total +
1	*L. monocytogenes*	0/4	0/4[a]	0/4[a]	0/4	2/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/48
	L. innocua	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/48
2	*L. monocytogenes*	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/48
	L. innocua	0/4	0/4	2/4	0/4	0/4	2/4	0/4	0/4	1/4	0/4	0/4	2/4	7/48
3	*L. monocytogenes*	0/4	0/4	0/4	0/4	0/4	0/4	ND	0/4	0/4	0/4	0/4	0/4	0/44
	L. innocua	0/4	0/4	0/4	0/4	0/4	2/4	ND	0/4	2/4	0/4	0/4	0/4	4/44
4	*L. monocytogenes*	0/4	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4	3/4	4/48
	L. innocua	0/4	0/4	0/4	0/4	0/4	1/4	0/4	0/4	1/4	0/4	0/4	0/4	2/48
Total		0/16	0/16	3/16	0/16	2/16	6/16	0/12	0/16	4/16	0/16	0/16	5/16	20/188
Total	*L. monocytogenes*	0	0	0	0	12.5	6.3	0	0	0	0	0	18.8	
pos.	*L. innocua*	0	0	18.8	0	0	31.3	0	0	25	0	0	12.5	
(%)	*L. spp.*	0	0	18.8	0	12.5	37.5	0	0	25	0	0	31.3	10.6

[a] = Number positive / total sample. ND = Not done.

Ftr = Feathers, SWO = scalding water overflow, CIU = chiller incoming water, FPDW = feather-picker drip water, CWO = chiller water overflow, RWCG = recycled water for cleaning gutters, NK = neck skin, LV = livers, HT = hearts, CC = ceca, LI = last part of the large intestine, MDM = mechanically deboned meat.

gloves of persons packaging poultry cuts were positive for *L. monocytogenes* and *Listeria* spp., respectively. Overall, 46.7% (35/75) of the handlers in the three stations studied harbored *Listeria* spp. in their hands and gloves, 34.7% (26/75) harbored *L. monocytogenes*, and 18.7% (14/75) harbored evidence of *L. innocua*. Five persons harbored both *L. monocytogenes* and *L. innocua*.

The above data demonstrated beyond doubt the potential contribution of handlers to cross-contamination of products by *Listeria* spp. Serological and phage typing of the isolated *Listeria* strains was not done.

DISCUSSION

In the last five years eight, outbreaks of human listeriosis attributed to food consumption have been reported (*1,3,15,21,29,30,34,38*). Meat, poultry, and their processed products have not been incriminated in listeriosis cases as

TABLE 4. *Prevalence of* Listeria *spp. in individual and packaged chicken parts at the end of the processing and after 4 d storage at 4°C.*

Trip	Micr.	Individual product			Packaged final product					
					Age 0 D			4 D		
		Livers	Wings	Legs	Livers	Wings	Legs	Livers	Wings	Legs
1	*L. monocytogenes*	0/4[a]	ND	ND	ND	3/5	4/5	ND	ND	ND
	L. innocua	0/4	ND	ND	ND	2/4	5/5	ND	ND	ND
2	*L. monocytogenes*	0/4	0/5	0/5	ND	0/5	3/5	ND	0/5	3/5
	L. innocua	0/4	0/5	0/5	ND	0/5	1/5	ND	0/5	1/5
3	*L. monocytogenes*	0/4	0/5	0/5	ND	5/5	1/5	ND	5/5	3/5
	L. innocua	0/4	0/5	0/5	ND	1/5	1/5	ND	1/5	2/5
4	*L. monocytogenes*	0/4	0/5	0/5	1/5	8/10	1/10	2/5	8/10	3/5
	L. innocua	0/4	0/5	0/5	3/5	1/10	8/10	3/5	1/10	10/10
5	*L. monocytogenes*	0/10	0/5	0/5	ND	5/5	2/5	ND	5/5	4/5
	L. innocua	2/10	0/5	0/5	ND	2/5	4/5	ND	2/5	4/5
	L. welshimeri	0/10	0/5	0/5	ND	0/5	1/5	ND	0/5	0/5
6	*L. monocytogenes*	0/5	ND	ND	4/10	ND	ND	4/10	ND	ND
	L. innocua	0/5	ND	ND	1/10	ND	ND	1/10	ND	ND
Total		2/31	0/20	0/20	7/15	22/30	24/30	9/15[b]	19/25	19/25
Total posit. %	*L. monocytogenes*	0.0	0.0	0.0	33.3	70.0	36.7	40.0	72.0	52.0
	L. innocua	6.5	0.0	0.0	76.7	20.0	63.3	26.7	16.0	68.0
	L. welshimeri	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3
	L. spp.	6.5	0.0	0.0	46.7	73.3	80.0	60.0	76.0	76.0

[a]= Number positive/total sample; b = total positive sampled do not match total individual samples because of the presence of more than one species of *Listeria* in certain samples; ND = not done; L.M. = *L. monocytogenes*, L.I. = *L. innocua;* L.W. = *L. welshimeri* L. spp. = *Listeria* spp.

TABLE 5. *Prevalence of* Listeria *spp. in the hands and gloves of poultry meat handlers in three stations (post chiller, drumcutters, wingcutters, and packaging) of the slaughterhouse.*

Trip	Station	*Listeria monocytogenes*				*Listeria innocua*				*Listeria* spp.
		Whole birds	Legs[b]	Wings	Total (%)	Whole birds	Legs	Wings	Total (%)	Total (%)
1	A	2/7			2/7[a]	0/7			0/7	2/7
2	A	0/5			0/5	1/5			1/5	1/8
3	A	0/8			0/8	1/8			1/8	1/8
Total	A	2/20			2/20 (10)	2/20			2/20 (10)	4/20 (20)
1	B		0/2	ND	0/2		0/2	ND	0/2	0/2
2	B		0/3	0/1	0/4		0/3	0/1	0/4	0/4
3	B		2/3	2/2	4/5		1/3	0/2	1/3	5/5
Total	B		2/8	2/3	4/11 (36.4)		1/8	0/3	1/11	5/11 (45.5)
1	C		1/7	ND	1/7		1/7	ND	1/7	2/7
2	C		2/6	4/4	6/10		1/6	0/4	1/10	7/10
3	C		6/15	7/12	13/27		8/15	1/12	9/27	17/27[c]
Total	C		9/28	11/16	20/44 (45.5)		10/28	1/16	11/44 (25)	26/44 (59)
Grand Total		2/20	11/36	13/19	28/75 (34.7)	2/20	11/36	1/19	14/75 (18.7)	35/75 (46.7)

Station A = post chilling handlers; a = positive samples/total samples.
Station B = leg and wing cutters; b = drumsticks; c = This total does not agree with the total numbers of samples positive of *Listeria monocytogenes* and *Listeria innocua* because a number of samples harbored both species.
Station C = final leg and wing packaging; ND = not done.

far as we know. Whether this is a coincidence or there are special ecological and product processing and handling reasons is not clear. A recent case-control study (*39*) attributed 20% of the risk of sporadic listeriosis to the consumption of undercooked chicken or uncooked hot dog. While epidemiologic associations do not establish causality, they are nevertheless of great value in the better understanding of a health problem, especially when the associations observed have biological explanations. In this respect the reported high prevalence of *L. monocytogenes* in retailed poultry meat (*5,6,12,16,17,26,33,42*), may indicate an increased risk if not for the general "healthy" public but at least for pregnant women, the elderly, and immunocompromised individuals exposed to undercooked poultry meat. In the case of consumption of uncooked hot dogs as a risk factor the justification may come from potential underprocessing, cross contamination post-processing from plant environment extensively contaminated with *Listeria*, as a recent study of 41 plants has shown (*45*), and from ability of *L. monocytogenes* to grow at ≥0°C in meat even under vacuum (*19*).

The finding of *Listeria* spp. in 40 to 53.3% and of *L. monocytogenes* in 10 to 22.2% of retailed fresh chicken parts is in agreement with figures published before. Studies from the 70's in the U.K. indicated prevalence of 33.3 to 51.7% for fresh chicken, 25% for turkeys, 100% for ducks (very few analyzed), and 50% for pheasants (*17,30*). In 1988 the presence of *L. monocytogenes* was reported (*16*) in 60% of raw chicken meat obtained from retail outlets. Data from Czechoslovakia indicated the presence of *L. monocytogenes* in 70.2% of 47 chickens purchased from 12 butcher shops (*12*). Data from Switzerland (*5*) indicated the presence of *Listeria* spp. in 55.3% and of *L. monocytogenes* in 25% of raw poultry samples. Recent data from New Zealand (*33*) reported the presence of *L. monocytogenes* in 48% (12/25) of retail display poultry portions collected over three sampling days. Data from the USA are scarce. A prevalence of 23 to 48% for *L. monocytogenes* in broiler chicken meat has been reported (*2,6*). The presence of *L. monocytogenes* in 15% of retailed fresh liver samples, and in 33% in packaged livers at the end of the processing line in the slaughterhouse, as shown in this study, is higher than the 11.1% (3/27) reported for fresh chicken livers from Canada (*14*).

Our study has demonstrated the significantly lower prevalence of *Listeria* spp. and *L. monocytogenes* in semi-frozen chicken parts than in fresh parts. Whether this is due to destruction of some *Listeria* cells during the freezing process or the inability of the recovery method for debilitated cells was not determined. In separate experiments we found that the freezing process decreased the numbers of two *L. monocytogenes* strains inoculated in BHI broth by 0.24-0.42 logs and storage of the frozen broth for 6 months by 0.24 to 1.44 logs.

In the U.K., Kwantes, and Isaac (*26*) reported the presence of *L. monocytogenes* in 64.1% (41/64) and Gitter (*17*) in 10.7% (7/56) of frozen chicken. In the present study we isolated *L. monocytogenes* from 6.6% and *Listeria* spp. from 10% of semi-frozen chicken legs, wings, and livers. These products arrived at the supermarket as fresh but were exposed to <0°C to extend the shelf life. At the time of purchasing they were semi-solid.

Data on the prevalence of *L. monocytogenes* in the various sites of a meat and poultry slaughterhouse is limited. The closest study to ours is the one reported recently by Skovgaard and Morgen (*42*). These authors used a pooled sample approach to evaluate the presence of *Listeria* in chicken neck skin and fecal material (25-30 g) from the cages in two slaughterhouses in Denmark. Pooled samples represented at least 10 birds each. *Listeria* spp., *L. monocytogenes,* and *L. innocua* were present in 94.1% (16/17), 47% (8/17), and 94.1% of composite neck skin samples, respectively. The corresponding numbers for cage material (feces and vomited fodder) were 33.3, 33.3, and 33.3% (5/15). *Listeria* spp. were present in 1/1 of giblet cooler water and 1/1 scalding tank filtered overflow water, and not in the two samples of spin chiller and one sample of scalding tank water.

The absence of *L. monocytogenes* from neck skin, livers, and hearts is understandable. The pathogen was present in 18.8% of mechanically deboned meat homogenate (MDM) which is a composite sample. *Listeria* spp. were also present in FPDW (18.8%), CWO (12.5%), and RWCG (37%). The significance of the defeathering machine, chillers, and recycled water in product cross-contamination and environmental contamination, as shown before for *Campylobacter jejuni* (*20,44*) is obvious. The presence of *L. monocytogenes* in poultry packing plant effluent at levels >16000 cells /L has been shown repeatedly in the U.K. (*43*).

Listeria spp. were not isolated from any of the feather composites or fecal samples. Feathers were evaluated as an indicator of environmental contamination of birds and intestinal contents as an indicator of endogenous infection. Absence of *Listeria* spp. in both feathers and intestinal contents may be due to the relative small number of samples analyzed. Dijkstra (*8,9*), in examining 2373 broiler intestines collected in the slaughterhouse, and representing 146 farms found that only 4.1% contained *Listeria*. When he examined intestinal contents of 3090 broilers aged 1 to 5 wk and coming from 1025 farms, he found 23.7% of the farms as having carrier birds. Based on these findings and the report of Skovgaard and Morgen (*42*) we can consider the birds as the reservoir of incoming *Listeria* to the plant, even though we failed to isolate the agent from fecal and feather samples. Ineffective sanitary practices in the plant may turn it into another major *Listeria* source.

The presence of *Listeria* in the feces of various human groups has been reviewed (*37*). Carriers in red meat and poultry plants ranged from 0.47 to 12.8% (*11,12,35,37*). The contribution of human fecal carriers to product contamination should be insignificant as these people may be considered victims of their exposure to *Listeria* originating from animals rather than being themselves a true fecal reservoir. In contrast, handlers may play a major role in spreading the contamination to poultry parts as they move

along the various stages of processing. This is supported by the data of Table 4 and 5. The prevalence of *Listeria* spp. from 6.5, 0.0, and 0.0% in livers, wings and legs, immediately after chilling increased to 46.7, 73.3, and 80.0% immediately after packaging, respectively (Table 4). While cross-contamination among poultry parts can contribute to the spreading of *Listeria*, hands or gloves of handlers once contaminated can more widely spread the agent because of the volume of parts handled. Such spreading is further amplified by the contribution of additional handlers along the line, like those hanging the birds after chilling, those cutting them into parts, and others packaging them. As Table 5 has shown, the prevalence of *L. monocytogenes* increased from 10 to 36.4 and then to 45.5% as the chicken carcasses and parts moved through station A, B, and C (hanging, cutting, packaging), respectively. The trend was also similar for *Listeria* spp. with the corresponding figures being 20, 45.5, and 59%. Overall, 46.7% of the product handlers carried *Listeria* spp. in their hands and gloves. An evaluation of four red meat plants in Czechoslovakia (*12*) over a two year period demonstrated the presence of *L. monocytogenes* in 25.6% (22/86) of the hands and 23.2% (20/86) of the gloves of meat handlers.

Of the nine combinations concerning three products (livers, legs, and wings) and three listerias (*Listeria* spp., *L. monocytogenes,* and *L. innocua*), in six there was increase in prevalence, in two there was slight decrease, and in one the prevalence remained the same when the products were kept at 4°C for 4 d (Table 4) as compared to initial prevalence. *L. monocytogenes* prevalence increased in all three products with storage. In the absence of *Listeria* counts in the chicken parts the question of whether growth of the agent took place during storage can not be answered with certainty. Increase in prevalence may reflect true growth from undetectable to detectable levels with *L. monocytogenes* having a shorter lag phase and being influenced less by the meat environment than *L. innocua*.

The potential of *Listeria* growth in raw meat at low temperatures has been addressed before. Growth or no growth at 4°C during 14 to 25 d storage, in the presence or absence of air was affected by the type of meat (whether lamb, pork, or beef) (*23,24,25*). Recently Grau and Vanderline (*19*) demonstrated the growth of *L. monocytogenes* in vacuum packaged beef stored at 0°C or 5.3°C. Growth was observed at 0°C. On low pH meat of 5.6, it started after 1-2 wk on fatty tissue, after 3 wk in the weep, and 9 wk on the lean tissue. On pH 6.0 meat, lag was very short on fat and 2 to 3 wk on lean meat and weep. At 5.3°C and low pH meat, it appeared to be little if any lag phase on fat and 5-6 d on lean meat.

Although *Listeria* spp. and *L. monocytogenes* were present in relatively high proportion of retailed packaged poultry parts, no indication of the numbers of the agent was obtained in this study. Gilbert and Pini (*16*) in their recent report indicated that in the U.K. poultry meat carried $<10^2$ *L. monocytogenes* cells/g.

In conclusion this study reinforced previous reports that raw poultry meat is a major source of *L. monocytogenes* though its direct incrimination to human disease still has to be proven. The study has also demonstrated that through improvement in certain practices at the slaughterhouse level there is a good possibility of minimizing the high *Listeria* prevalence in the finished products. This can be accomplished through strict adherence to Good Manufacturing Practice standards (*45*) and new innovations in type and schedule of hand and glove sanitizing.

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