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Occurrence and Characterization of *Salmonella* from Chicken Nuggets, Strips, and Pelleted Broiler Feed

O. BUCHER,[1] R. A. HOLLEY,[1]* R. AHMED,[1,2] H. TABOR,[2] C. NADON,[2] L. K. NG,[2] AND J.-Y. D'AOUST[3]

[1]*Department of Food Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2;* [2]*National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada R3E 3R2; and* [3]*Bureau of Microbial Hazards, Sir F. G. Banting Research Centre, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2*

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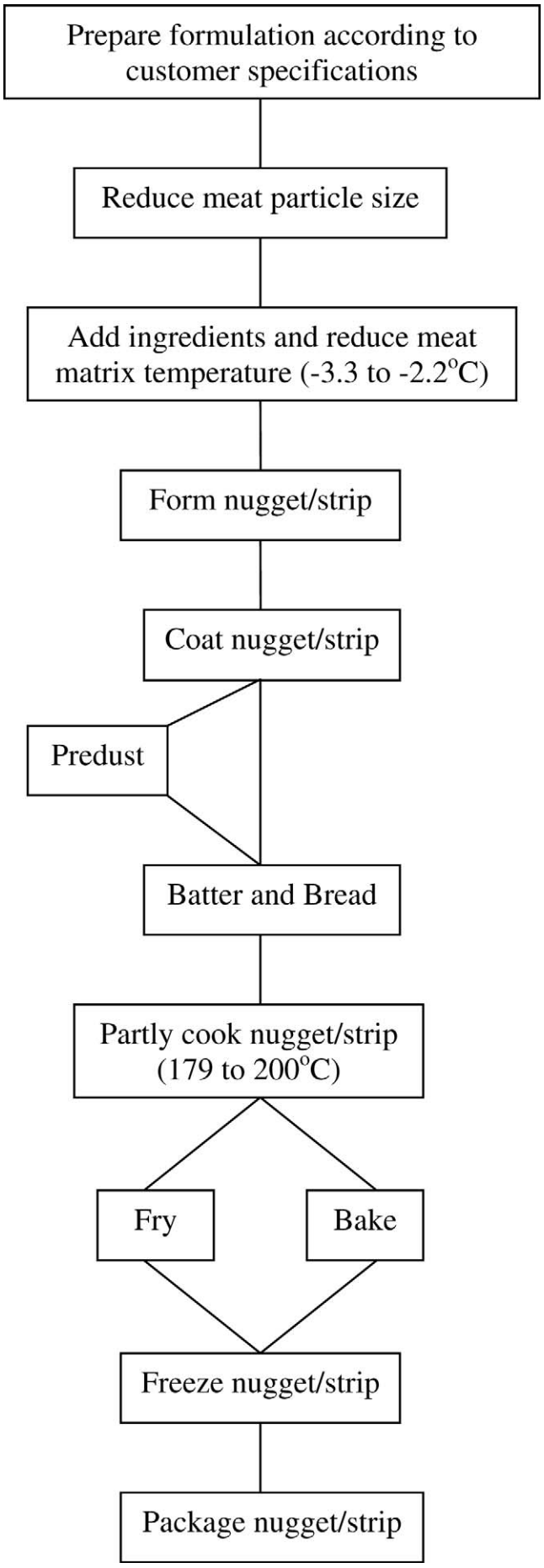
ABSTRACT

Raw, frozen chicken nuggets and strips have been identified as a significant risk factor in contracting foodborne salmonellosis. Cases of salmonellosis as a result of consuming partly cooked chicken nuggets may be due in part to *Salmonella* strains originating in broiler feed. This study was undertaken to determine the occurrence and characterize the strains of *Salmonella* contaminating chicken nuggets, strips, and pelleted feeds, in an attempt to demonstrate whether the same *Salmonella* strains present in broiler feed could be isolated from raw, frozen chicken nuggets and strips available for human consumption. Salmonellae were recovered using the Health Canada MFHPB-20 method for the isolation and identification of *Salmonella* from foods. Strains were characterized by serotyping, phage typing, antimicrobial resistance typing (R-typing), and by pulsed-field gel electrophoresis (PFGE). Salmonellae were isolated from 25-g samples in 27% ($n = 92$) of nugget and strip samples, 95% ($n = 20$) of chicken nugget meat samples, and from 9% ($n = 111$) of pelleted feed samples. *Salmonella* Heidelberg, *Salmonella* Enteritidis, and *Salmonella* Orion were the most commonly isolated serovars from chicken nuggets and strips, nugget and strip meat, and pelleted broiler feeds, respectively. *Salmonella* Enteritidis phage type (PT) 13a with PFGE pattern SENXAI.0006 and R-type sensitive as well as *Salmonella* Enteritidis PT13a with PFGE pattern SENXAI.0068 and R-type sensitive were isolated from pelleted feed, and chicken nugget and strip meat in two separate instances. Data showed that *Salmonella* strains isolated from broiler feed were indistinguishable from strains isolated from packaged raw, frozen chicken nuggets and strips. However, results did not rule out the possibility that breeding stock or contamination during processing may have contributed to chicken meat contamination by *Salmonella.*

Raw, frozen chicken nuggets and strips purchased at retail stores and prepared for consumption at home have been identified as a significant risk factor in contracting foodborne salmonellosis. A 1998 investigation into an outbreak of *Salmonella* Typhimurium phage type (PT) 12 infection in Australia, as well as a 2003 investigation into a family cluster of *Salmonella* Heidelberg PT26 infections in British Columbia, Canada, and case-control studies conducted in 2002 in Quebec, Canada, and in 2003 across Canada, have highlighted this problem *(7, 18, 20).* With the exception of a few precooked, microwave-ready products, most frozen chicken nuggets and strips sold in stores are not fully cooked. During processing, the product undergoes a partial-frying step to ensure its shape is maintained and to provide the batter and breading coating a golden brown color before it is frozen and packaged (Fig. 1). Since the resulting nugget or strip neither looks nor smells raw, increased risk of contracting salmonellosis from these products has been attributed to uncertainty as to whether they are cooked *(18).* A case-control investigation across Canada in 2003 found that consumption of home-prepared chicken nuggets and strips was a leading risk factor for *Salmonella* Heidelberg infection, with 34% of all laboratory-confirmed cases of *Salmonella* Heidelberg infection attributable to these products *(7).* In addition, one-third of the respondents participating in another 2003 case-controlled study in British Columbia believed the frozen nuggets were precooked, requiring reheating only, with 27.3% indicating they either always or sometimes used a microwave oven to heat these products, despite voluntary labeling advising against this method of cooking *(20).*

Pelleted and mash poultry feeds have long been recognized as vectors for *Salmonella* contamination of commercial poultry production systems. In particular, ingredients of animal origin have been shown to have the highest frequencies of contamination, though ingredients of vegetable origin have also been reported to harbor the organism *(3, 6, 10, 15, 24, 27).* Recent work conducted in the southern United States found that 8.8% of mash feed samples and 4.2% of pelleted feed samples were contaminated with *Salmonella,* suggesting that the pelleting process reduces *Salmonella* isolations from poultry feed but does not eliminate the organism *(17).* In addition, it was also mentioned that contrary to other suggested temperatures (80°C), a temperature of 85°C is required to produce *Salmonella*-free feed. Current European Union legislation requires that feed manufacturers adopt hazard analysis and critical control point (HACCP) procedures to control *Salmonella* and other pathogens in feeds *(17).* Currently, Canada does not have a standard for feed pelleting time and temperature treat-

* Author for correspondence. Tel: 204-474-9601; Fax: 204-474-7630; E-mail: rick_holley@umanitoba.ca.



ment. Even though poultry feeds have been suggested as significant vectors for *Salmonella* contamination of broiler flocks, the extent to which foodborne outbreaks have been linked back to the consumption of poultry products manufactured from flocks fed contaminated feed is unknown *(3).* Nevertheless, it has been reported by primary breeders that close to 80% of *Salmonella* serovars isolated from feeds and feed ingredients were the same serotypes isolated weeks later in breeding flocks and their offspring *(17).*

This work was undertaken to determine whether the same strains of *Salmonella* could be isolated from pelleted broiler feed and raw, frozen chicken nuggets and strips. The objectives of this work were two-fold: (i) to determine the frequency of *Salmonella* occurrence in pelleted feeds and chicken nuggets and strips, and (ii) to characterize the strains isolated by serotyping, PT, antimicrobial resistance typing (R-typing), and pulsed-field gel electrophoresis (PFGE) analysis to establish whether the same strains could be found in both feed and in nuggets and strips.

MATERIALS AND METHODS

Sample collection and handling. A total of 92 raw, frozen, chicken nugget and strip samples representing 58 different lots prepared at nine different establishments were collected. Chicken nuggets and strips used in this study were identical products, differing only in their shape. Of these, 57 (wholesale samples) were couriered to the University of Manitoba (Winnipeg, Manitoba, Canada) laboratory by the two major manufacturers of chicken nuggets and strips in Canada (from three different processing plants in Ontario). The remaining 35 were obtained from four local supermarkets (Winnipeg). In addition to chicken nuggets and strips, 20 samples representing one lot of chicken nugget and strip meat (destined for nugget and strip manufacture) was also provided by one of the major manufacturers. All samples were transported frozen and kept at −28°C on arrival until analysis. Samples were typically analyzed on receipt or within 2 weeks after arrival.

A total of 111 samples of pelleted broiler feed from different lots were couriered to the laboratory from the major manufacturer of pelleted poultry feed in Canada (southern Ontario). Of the samples provided, 13 contained ingredients of animal origin, while 98 were made from ingredients of vegetable origin only. Samples were kept under refrigeration (5°C) or briefly at room temperature (22°C) until analysis. Samples were analyzed within 2 weeks after arrival.

Although samples were collected from the major manufacturers of both pelleted feed and raw, frozen chicken nuggets and strips in Canada, it was not possible to determine whether the lots of feed analyzed were fed to broiler hens used in the production of the lots of chicken nuggets and strips sampled for *Salmonella* in this study.

***Salmonella* isolation from pelleted broiler feed.** Sampling was done in duplicate and followed the Health Canada MFHPB-20 method for the isolation and identification of *Salmonella* from foods *(8).* Isolation from pelleted feed was done by mixing the contents (about 500 g) of the sample containers by hand for 30 s before aseptically weighing 25 g into 225 ml of buffered peptone

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FIGURE 1. Process flow diagram of chicken nugget and strip manufacture. Adapted from Owens (22).

TABLE 1. *Serotype descriptions (antigenic formulae[a]) of the* Salmonella *serovars isolated from pelleted feed or raw, frozen chicken nuggets and strips and nugget meat*

Serovar	Somatic (O) antigen[b]	Flagellar (H) antigen	
		Phase 1	Phase 2
Enteritidis	1,9,12	[f],g,m,[p][c]	[1,7]
Hadar	6,8	z_{10}	e,n,x
Heidelberg	1,4,[5],12	r	1,2
Indiana	1,4,12	z	1,7
Infantis	6,7,14	r	1,5
Kentucky	8,20	i	z_6
Mbandaka	6,7,14	z_{10}	e,n,z_{15}
Orion	3,10,[15],[15,34]	y	1,5

[a] Serotypes are classified by the Kauffman-White scheme outlined by LeMinor and Popoff *(19)*.

[b] Underlined antigenic factors indicate bacteriophage conversion. These antigens are present only when the culture is lysogenized.

[c] Brackets indicate O or H antigens that may be present independent of bacteriophage conversion. H factors in brackets denote antigens that are occasionally found in wild strains.

water (Difco, Becton Dickinson, Sparks, Md.). Samples were soaked at 22°C for 1 h, treated in a stomacher (Bagmixer 400, Interscience, Bois Arpent, France) for 30 s and incubated for 24 h at 35°C. Following incubation, 1 ml of sample was pipetted into 9 ml of selenite cystine (Fluka BioChemika, Buchs, Switzerland) and tetrathionate brilliant green (Fluka BioChemika) broth tubes. Tubes were vortex mixed and incubated for 24 h at 35 and 43°C, respectively. Following incubation, a loopful of selenite cystine and tetrathionate brilliant green sample was streaked onto duplicate plates of brilliant green sulfa (Difco, Becton Dickinson) and bismuth sulfite (Difco, Becton Dickinson) agar. The plates were incubated at 35°C for 24 and 48 h, respectively. Plates with suspect *Salmonella* colonies were counted, and a number (the square root) of those colonies was streaked on MacConkey agar (Difco, Becton Dickinson) plates and incubated for 24 h at 35°C. Single colonies from MacConkey agar plates were then stab and surface inoculated into triple sugar iron (Oxoid, Ltd., Basingstoke, UK), lysine iron (Oxoid, Ltd.), and urea (Difco, Becton Dickinson) agar slants. Slants were loosely capped and incubated for 24 h at 35°C. Presumptive-positive slants were confirmed as *Salmonella* with the *Salmonella* Rapid Latex Agglutination Test Kit (Oxoid, Ltd.) according to the manufacturer's instructions. Samples confirmed as *Salmonella* were stab inoculated into nutrient agar (Oxoid, Ltd.) tubes and delivered to the National Microbiology Laboratory (Winnipeg) for characterization by serotyping, PT, R-typing, and PFGE. Serotypes were described by somatic (O) and flagellar (H) antigens according to the antigenic formulae (Table 1) outlined by LeMinor and Popoff *(19)*. PT was done per the standard methods described by Anderson and Williams *(1)*. R-typing was performed according to Clinical and Laboratory Standards Institute (formerly NCCLS) guidelines *(21)*. Table 2 contains ranges, and sensitivity and resistance breakpoint concentrations of the antimicrobials used for antimicrobial resistance profiling. PFGE analyses were performed by PulseNet Canada according to internationally standardized procedures *(5)*.

***Salmonella* isolation from nuggets and strips, batter coating, and meat.** Sampling of raw, frozen chicken nuggets and strips was done in triplicate to account for variance in contamination between individual chicken nugget and strip pieces. Samples in containers (ranging from 0.8 to 3 kg, containing between 20 and 50 individual nuggets and strips) were mixed for 30 s before aseptically weighing 25 g of sample into 225 ml of buffered peptone water. Samples were thawed at 22°C for 1 h, treated for 30 s in a stomacher, and incubated for 24 h at 35°C. Subsequent isolation and characterization steps were carried out as noted in the previous section.

TABLE 2. *Antimicrobials and susceptible ranges used for R-typing* Salmonella *serovars isolated[a]*

Antimicrobial	Susceptible range (μg/ml)	Intermediate range (μg/ml)	Resistant range (μg/ml)
Amikacin	≤16	32	≥64
Amoxicillin–clavulanic acid	≤8/4	16/8	≥32/16
Ampicillin	≤8	16	≥32
Cefoxitin	≤8	16	≥32
Ceftiofur	≤2	4	≥8
Ceftriaxone	≤8	16–32	≥64
Chloramphenicol	≤8	16	≥32
Ciprofloxacin	≤1	2	≥4
Gentamicin	≤4	8	≥16
Kanamycin	≤16	32	≥64
Nalidixic acid	≤16	—	≥32
Streptomycin	≤32	—	≥64
Sulfisoxazole	≤256	—	≥512
Tetracycline	≤4	8	≥16
Trimethoprim-sulfamethoxazole	≤2/38	—	≥4/76

[a] Antimicrobial susceptibility testing was performed according to CLSI (formerly NCCLS) guidelines *(21)*.

Unbreaded, frozen chicken nugget meat received directly from the manufacturer was sampled as above by aseptically weighing 25 g of partly thawed meat into 225 ml of buffered peptone water. Samples were held at 22°C to thaw for 1 h, pummeled for 30 s in the stomacher, and incubated for 24 h at 35°C. Subsequent *Salmonella* isolation and characterization steps were carried out as noted previously.

Chicken nugget coating (batter and breading) samples were analyzed separately from a package of *Salmonella*-positive chicken nuggets after partial thawing. The coating was removed, the surface previously exposed to the meat was treated with 95% ethanol, and the excess alcohol burned away. Twenty 25-g samples of coating were aseptically weighed separately into 225 ml of buffered peptone water, treated in the stomacher for 30 s, and incubated for 24 h at 35°C, with subsequent isolation and characterization steps carried out as noted previously.

RESULTS

Occurrence of *Salmonella* in pelleted feed. *Salmonella* was found in 9% of the 111 samples or lots tested (Table 3). Feed manufactured from ingredients of both animal and vegetable origin had a higher percentage of *Salmonella*-positive samples (15%) compared with feed manufactured from ingredients of only vegetable origin (8%). *Salmonella* Enteritidis had somatic and flagellar antigen profiles typical for this organism (Table 1), while *Salmonella* Orion had somatic antigens O15 and O34, indicating the culture had become lysogenized. *Salmonella* Orion was the most common serovar isolated and was found in 8 of

TABLE 3. *Occurrence of* Salmonella *in pelleted broiler feed made from ingredients of animal plus vegetable origin (mixed) as well as from vegetable origin only*

Ingredient origin	No. of samples	No. of lots	No. (%) of positive samples/lot
Vegetable	98	98	8 (8)
Mixed	13	13	2 (15)
Total	111	111	10 (9)

10 positive samples (Table 4). All of the *Salmonella* Orion isolates had PFGE pattern OrionXAI.0002 and were susceptible to all the antimicrobials tested. Two strains of *Salmonella* Enteritidis were isolated (having different PFGE patterns), but were both PT13a and were sensitive to all the antimicrobials tested.

Occurrence of *Salmonella* in chicken nuggets and strips. The results from analysis of 92 chicken nugget and strip samples are shown in Table 5. Sampling of chicken nuggets and strips from local retail stores revealed that 31% were contaminated with *Salmonella,* while 25% of wholesale samples were found to be positive for *Salmonella.* Overall, 27% of samples were positive for *Salmonella.* Table 6 indicates the different strains of *Salmonella* isolated. Both retail and wholesale samples had similar diversity among the salmonellae isolated, with 12 different strains isolated from retail sources and 13 different strains isolated from wholesale sources. All 12 of the retail isolates except for *Salmonella* Heidelberg PT29 were isolated from different lots. Salmonellae were absent from retail product from three establishments, while one plant was a source of each of the different isolates except for *Salmonella* Hadar and Infantis. The 13 isolates from wholesale samples were all found in different lots except for *Salmonella enterica* subsp. *enterica* 6,8:−e,n,x. Another establishment served as a source of all serovars isolated except for *S. enterica* subsp. *enterica* 6,8:−e,n,x, *Salmonella* Hadar, and *Salmonella* Mbandaka. All nugget and strip isolates had typical somatic and flagellar antigen profiles except serovars Heidelberg, Infantis, and Kentucky. Two *Salmonella* Heidelberg isolates from wholesale samples had an O1 antigen, indicating the strains had become lysogenized. All strains of *Salmonella* Kentucky had an O20 antigen, while the single *Salmonella* Infantis strain had an O14 antigen, indicative of lysogenization. *Salmonella* Heidelberg was the most frequently isolated serovar in both sets of samples and showed the most diversity among the strains isolated, with six different strains isolated from both retail and wholesale samples. Five different PTs (19, 19b, 26, 29, and 54) and four different R-types and PFGE patterns were identified in isolates from retail samples, while four PTs (4, 19, 29, and atypical), two R-types (in common with retail isolates), and three PFGE patterns (two different from retail isolates) were found in isolates from wholesale samples. *Salmonella* Kentucky was the second most frequently isolated serovar, with two strains isolated from retail samples and four strains isolated from wholesale samples. These isolates from retail samples had the same R-type (sensitive) but differed in their PFGE patterns, whereas isolates from wholesale samples had four different R-types and PFGE patterns. *Salmonella* Hadar was the only other serovar isolated from both retail and wholesale samples, though strains differed in PT, R-type, and PFGE patterns. *Salmonella* Enteritidis was only isolated from retail samples, with the same strain (PT13, R-type sensitive, PFGE pattern SENXAI.0038) appearing in three different samples. Single isolates of *Salmonella* Indiana and *Salmonella* Infantis were found in retail samples. Strains isolated only from wholesale samples were *S. enterica* ssp. *enterica* var. 6,8:−e,n,x (R-type Te, PFGE SHAXAI.0037) and a single strain of *Salmonella* Mbandaka.

TABLE 4. *Serotype, PT, PFGE pattern, and R-type of* Salmonella *isolated from pelleted broiler feed of animal plus vegetable origin (mixed) and from vegetable ingredients only*

Feed type	Serovar	PT[a]	PFGE pattern[b]	R-type[c]	No. of contaminated samples (n = 10)
Mixed	Enteritidis	13a	SENXAI.0068	Sensitive	1
	Orion	—	OrionXAI.0002	Sensitive	1
Vegetable	Enteritidis	13a	SENXAI.0006	Sensitive	1
	Orion	—	OrionXAI.0002	Sensitive	7

[a] There is no PT scheme available for *Salmonella* Orion.
[b] Pulse types (PFGE) were determined using *Xba*I nuclease.
[c] Antimicrobials tested are listed in Table 2.

***Salmonella* in chicken nugget meat and nugget coating.** Chicken nugget meat and chicken nugget coating were sampled to determine whether these components were possible sources of *Salmonella. Salmonella* was present in 95% of chicken nugget meat samples analyzed, but were absent from the coating (Table 5). Three different *Salmonella* strains were isolated from chicken nugget meat: two were *Salmonella* Enteritidis and one was *Salmonella* Heidelberg (Table 6). All strains showed typical somatic and flagellar antigen profiles (Table 1). *Salmonella* Enteritidis was the most common, and 19 strains were isolated from 18 of the 19 samples tested, whereas *Salmonella* Heidelberg was isolated from only 1 of the samples. The *Salmonella* Enteritidis strains were all PT13a, with 11 of the strains having

TABLE 5. *Occurrence of* Salmonella *in chicken nuggets and strips sampled from retail stores and manufacturing plants (wholesale), plus chicken nugget meat used in nugget and strip manufacture and chicken nugget coating*

Sample type	No. of samples	No. of lots	No. (%) of positive samples	No. (%) of positive lots
Retail	35	20	11 (31)	9 (45)
Wholesale	57	38	14 (25)	13 (34)
Total	92	58	25 (27)	22 (38)
Nugget meat	20	1	19 (95)	1 (100)
Nugget coating	20	1	0 (0)	0 (0)

TABLE 6. *Serotype, PT, PFGE patterns, and R-type of* Salmonella *isolated from chicken nuggets and strips sampled from retail and manufacturing plants (wholesale)*

Sample type	Serovar/serotype	PT	PFGE pattern[a]	R-type[b]	No. of contaminated samples (n = 44)[c]
Retail	Heidelberg	19	SHEXAI.0121	GeStSu	1
		19b	SHEXAI.0068	Am	1
		26	SHEXAI.0141	Sensitive	1
		26	SHEXAI.0001	Sensitive	1
		29	SHEXAI.0001	AcAmCeCf(Cx)	2
		54	SHEXAI.0001	Sensitive	1
	Kentucky	—[d,e]	KenXAI.0002	Sensitive	1
		—[d,e]	KenXAI.0004	Sensitive	1
	Hadar	23	SHAXAI.0003	StTe	1
	Indiana	—[d]	IndiXAI.0003	St	1
	Infantis	5	SINXAI.0028	AcAmCeCf(Cx)	1
	Enteritidis	13	SENXAI.0038	Sensitive	3
Wholesale	Heidelberg	4	SHEXAI.0007	AcAmCeCf(Cx)	2
		4	SHEXAI.0129	AcAmCeCf(Cx)	1
		19	SHEXAI.0001	Sensitive	1
		29[e]	SHEXAI.0001	Sensitive	3
		29	SHEXAI.0007	Sensitive	2
		Atypical[e]	SHEXAI.0001	AcAmCeCf(Cx)	1
	Kentucky	—[d,e]	KenXAI.0019	AcAmCeCf(Cx)	1
		—[d,e]	KenXAI.0018	AcAmCeCf	1
		—[d,e]	KenXAI.0013	StTe	1
		—[d,e]	KenXAI.0021	Te	1
	Hadar	Atypical	SHAXAI.0037	Te	1
	6,8:-e,n,x	—[d]	SHAXAI.0037	Te	2
	Mbandaka	—[d]	MbaXAI.0009	Sensitive	1
Nugget meat	Enteritidis	13a	SENXAI.0006	Sensitive	11
	Enteritidis	13a	SENXAI.0068	Sensitive	8
	Heidelberg	40	SHEXAI.0141	Sensitive	1

[a] Pulse types were determined using *Xba*I nuclease.

[b] Antimicrobials in parentheses showed intermediate resistance. Ai, amikacin; Ac, amoxicillin–clavulanic acid; Am, ampicillin; Ce, cefoxitin; Cf, ceftiofur; Cx, ceftriaxone; Ch, chloramphenicol; Ci, ciprofloxacin; Ge, gentamicin; Ka, kanamycin; Na, nalidixic acid; St, streptomycin; Su, sulfisoxazole; Te, tetracycline; Tm, trimethoprim-sulfamethoxazole.

[c] Three retail samples, four wholesale samples, and a single sample of nugget meat contained two or more different *Salmonella* strains differing by serovar, PT, or PFGE pattern.

[d] No PT scheme is available to subtype these organisms.

[e] Lysogenic *Salmonella* strains.

PFGE pattern SENXAI.0006 and the remaining 8 having pattern SENXAI.0068. Although PFGE can have limited discriminatory power for *Salmonella* Enteritidis, SENXAI.0006 and SENXAI.0068 are relatively rare PFGE patterns for this serovar in Canada. The *Salmonella* Heidelberg strain was found to be PT40 with PFGE pattern SHEXAI.0141. Strains isolated were found to be susceptible to all the antimicrobials tested.

DISCUSSION

Occurrence of *Salmonella*. It should be noted that because feed analyzed was from one commercial mill and because of the restricted number of different lots of chicken nuggets and strips sampled, the results presented may not truly represent the overall industry situation. Nonetheless, the samples were obtained from the largest commercial concerns active in the industry in Canada, and they demonstrated that *Salmonella* was present in these products. The observation here that *Salmonella* was present in 9% of pelleted feed samples is higher than the 0 to 6.6% incidence found in other reports *(3, 6, 27).* Hacking et al. *(16)* investigated the presence of *Salmonella* from a single feed mill in Ontario (Canada) and found *Salmonella* in 3% of pelleted feed samples. Differences in results may be attributed to different sensitivities in isolation protocols used. Previous difficulty in isolating *Salmonella* from feed has been thought to stem from the nonuniform distribution of the organism among samples, as well as the effect of stress on the organisms from processing operations in feed mills, which may injure the few cells (10 to 100 CFU/g) that are present *(17, 24, 27).* Jones and Richardson *(17)* found that feed mill management practices were related to *Salmonella* contamination, and so differences in contamination seen here may reflect this influence. In addition to differences among different feed mills, differences in regulatory guidelines in different countries may also affect the frequency of reported *Salmonella* contamination. In Canada, there are no strict guidelines for minimum time and temperature treat-

ments when pelleting feed, and lower processing temperatures (57 and 80°C are not uncommon) may be used when thermosensitive enzyme supplements are added *(27).* When feeds containing ingredients of animal origin were compared with those containing only vegetable ingredients, a higher incidence of *Salmonella* was found in the former. Though the number of feed samples containing ingredients of animal origin was small, the present results are consistent with others that show feeds with ingredients of animal origin tend to have higher rates of *Salmonella* contamination *(3, 6, 10, 15, 24, 27).*

Salmonellae were isolated from 27% of chicken nuggets and strips in the present work, and to our knowledge this is the first study to provide data on the occurrence of *Salmonella* in these products. During manufacture of nuggets, whole breast muscle and chunks of white and/or dark meat, ground breast meat with and without mechanically deboned meat plus skin can be used, depending on customer specifications *(2).* Whole pieces or chunks of meat must be size reduced to improve protein extraction and facilitate binding of meat pieces to prevent development of loose texture *(22).* Thus, similar to ground beef with internalized *E. coli* O157:H7, chicken nuggets and strips are non-intact meats, with the potential to have contaminating *Salmonella* distributed throughout the meat matrix *(20).* Since meat used in the manufacture of these products can come from a number of sources, the likelihood of obtaining meat contaminated with *Salmonella* is increased. Thus, comparisons between the present results and those found with ground or mechanically deboned chicken meat seem fitting. When random samples from large, small, and very small federally inspected processing facilities in the United States were taken during 1998 to 2000, it was found that the overall prevalence of *Salmonella* in ground chicken meat was 14.4%. In comparison, *Salmonella* contamination on broiler carcasses was found to be 10.2% *(23).* The latter result is slightly lower than the results in the present study, which may be a result of the implementation of the pathogen-reduction HACCP rule, since a general decline was observed in *Salmonella* prevalence over the course of the sampling period. Other work in São Paulo, Brazil, found that of 60 mechanically deboned meat samples, 15 were *Salmonella* positive, whereas only 6 of 45 samples of broiler carcass rinse water contained the organism *(4).* The present results showed a similar prevalence of *Salmonella.*

***Salmonella* isolate characterization.** In 2002 and 2003, laboratory surveillance data for enteric pathogens in Canada showed that *Salmonella* Senftenberg was the most prevalent serovar isolated from animal feed and feed ingredients, followed by *Salmonella* Montevideo *(9).* In the present study, three different strains of *Salmonella* from two different serovars (Enteritidis and Orion) with single PT and R-types and three different PFGE patterns were found in pelleted chicken feed, but *Salmonella* Senftenberg and *Salmonella* Montevideo were absent. Previously, *Salmonella* Orion and *Salmonella* Enteritidis were isolated from raw materials used in feed manufacture as well as mash feeds, but to our knowledge these serovars have not been isolated from pelleted feeds *(3, 6, 16, 24, 27).* Although thermal treatment can drastically reduce *Salmonella* in feeds, the pelleting processes actually used in different pelleting mills can be highly variable in terms of time, temperature and feed moisture *(3, 6, 24).*

Both chicken nugget meat and chicken nugget batter and breading were sampled for *Salmonella* to provide an indication of the source of chicken nugget and strip contamination. While three strains of *Salmonella* were isolated from chicken nugget meat, there was no *Salmonella* isolated from chicken nugget coating, suggesting that the source of *Salmonella* in chicken nuggets and strips was the meat. This result is not surprising, since most of the chicken nuggets and strips purchased in stores are often partially fried in oil for 20 to 30 s at temperatures of 179 to 200°C before freezing and packing *(2, 22).* Though it is established that chicken is a significant reservoir of *Salmonella* in food *(7, 9, 20),* there are other potential sources of contamination including spices and seasonings, which are added to the meat after particle size reduction *(25).* The three contaminating *Salmonella* strains found in nugget meat were two strains of *Salmonella* Enteritidis and one strain of *Salmonella* Heidelberg. This result is somewhat surprising, since preliminary surveillance results from three Canadian provinces in 2005 indicate that *Salmonella* Heidelberg can make up anywhere from 24 to 46% of *Salmonella* isolates from retail chicken samples, whereas *Salmonella* Enteritidis has been found in much lower percentages of 4 to 5% *(12).* While it is recognized that the meat used in the present tests was from a single lot, it is important to note that the two strains of *Salmonella* Enteritidis isolated from the nugget meat were indistinguishable, both phenotypically and genotypically, from those isolated from pelleted feed. This finding is significant, since the same strains of *Salmonella* Enteritidis were found in both feed and chicken nuggets and strips. However, since it could not be determined if the feed contaminated with these two strains of *Salmonella* Enteritidis was fed to broilers used to manufacture the sampled nuggets that were also contaminated with these same strains, there is no direct evidence to suggest their horizontal transfer.

Salmonella Heidelberg has been identified as a significant source of foodborne illness associated with consuming under-cooked chicken nuggets and strips. The present findings support conclusions regarding the involvement of this serovar since just over half of the *Salmonella*-positive samples contained *Salmonella* Heidelberg. Even though two of these *Salmonella* Heidelberg isolates were the same PT (26) as isolates from a family cluster outbreak in British Columbia in 2003 from patients and uneaten nuggets, isolates in the present study were different because they all were able to produce H_2S *(20).* The second most commonly isolated serovar from the present study was *Salmonella* Kentucky. This is in agreement with national findings indicating that after *Salmonella* Heidelberg, *Salmonella* Kentucky was the most frequently isolated serovar from chicken from 1999 to 2003 *(9).*

Serotyping isolates from the present study yielded lysogenic strains of *Salmonella* Orion, *Salmonella* Heidel-

berg, *Salmonella* Infantis, and *Salmonella* Kentucky. Lysogenized host cells have often been shown to demonstrate an increased resistance to environmental stresses *(14),* and the presence of bacteriophages in foods has been demonstrated *(13),* so the presence of these strains in chicken nuggets and strips is not surprising. It is of interest that in the present study 60% of salmonellae isolated from nuggets and strips was resistant to at least one antimicrobial, and that 40% was resistant to multiple antimicrobials. Though previous investigations into foodborne illness associated with consuming chicken nuggets and strips *(7, 20)* did not mention salmonellae with antimicrobial resistance, multidrug-resistant nontyphoidal salmonellae have been isolated from ground chicken meat sampled in the greater Washington, D.C., area *(26).* In the present study, the isolation of ceftriaxone-resistant *Salmonella* Heidelberg is notable. Antibiotics are not regularly used to treat *Salmonella* infections; although for people with an invasive infection they may be lifesaving. Ceftriaxone is a broad-spectrum cephalosporin commonly used to treat *Salmonella* infections in children *(11),* and so the finding of ceftriaxone-resistant *Salmonella* Heidelberg in chicken nuggets and strips is of great concern.

Despite its general acceptance that processing at 80°C for 1 min is satisfactory for killing salmonellae during poultry feed pelleting *(24, 27),* it is clear that *Salmonella* is still capable of contaminating pelleted broiler feed, perhaps by aerosolized dust in the pelleting mill. Recent work has found that pelleting at 85°C is more reliable for eliminating the organism *(17).* It is possible that the *Salmonella* serovars isolated from pelleted poultry feed had enhanced thermal resistance, and this is being examined. Additional comparisons between strains isolated from human clinical sources and those isolated from poultry feed and chicken nuggets and strips should provide incentive to re-examine guidelines regarding poultry feed processing and pelleting, and generate data on the extent to which foodborne illness can be traced back to contaminated animal feeds. The finding in the present study of two *Salmonella* Enteritidis strains in pelleted feed, indistinguishable from two *Salmonella* Enteritidis strains in chicken nugget meat, provides some evidence that *Salmonella* contaminating pelleted feed may be passed along the production chain to food for human consumption. Since it is unknown whether *Salmonella* was present in the lots of poultry feed used for rearing animals from which sampled nugget/strip meat was obtained, evidence provided for this source of meat contamination is only circumstantial.

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