

# Incidence of Salmonellae in Dressed Broiler-Fryer Chickens<sup>1</sup>

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## ABSTRACT

WOODBURN, MARGY (Purdue University, Lafayette, Ind.). Incidence of salmonellae in dressed broiler-fryer chickens. *Appl. Microbiol.* 12:492-495. 1964.—Salmonellae were isolated from 72 of 264 broiler-fryer type chickens that had been purchased in retail stores in the Lafayette, Ind., area in 1963. Meat from the tail area and giblet portions were used in sampling. Equal numbers of dressed whole and cut-up birds were positive for salmonellae. Thirteen different serotypes were identified, the more common being *Salmonella infantis*, *S. reading*, and *S. blockley*. Incubation at 43 C of the blended sample in Selenite-F Enrichment broth containing cystine gave a larger number of recoveries than did incubation at 37 C. There was no significant difference between the means for the birds that yielded salmonellae and those that did not in the locally processed group, when compared for numbers of aerobic microorganisms at 37 C, coliforms, or most probable number of enterococci. In a comparison of poultry processed in-state by the five processors included in the study with that processed out-of-state, there was a general trend for a larger number of positive specimens in the locally produced group. The fall season was an exception.

The incidence of salmonellae serotypes on dressed frying chickens in retail markets in the Lafayette, Ind., area was studied for four seasons as one part of a regional poultry marketing project. Several techniques were used to obtain a more complete recovery than a single method might offer. Poultry and poultry products were referred to by Quist (1963) as the sources most often incriminated in foodborne outbreaks of salmonellosis. The source of the salmonellae may be the bird itself or contamination during processing, or handling at the retail level or in the kitchen.

One approach was the study of birds at various stages of processing. Galton et al. (1955) did not find salmonellae in the birds removed from the processing line before evisceration, but did from the processing environment and from the edible viscera and iced carcasses. Sadler et al. (1961) recovered salmonellae from 3% of the birds examined in California plants. Brobst, Greenberg, and Gezon (1958) in Pennsylvania found birds that were positive for salmonellae in one of eight plants not federally inspected and on 1 of 5 days. In an Iowa study in a federally inspected location, Morris and Ayres (1960) recovered salmonellae from one-third of the eviscerated carcasses in two surveys, as well as from water after the final rinse and

drainage from chilled giblets. Earlier Walker and Ayres (1956) did not find salmonellae during a study of birds in six firms.

Since dressed poultry may contain viable salmonellae as it leaves the processing plant, other investigators studied the incidence on birds at the retail level. Felsenfeld, Young, and Yoshimura (1950) reported that 5% of the poultry that they purchased in Chicago markets from 1943 to 1949 were positive for salmonellae. Thatcher and Loit (1961), in a study of dressed broiler chickens from retail stores in three Canadian cities, isolated salmonellae from 14% of fresh birds that had not been treated with chlortetracycline. There was little evidence of seasonal variation. Wilson et al. (1961), in a study of retail poultry in the Cincinnati area, found that 24% of chicken giblets and 13 to 21% of parts tested contained salmonellae.

## MATERIALS AND METHODS

A total of 264 dressed broiler-fryer chickens were purchased at 19 local retail stores during the four seasons of 1963. A lot was purchased from each store once in each season and included both stores using birds produced within the state and those using out-of-state (chiefly southern) products. At least one whole bird and one cut-up bird were obtained at each market visit, and for three seasons two of each were purchased. At the time of purchase, information as to source and inspection was obtained. None of the carcasses had been antibiotic-treated, as reported by the seller. After storage in a refrigerator overnight, the birds were sampled by use of aseptic techniques. One sample consisted of 20 g of meat and skin from the tail area; the second, 25 g from the liver, heart, and gizzard of the same bird.

The procedure recommended by Montford and Thatcher (1961) was used. This included a 2-hr incubation of the blended slurry (sample plus an equal weight of peptone diluent) at 37 C, the addition of an equal amount of double-strength Selenite-F Enrichment broth (BBL) containing cystine, and incubation at 37 C for 18 to 24 hr. One loopful of the resultant culture was then streaked on Brilliant Green Agar (Difco) and on Brilliant Green Agar containing 0.1% sulfapyridine. After incubation at 37 C for 24 hr, typical colonies were transferred to Plate Count Agar slants (Difco) and were stored until subcultured to MacConkey Agar (Difco). It was not generally possible to choose more than one colony from each plate. If colony

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appearances differed, then a representative colony of each was chosen. Lactose-negative colonies were used to inoculate Triple Sugar Iron Agar (Difco) slants and one tube each of Gillies medium I and II (BBL). Cultures characteristic of salmonellae were tested for agglutination by polyvalent diagnostic antisera (Lederle Laboratories, Pearl River, N.Y.). Confirmation and serotyping of these cultures were then done in the Bureau of Laboratories of the Indiana State Board of Health through the courtesy of A. A. Hajna.

For the last three seasons, a 1:100 dilution of the Selenite-F culture was also made and was used to streak the two selective agar media. In addition, a portion of the inoculated enrichment broth was removed, incubated for 18 to 24 hr at 43 C (Georgala, 1962), and used on the two agar media.

The total count of aerobic microorganisms was determined from swab samples of two exterior and two interior areas (breast, thigh, and abdominal cavity to total 3 in.<sup>2</sup>). Appropriate dilutions were plated with Plate Count Agar (Difco) and were incubated for 24 hr at 37 C. Coliforms were determined by plating on Violet Red Bile Agar (Difco); enterococci were determined by the most probable number (MPN) method with triplicate tubes of ethyl violet azide broth (Difco) for each of three dilutions. Positive tubes were used to inoculate Azide Dextrose Broth (Difco). Tubes were read after 48 hr at 37 C. Two birds were used each day for this series. Statistical comparisons of means by the *t*-distribution test were made for total plate counts at 37 C, coliform numbers, and MPN of enterococci, for the birds produced in-state from which salmonellae were and were not recovered. Logarithmic transformations of counts and MPN were used in the calculations. These were not done for the out-of-state birds because of the small number in the salmonellae-positive group.

RESULTS

Salmonellae serotypes were isolated from 72 (27%) of the 264 dressed frying chickens that had been purchased on the retail market over a 1-year period (Table 1). Of the birds from which salmonellae were recovered, 22 were positive from meat samples only, 20 from giblet samples, and 30 from both. A marked increase in isolates from chickens processed out of state, and thus in the total, occurred in the fall. If data from the one method of recovery used for the entire year are compared, 30% were found for fall, as compared with 10, 7, and 5% for winter, spring, and summer, respectively. Data for the last three seasons indicated that the fall period also had the highest percentage (48%) of recoveries. One or more birds having positive cultures were found in 35 (45%) of the 77 days on which purchases were made. On the basis of market form, 36 of the dressed whole fryers and 36 of the cut-up type were positive.

For the year, salmonellae were recovered from a higher

percentage (33%) of birds produced within the state than from those produced out-of-state (18%; Table 2). Those supplied by one processor (A, Table 2) were more frequently positive than were those from the other four. However, in the fall season, there were higher percentages of positive birds and of stores supplying these birds in the out-of-state group. Of the 48 purchases of two to four birds in stores selling locally grown birds, 26 (54%) were found to yield salmonellae in one or more samples; of 29 purchases from stores selling birds produced in other states, 9 (31%) yielded salmonellae in one or more samples. Poultry from which salmonellae were recovered were purchased in at least one of the four seasons from 16 of the 19 retail stores. Of the three others, one was a small private

TABLE 1. Source of sample and technique of recovery of dressed frying chickens yielding salmonellae

Season	No. of birds	Salmonellae-positive		Selective agar medium <sup>a</sup>	Meat sample			Giblets		
		No.	Per cent		37 C	37 C <sup>b</sup>	43 C	37 C	37 C <sup>b</sup>	43 C
Winter...	68	7	10	BG BGS	2 <sup>c</sup> 2	— —	— —	1 2	— —	— —
Spring....	72	17	24	BG BGS	2 1	1 0	10 0	1 0	3 0	6 4
Summer..	64	19	30	BG BGS	1 0	1 0	13 5	2 0	1 0	11 10
Fall.....	60	29	48	BG BGS	7 7	7 6	12 8	6 7	8 8	15 17
Total....	264	72	27		22	15 <sup>d</sup>	48 <sup>d</sup>	19	20 <sup>d</sup>	63 <sup>d</sup>

<sup>a</sup> BG, Brilliant Green Agar (Difco); BGS, BG plus 0.1% sulfapyridine.

<sup>b</sup> At 1:100 dilution.

<sup>c</sup> Figures indicate number of chickens yielding salmonellae after incubation of samples in Selenite-F Enrichment broth containing cystine.

<sup>d</sup> Technique used on only 74% (196 of 264) of the birds.

TABLE 2. Comparison of salmonellae-positive dressed frying chickens by production area and processor for in-state and by season\*

Area	No. of birds purchased				No. positive for salmonellae			
	W	Sp	S	F	W	Sp	S	F
Indiana processor								
A.....	12	16	12	12	5	8	6	9
B.....	4	0	2	4	0	0	1	0
C.....	12	10	12	8	0	3	3	3
D.....	12	14	8	12	1	4	2	5
E.....	4	6	4	2	0	0	4	0
Total.....	44	46	38	38	6	15	16	17
Out-of-state.....	24	26	26	22	1	2	3	12
Total.....	68	72	64	60	7†	17	19	29

\* Abbreviations: W, winter; Sp, spring; S, summer; F, fall.

† Recovery procedure utilized only two basic techniques; in other seasons, six techniques were used.

grocery selling locally grown poultry and two were supermarkets, both selling out-of-state inspected poultry. Only one store provided positive samples in each of the four purchase periods. This was a supermarket selling birds processed in Indiana.

Thirteen different serotypes were recovered (Table 3).

TABLE 3. *Salmonella* serotypes recovered from dressed frying chickens purchased in local markets

<i>Salmonella</i> serotype	Season	No. of positive birds		
		Total	Sample source	
			Meat	Giblets
<i>S. anatum</i>	Summer	2	2	0
	Fall	4	3	2
<i>S. blockley</i>	Winter	2	1	1
	Spring	4	3	4
	Summer	2	0	2
<i>S. braenderup</i>	Fall	6	3	5
	Spring	1	1	0
<i>S. derby</i>	Summer	3	3	0
	Spring	1	1	0
<i>S. heidelberg</i>	Winter	1	0	1
	Spring	1	0	1
<i>S. infantis</i>	Summer	1	1	0
	Fall	6	5	3
	Spring	2	1	1
<i>S. litchfield</i>	Summer	6	6	6
	Fall	13	7	10
	Spring	1	0	1
<i>S. montevideo</i>	Summer	1	1	0
	Fall	3	1	2
<i>S. norwich</i>	Spring	1	0	1
	Fall	2	0	2
<i>S. reading</i>	Spring	3	2	1
	Winter	3	2	1
<i>S. saint-paul</i>	Spring	4	2	4
	Summer	5	3	5
<i>S. thompson</i>	Fall	4	3	1
	Winter	1	1	0
<i>S. typhimurium</i>	Spring	1	0	1
	Fall	2	1	2

TABLE 4. Comparison of means of total aerobic counts, coliforms, and enterococci for birds grouped as salmonellae-negative and -positive

Determination of surface contamination per in. <sup>2</sup>	Salmonellae present	No. of birds*	Mean of log transformations	t Value†
Total aerobic microorganisms at 37 C (Plate Count Agar)	+	53	5.20	1.38
	-	105	5.38	
Coliforms (Violet Red Bile Agar)	+	32	2.61	0.95
	-	58	2.81	
Enterococci (most probable number)	+	32	2.83	1.15
	-	58	2.64	

\* Only the poultry produced and processed in-state were included.

† All values given were not significant at the 5% level.

*S. infantis* was the predominant serotype isolated in the fall, and was recovered from the largest total number of birds. *S. reading* and *S. blockley* were next in frequency. *S. typhimurium* was isolated from three birds. More than one serotype were recovered from 13 birds. Birds purchased on 1 day were found to have a common serotype in six of the positive lots. However, contamination with two types was observed on ten lots from single days and with three to four types on 3 days.

The greatest gain in recovery appeared to be from the addition of Selenite-F Enrichment broth containing cystine, which had been incubated at 43 C (Table 1). Of the 29 meat or giblet samples that yielded only one of a possible six plates from which salmonellae were later confirmed, 16 were those from the series at 43 C, 9 from the standard 37 C, and 4 from the 1:100 dilution of the enrichment broth incubated at 37 C.

The total numbers of microorganisms recovered on Plate Count Agar at 37 C did not differ significantly when the means for birds from which salmonellae had been recovered were compared with those from negative birds (in-state only; Table 4). Means of coliform numbers and MPN of enterococci were also similar for the two groups.

#### DISCUSSION

The proportion of dressed fryers from which salmonellae were isolated was comparable to that reported by others, except for a high percentage in the fall. In the Chicago area, 32 of 643 birds that had been purchased in local markets were positive for salmonellae (Felsenfeld et al., 1950). In two more-recent studies, 14 of 100 dressed broiler chickens from sources in three Canadian cities (Thatcher and Loit, 1961), and 13 to 21% of parts and 22 of 93 giblets from purchases in the Cincinnati area (Wilson et al., 1961), yielded salmonellae. In the present study, salmonellae were recovered from 27% of the samples, including the fall group.

In general, the out-of-state birds were less frequently contaminated with salmonellae than were the in-state chickens. Only one of the in-state sources used the federal inspection service; the total number of birds from this single source was too small for comparisons. The difference in incidence between poultry from five in-state plants and poultry from federally inspected out-of-state plants may reflect a difference in equipment and plant sanitation or in detection of diseased birds. Sadler et al. (1961) observed that of the 21 chicken fryers testing positive, 16 of the carcasses had shown pathological changes warranting condemnation. Numerous studies confirmed the possible contamination of plant equipment and other surfaces from carcasses. In two previous studies, there was a greater percentage of birds yielding salmonellae from uninspected birds than from the U.S. Inspected ones. Felsenfeld et al. (1950) reported that 1% (3 of 327) of U.S. Inspected poultry and 9% (29 of 316) of uninspected poultry purchased in local shops yielded salmonellae.

Although Wilson et al. (1961) recovered salmonellae from 24% of poultry from local packer-processors and 13% from regional sources, the percentage expressed in terms of markets that supplied positive poultry was the same (39%) for both.

Sanitation ratings for the shops were reported by Wilson et al. (1961), but did not seem to be related to salmonellae recoveries. The effect of sanitation in the local market would appear to be small in the present study, since no greater recovery was found from cut-up birds than from whole birds.

The presence of viable salmonellae on poultry is of concern to the homemaker as a potential source of contamination of foods and kitchen work surfaces. Since the meat is usually cooked to the well-done stage, the consumption of poultry contaminated as the raw bird is less of a problem than the possible cross-contamination of the cooked product from the raw. Evidence from epidemiological studies for this possibility was presented by Sanborn (1963). Cross-contamination is also a hazard in the retail store, since the chicken may be cut on the same chopping block used in slicing ready-to-serve meats, or hands may carry the organisms. One of the stores selling inspected birds that yielded one or more positive samples in three of the four seasons also sold locally produced stewing hens obtained from a processor that frequently had fryers positive for salmonellae.

The serotypes recovered reflected current incidence, and included those that had also been isolated in other studies. *S. typhimurium* was the most prevalent from the chicken-processing plant specimens collected by Galton et al. (1955). Of the four serotypes isolated in the Canadian study (Thatcher and Loit, 1961), only *S. typhimurium* was also found in this investigation. Sadler et al. (1961) identified four different serotypes, including *S. infantis* from four of five positive flocks and *S. heidelberg* from one. Wilson et al. (1961) recovered *S. typhimurium*, *S. heidelberg*, *S. montevideo*, *S. anatum*, and *S. blockley* most commonly from poultry. Of the ten most common serotypes received at the Communicable Disease Center between 1947 and 1958, five were shared by man and poultry (Quist, 1963). Of these, *S. typhimurium*, *S. anatum*, and *S. montevideo* were also found in the present series.

As was suggested by others, incubation of the Selenite-F Enrichment broth containing cystine at 43 C appeared to favor the growth of the salmonellae. Harvey and Phillips (1961) reported the use of this incubation temperature for swab samples from the equipment and other surfaces of bakehouses and abattoirs. The medium they used was a Selenite-F broth containing Brilliant Green. Georgala (1962) discussed the increased toxicity at 43 C of selective factors to organisms other than salmonellae. A part of the effectiveness may be explained by the failure of the psychrotrophic Enterobacteriaceae (Mossel and Zwart, 1960), as well as other members of the psychrophilic flora, to grow at this temperature.

Undoubtedly, sampling from two rather than one broth samples, and the use of two selective agars, increased the number of positive samples (Galton, Lowery, and Hardy, 1954). Even so, the recovery was probably not complete, but represents an underestimate of both positive birds and serotypes. In most cases, a single typical colony was picked from each plate. The value of picking multiple colonies was recently illustrated in a family outbreak of salmonellosis (Communicable Disease Center, 1964).

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