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Survey of Market Poultry for *Salmonella* Infection[1]

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Poultry products were the source of infection in 16 per cent of the salmonellosis and 27 per cent of the gastroenteritis of unknown etiology reported to the National Office of Vital Statistics in 1958 (Dauer and Davids, 1959). Those authors pointed out that "since only a fraction of the outbreaks caused by staphylococci and salmonellae and other organisms appear to be reported, the real extent of the foodborne diseases is unknown." The accepted role of poultry in the epidemiology of human salmonellosis is evidenced by the statements of Edwards, Bruner, and Moran (1948) that fowls are the greatest reservoir of *Salmonella* in this country and that a high incidence of a given type of *Salmonella* in a certain locality is accompanied by a high incidence of the same type in man.

Frequent occurrence of *Salmonella* on carcasses and equipment in chicken and turkey processing plants has been adequately demonstrated by such studies as those of Browne (1949), Schneider and Gunderson (1949), Gunderson, McFadden, and Kyle (1954), and Galton *et al.* (1955). Isolation of this organism from carcasses in retail stores has been reported by Felsenfeld, Young, and Yoshimura (1950). Walker and Ayers (1956), however, were unable to isolate *Salmonella* from outer skin and body cavity surfaces. They agreed with Gunderson *et al.* (1954) that the most probable sources of these organisms are the viscera and intestinal contents. Browne (1949) demonstrated that infected birds grossly contaminated the environment during processing, with resultant contamination of carcasses. The methods of most investigators have not been critical enough to identify the source of *Salmonella* on equipment or carcasses—whether from human carriers, rodents, previously processed birds, or birds actually being sampled.

Edwards (1958) pointed out that, in any consideration of preventing transmission of a disease from animals to man, it is desirable first to establish the incidence of the condition in man and in the animal reservoir. Any attempt to deduce incidence in fowl from the reports of diagnostic laboratories produces invalid data, for obvious reasons. The only practical method of acquiring adequate data on incidence in a population of meat birds is by random samplings of poultry being processed for human consumption, with the method designed to detect not contamination, but active infection or a carrier state in individual carcasses.

Galton *et al.* (1955) reported two small random samplings: one of tissues and cecal feces of 129 chicken fryers (negative results) and one of cloacal swabs of 53 hens (one isolation). Brobst, Grunberg, and Gezon (1958), from what appears to be the only other similar sampling, reported negative results from cloacal swabs of 263 chickens and one isolation from cloacal swabs of 28 ducks.

Herein are the results of a survey designed to determine the incidence of *Salmonella* infection in poultry at the time of slaughter.

MATERIALS AND METHODS

Samplings were made over a period of 20 months in eight large processing plants in the Sacramento and San Joaquin valleys of California, and one in the San Francisco area. The plants process fryer chickens, hens, and turkeys from all areas of northern California, and turkeys from Nevada. The plant visited for each of the 53 samplings was randomly selected.

For a number of reasons, true randomness could not

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always be achieved in the selection of individual samples. Routine procedure was to sample every 100th carcass as it passed the official inspection point. In addition, some carcasses showing evidence of disease were sampled; in such cases the 10th normal carcass following was also sampled. The entire liver and a section of the intestines, including the cecum, were carefully excised from each sample carcass; the samples were placed in labeled half-pint ice cream cartons, which were then sealed and covered with ice in aluminum canisters for transportation to the laboratory at Davis. Six "mass" samplings of intestines only were made in turkeys. These were accomplished with every 2nd to 4th carcass either by excising the cecal area of the intestine or by inserting a sterile cotton swab deep into the intestine via the vent and immediately placing the swab in a tube of enrichment broth. The instruments used were washed in water and immersed in alcohol between each sampling.

The tissues were cultured either immediately after arrival at the laboratory (4-hr interval) or after holding overnight at 4 C (24-hr interval). This holding period was found not to adversely affect the recovery of *Salmonella* (Yamamoto *et al.*, 1961).

For recovery of *Salmonella* from the intestine, the routine procedure was the aseptic removal of pieces of cecal tonsil and feces from each sample for incubation in enrichment broth. After 18 to 24 hr at 37 C, all broth cultures were subcultured in a selective agar medium. Lactose-negative colonies developing on agar medium within 24 to 48 hr were identified by routine procedures. Cultures giving biochemical reactions characteristic for *Salmonella* and agglutinated by polyvalent *Salmonella* antiserum were sent to the California State Department of Public Health for typing.

At first, both selenite broth and SS (*Salmonella-Shigella*) agar[2] were used for isolation of *Salmonella*. Various other media were tested, however, in an attempt to improve recovery rate and reduce the incidence of *Proteus* sp., which appeared constantly. The media that appeared most satisfactory were selenite-cystine broth for enrichment and brilliant green agar as selective agar (Yamamoto *et al.*, 1961). These were therefore used for the bulk of the isolations.

When paired cecal and liver samples were taken from a given carcass, the liver was seared and bits of tissue were obtained well below the sterilized surface and seeded onto (a) blood agar, (b) tryptose broth enriched with horse serum (2 per cent), and (c) thioglycollate medium. Routine procedures were used to identify the organisms that developed in these media.

About 45 paired samples were taken for each sampling day except for six mass samplings of intestines (in turkeys only). The number of lots (flocks) processed during any one sampling varied from one to four. On 21 of the sampling days only turkeys were processed in the plant selected; on 14 days only fryer chickens; on 12 days only chicken hens. Both turkeys and fryer chickens were processed in the same plant on 1 day; both turkeys and chicken hens on 1 day; and both fryer chickens and hens on 4 days.

[2] Difco Laboratories, Inc., Detroit, Michigan.

Results and Discussion

Species of *Salmonella* were isolated from the intestinal tracts of infected birds being processed on 16 (30 per cent) of the 53 sampling days. They were found in turkeys on 10 (43 per cent) of the 23 days they were being examined; in chickens on 5 (19 per cent) of 19 days; and in hens on 2 (12 per cent) of 17 days. Assuming that the methods gave a suitable degree of randomness, one might conclude that these figures suggest the probable percentage of time in days during which carcasses being processed are subject to contamination by *Salmonella* from within the birds themselves or from birds processed concurrently. Since multiple flocks were processed on some of the sampling days, a total of 94 flocks were sampled; with some individual flocks, however, the adequacy of the sample remains a question. Twenty-one flocks (22 per cent) were found to contain infected birds; 13 (37 per cent) of 35 turkey flocks, 5 (18 per cent) of 28 fryer flocks, and 3 (10 per cent) of 31 chicken hen flocks.

The positive sampling results are detailed in table 1 for chicken fryers and hens, and in table 2 for turkeys. Total samples and the per cent positive are listed both for sampling days and for individual lots or flocks. Where both chicken fryers and hens were in the same sample, only the isolation percentage for the respective class is listed. Each isolate listed represents an individual carcass sample and not a replicate culture from a single sample. In no instance was *Salmonella* isolated from a liver; two lots of fryer chickens, however, yielded the organism from the pericardial exudate as well as from the intestines; gross pathology in these fryers was typical of colibacillosis with extensive fibrinous pericarditis and perihepatitis. Hearts, although not routinely sampled, were sampled in these two lots since extensive gross lesions were observed. Several lots of fryers and fowl yielded *Paracolobactrum* Sp., but none were typed as being in the Arizona group.

Table 2 lists the results from sampling turkeys by two different methods. The first group of 17 samplings (6 positive) was made by routine procedures described above for all three classes of carcasses. The second group of six (four positive for *Salmonella* and a fifth positive for Arizona type *Paracolobactrum*) were "mass" samplings made by taking cecal tonsils from series YY and cloacal swabs from the other five. Paired samples, consisting of (a) cloacal swabs and (b) cecal tonsils and feces, had been taken during several earlier samplings

(three positive isolations). Although isolations were not always made from the same carcass by the two methods employed, the total isolations were the same by each method in the respective sampling series (1 isolation by each method from 30 samples, 4 by each from 65, and 2 by each from 50). Therefore, it was felt that data derived by either method could be considered of equal validity. Cloacal swabs were made without reference to the presence or absence of gross pathology.

Salmonella typhimurium was the type most frequently isolated from turkeys (43 of 57 isolates), as expected, but, surprisingly, also was found in 2 of the 3 positive flocks of chicken hens. *Salmonella infantis* and *Salmonella bredeney* predominated in the fryer isolates, with the former being present in 4 of the 5 positive flocks. Numerous *Paracolobactrum* were isolated from the 35 turkey flocks, but only 1 flock yielded Arizona type. The carcasses from which intestinal isolates were made showed gross pathology warranting condemnation of 16 of 21 chicken fryers (76 per cent) and 3 of 6 fowl (50 per cent), but only 2 of 17 turkeys (12 per cent).

The statistical adequacy of a sample depends on the probability of occurrence of an event in a population to be sampled and the level of confidence desired. If one arbitrarily estimates an incidence of 1 per cent, the number of samples required will be 299 for a 95 per cent confidence level and 459 for a 99 per cent level. Limitations of laboratory facilities, time, and personnel prevented taking such large samples routinely. "Mass" samplings were made in 6 instances, however, with

TABLE 1

Isolations of Salmonella from intestines of chicken carcasses randomly selected during commercial processing

Sample Series*	Carcasses Tested per Series			Carcasses Tested per Flock		*Salmonella* Infections†
	Number	Per cent positive	Flock	Number	Per cent positive	
Hens						
NN	35	11.4	1	18	0	
			2	17	23.5	*S. infantis*, 4 strains
AA	47	4.3	1	—‡	+	
			2	14	7.1	*S. typhimurium*, 1 strain
			3	33	3.0	*S. typhimurium*, 1 strain
(15)§	437	0	(27)			
17	519	1.16	31			
Fryers						
T	56	26.8	1	25	0	
			2	31	48.4	*S. bredeney*, 3 strains; *S. infantis*, 1 strain; *Salmonella* sp., 11 strains
				(13)¶	(46.2)	*S. bredeney*, 5 strains; *Salmonella* sp., 1 strain
AA	16	12.5	1	16	12.5	*S. infantis*, 1 strain; *S. worthington*, 1 strain
			2	—‖	+	
			3	—‖	+	
V	71	1.4	1	31	0	
			2	13	7.7	*S. infantis*, 1 strain
				(11)¶	(9.1)	*S. infantis*, 1 strain
			3	27	0	
CC	58	3.4	1	32	6.3	*S. infantis*, 2 strains
			2	4	0	
			3	22	0	
V	50	2.0	1	20	0	
			2	30	3.3	*S. heidelberg*, 1 strain
(14)§	560	0	(17)			
19	811	2.59	28			

* Random sampling made on a given day.
† Each isolate is from an individual carcass.
‡ Fryers processed on same day.
§ Number of series with negative results.
¶ Hearts sampled.
‖ Hens processed on same day.

sample numbers ranging from 98 to 386. Four of these 6 yielded Salmonella, and 5 of the 6 yielded either *Salmonella* or Arizona type *Paracolobactrum*. In the 17 turkey samplings with small sample numbers, in contrast, only 6 yielded *Salmonella* or *Parcolobactrum* of this type. It may be noted that the only negative "mass" sample is the one that had a sample number of only 98. Seventeen isolations were made from 674 turkey intestinal samples taken by routine small-sample procedures for an incidence of 2.52 per cent and 42 (counting 2 Arizona type *Paracolobactrum*) isolations were made from 1,706 intestinal or cloacal samples taken by mass sampling techniques for an incidence of 2.34 per cent. The comparability of these two incidence rates suggests that some degree of randomness was achieved and that the lower percentage of flocks or sample days from which *Salmonella* was isolated by the small-sample method could be attributed to inadequacy of sample. One might further reason that, if the size of the samples had been raised to the range necessary for a 99 per cent confidence level, an even greater percentage of positive sampling periods (approaching 100 per cent) would have resulted.

Summary

Random sampling of market meat birds was conducted by methods designed for the detection of *Salmonella* infection in individual birds. *Salmonella* was usually associated with disease processes in fryer chickens but seldom in turkeys. Infected or carrier birds were found being processed on 43 per cent of the sampling days for turkeys, 26 per cent for chicken fryers, and 12 per cent for hens. With turkeys, *Salmonella*-infected birds were detected on twice as many

TABLE 2

Isolation of Salmonella from intestines of turkey carcasses randomly selected during commercial processing

Sample Series*	Carcasses Tested per Series			Carcasses Tested per Flock		*Salmonella* Isolations†
	Number	Per cent positive	Flock	Number	Per cent positive	
Routine Procedures						
K	48	8.3	1	6	0	
			2	8	12.5	*S. typhimurium*, 1 strain
			3	34	8.8	*S. typhimurium*, 3 strains
CCC	50	8.0				*S. give*, 2 strains; *S. anatum*, 2 strains
OO	39	7.7				*S. typhimurium*, 3 strains
I	49	6.1				*S. typhimurium*, 3 strains
H	45	4.4	1‡	—	0	
			2	45	4.4	*S. typhimurium*, 2 strains
P	59	1.7	1	7	14.3	*S. san diego*, 1 strain
			2	52	0	
(11)§	384	0	(14)			
17	674	2.52	23			
Mass Samplings						
YY	370	5.1				*S. typhimurium*, 14 strains; *S. anatum*, 4 strains; *S. derby*, 1 strain
AB	320	6.3	1	21	4.8	*S. san diego*, 1 strain
			2	89	1.1	*S. bredeney*, 1 strain
			3	129	0	
			4	81	21.0	*S. typhimurium*, 17 strains
AD	205	0.5				*S. javiana*, 1 strain
BBB	386	0.26				*S. panama*, 1 strain
AC	327	0 (0.61)¶				(Arizona type *Paracolobactrum*, 2 strains)
AAA	98	0	(4)			
6	1,706	2.34 (2.46)‡	12			
23	2,380	2.40 (2.48)‡	35			

* Random sampling made on a given day.
† Each isolate is from an individual carcass.
‡ Fryer chickens processed the same day.
§ Number of series with negative results.
¶ Including 2 Arizona type *Paracolobactrum*.

sampling days with mass sampling as with small-sample techniques.

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Comparison of Media and Methods for Recovering *Salmonella Typhimurium* from Turkeys[1]

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Edwards and Ewing (1955) make the statement in an authoritative text on the isolation and identification of *Enterobacteriaceae* that the "enrichment and plating medium to be used are dictated by the particular circumstances under which one is working." Browne (1949) had found this to be true in his development of methods to isolate *Salmonella typhimurium* from turkey feces and tissues. In a survey of market poultry for *Salmonella* (Sadler *et al.*, 1961) and a study of *Salmonella* infection in adult turkeys (Yamamoto *et al.*, 1961) it became apparent that the validity of the results would be greatly influenced by the media and techniques used for isolation. This paper reports the results of comparisons of some recently described, as well as older media in the isolation of *S. typhimurium* under the conditions of the survey and experiments mentioned above.

MATERIALS AND METHODS

The source of material for this study and the quantitative methods used are described by Yamamoto *et al.* (1961).

Efficacy was compared for one specimen preservative, four enrichment broths, and four different agar media. The specimen preservative used has been reported by Hajna (1955); it yields a greater number of isolations of *Salmonella* from stool specimens than is yielded by saline or buffered-glycerol-saline. He attributed the efficacy of this solution as possibly due to inhibition of growth of extraneous bacteria and thus concurrent maintenance of the enteric pathogens at a constant level.

The four enrichment media were: (a) tetrathionate broth[2] modified by the addition of brilliant green (Galton, Scatterday, and Hardy, 1952); (b) selenite broth[2] modified by the addition of cystine (North and Bartram, 1953); (c) selenite-brilliant-green sulfapyridine broth prepared according to the formula of Osborne and Stokes (1955); (d) tryptose phosphate broth.[2] The last named broth was used as a primary enrichment to test whether the usefulness of a selective-enrichment broth may not be due so much to its selective ingredients as to its fluid quality (Thomson, 1955). The agar media consisted of brilliant green (BG) agar,[2] bismuth sulfite (BS) agar,[2] SS (*Salmonella-Shigella*) agar,[2] and brilliant green agar plus 0.1 per cent sulfapyridine (BGS) (an amendment recommended by Osborne

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[2] Difco Laboratories, Inc., Detroit, Michigan.