Culture Survey of *Salmonella* at a Broiler-Raising Plant

ARNOLD F. KAUFMANN, D.V.M., and JAMES C. FEELEY, B.S.

EFFECTIVE field evaluation of sources and vehicles of *Salmonella* contamination in domestic poultry has been hampered by the multiplicity of sources of feeds and hatching eggs used by the poultry industry. However, the shift of the industry to vertical integration, a system in which a single firm maintains and controls all phases of its operation, from feed production to broiler processing, makes controlled epidemiologic studies possible. We studied a large bird population of known parentage which was fed a standard diet and kept under standard conditions.

The study was conducted in the Southeast at the vertically integrated plant of a large broiler-raising corporation, which included hatcheries, farms for raising broilers and producing hatching eggs, a feed mill, processing plants, and rendering plants. Bacteriological culturing for salmonellae was conducted over 2 years. We visited the corporation's facilities in July 1965, June 1966, and December 1966. Surveillance was carried out on specified samples collected and shipped by the poultry firm to the Public Health Service's National Communicable Disease Center (NCDC).

The purpose of the sampling was to determine the extent and sources of *Salmonella* contamination in the hatcheries, feed and its components, breeding-hen flocks, broiler flocks, rendering plants, and processing plants.

Dr. Kaufmann is a veterinary epidemiologist and Mr. Feeley is a microbiologist with the Epidemiology Program, Public Health Service's National Communicable Disease Center, Atlanta, Ga.

Materials and Methods

Feeds contained poultry meal, feather meal, fish meal, soy bean meal, alfalfa meal, residue of a corn fermentation process, shelled corn, supplemental vitamins and minerals, animal fat, vegetable oil, and salt in varying proportions. Samples of these ingredients were collected at the feed mill; samples of mixed feed were collected at both the feed mill and the chicken farms.

Chicken flocks of different ages were selected. Eight broiler flocks were sampled on each of our three visits, a total of 24 different flocks. Two breeding-hen flocks were also sampled in each of the first two surveys, a total of four flocks. The sampling consisted of 50 fresh fecal droppings from each of the 28 flocks.

The two rendering plants were adjacent to the two processing plants and received raw products (offal and feathers) from them by screw-type conveyors. Both rendering plants were small, and there was no physical separation of raw and finished products. Employees with equipment circulated freely from raw- to finished-product areas. The older plant was visited in July 1965 and December 1966; the new plant had just been built when it was sampled in December 1966. Over the 2-year period 162 samples were taken at the old plant; only 15 samples

Table 1. Distribution of *Salmonella* serotypes by source, 1965 and 1966

Serotype (group)	Broilers	Processing plant	Rendering plant	Poultry meal	Feather meal	Fish-meal	Finished feed	Hatchery	Total
1965	85	19	53	30	2	5	14	0	208
abortus-bovis (B)	0	0	0	1	0	0	0	0	1
california (B)	6	0	8	12	0	0	2	0	28
typhimurium (B)	0	0	1	0	0	0	0	0	1
typhimurium v. copenhagen (B)	0	1	2	0	0	0	0	0	3
livingstone (C–1)	0	0	2	0	0	0	0	0	2
oranienburg (C–1)	3	0	3	0	0	0	0	0	6
muenchen (C–2)	1	0	0	0	0	0	0	0	1
albany (C–3)	0	0	1	0	0	0	0	0	1
kentucky (C–3)	6	5	21	2	0	0	0	0	34
eimsbuettel (C–4)	39	0	1	1	0	4	6	0	51
anatum (E–1)	0	0	1	0	0	0	0	0	1
give (E–1)	0	0	0	0	0	0	1	0	1
orion (E–1)	0	0	0	1	0	0	0	0	1
binza (E–2)	3	0	3	2	0	0	0	0	8
manila (E–2)	2	0	3	0	0	0	0	0	5
new-brunswick (E–2)	0	0	1	0	0	1	0	0	2
newington (E–2)	0	0	1	2	0	0	1	0	4
arkansas (E–3)	0	0	0	1	0	0	0	0	1
canoga (E–3)	0	0	0	0	0	0	1	0	1
thomasville (E–3)	0	0	0	1	1	0	3	0	5
senftenberg (E–4)	0	0	0	1	0	0	0	0	1
westerstede (E–4)	7	7	0	0	0	0	0	0	14
cubana (G–2)	0	0	0	1	0	0	0	0	1
cerro (K)	2	2	0	0	0	0	0	0	4
siegburg (K)	0	0	0	1	1	0	0	0	2
minnesota (L)	16	4	3	2	0	0	0	0	25
alachua (O)	0	0	1	1	0	0	0	0	2
untypable	0	0	1	1	0	0	0	0	2
1966	80	0	5	23	6	12	19	1	146
california (B)	0	0	0	5	3	0	0	0	8
heidelberg (B)	0	0	3	0	0	0	1	0	4
typhimurium (B)	3	0	0	0	0	0	0	0	3
typhimurium v. copenhagen (B)	1	0	0	0	0	0	0	0	1
bareilly (C–1)	5	0	0	0	0	0	4	0	9
braenderup (C–1)	0	0	0	0	0	0	0	1	1
montevideo (C–1)	27	0	0	0	0	0	1	0	28
oranienburg (C–1)	0	0	0	0	1	0	0	0	1
tennessee (C–1)	0	0	0	0	0	1	0	0	1
blockley (C–2)	0	0	0	0	0	1	0	0	1
lindenburg (C–2)	0	0	0	1	0	0	0	0	1
kentucky (C–3)	2	0	1	0	0	0	0	0	3
eimsbuettel (C–4)	31	0	1	2	2	5	3	0	44
lexington (E–1)	1	0	0	0	0	0	3	0	4
meleagridis (E–1)	0	0	0	4	0	0	1	0	5
orion (E–1)	0	0	0	1	0	0	0	0	1
westhampton (E–1)	0	0	0	1	0	0	0	0	1
binza (E–2)	1	0	0	0	0	0	0	0	1
newington (E–2)	2	0	0	0	0	0	1	0	3
thomasville (E–3)	0	0	0	0	0	0	1	0	1
senftenberg (E–4)	0	0	0	9	0	0	1	0	10
cubana (G–2)	0	0	0	0	0	2	0	0	2
minnesota (L)	7	0	0	0	0	1	2	0	10
johannesburg	0	0	0	0	0	1	1	0	2
untypable	0	0	0	0	0	1	0	0	1

were collected at the new plant. Cultures were made primarily from swabs of the machines, pipes, walls, and floor. Affluents and effluents were monitored by means of dry swabs dipped into the moving water. Bulk samples of poultry meal and feather meal were gathered at each visit, and samples were sent to NCDC at monthly intervals.

Only the older processing plant was surveyed in July 1965. A total of 145 samples were taken stepwise along 17 points of the processing line. The specimens were 130 swabs and 15 water samples from the washing tanks and chill tanks.

The corporation's two hatcheries underwent continuous fumigation with formaldehyde of an undetermined concentration during the hatching period. Three sampling techniques were used to monitor the hatcheries—refrigerator jars containing tetrathionate broth were left open overnight in hatchers in which chicks were actively pipping, cultures were made from infertile eggs externally and internally, and eggshell fragments from which chicks had just hatched were collected and cultures made from them. Their inner wet surfaces caught any fluff or dust particles settling out of the air. All three methods were used during the first visit. However, samples from subsequent visits and samples mailed to NCDC were collected only by the third method.

Swab samples were sterile cotton swabs rubbed over the item selected for culturing and broken off aseptically into plastic, screw-cap tubes containing 10 ml. of tetrathionate broth. Water samples, also collected in plastic, screw-cap tubes, consisted of 10 ml. of water added to 10 ml. of double strength tetrathionate broth.

Table 2. Distribution of *Salmonella* contamination by source, 1965–66

Source	Number samples	Number contaminated	Percent contaminated
Chicken feces	1,400	164	18.2
Feed	170	27	15.9
Feed ingredients	154	68	44.2
Rendering plant environment	95	46	48.4
Hatcheries	117	1	.9
Processing plant	145	12	8.3
Total	2,081	318	15.3

Eggshells, feeds, and feed ingredients were gathered in bulk and put aseptically into plastic, whirl-pack bags with the aid of sterile wooden tongue blades. Later at the laboratory, 30-gm. aliquots of feed and feed ingredients were dispensed into 100 ml. of tetrathionate broth. Eggshells were crushed, and the pieces put into 100 ml. of tetrathionate broth without regard to weight. The tetrathionate broth cultures were incubated at 37° C. for 48 hours and then streaked onto brilliant green agar with added sulfadiazine at a concentration of 80 mg. per 100 ml. Two *Salmonella*-suspect colonies were picked from these plates after 24 hours' incubation at 37° C. and put on triple sugar iron agar slants. Complete identification of cultures followed according to standard methods (*1*).

Egg contents were treated somewhat differently. Initially, the external surfaces were swabbed for *Salmonella* detection. Cleansing with 70 percent alcohol followed. Then the eggs were cracked and the contents aseptically dropped into 100 ml. of nutrient broth. After 48 hours' incubation at 37° C., transfers were made to tetrathionate broth and brilliant green agar and further processed by methods already described.

Results

The serotypes isolated by source in the 2-year period are shown in table 1. Twenty-seven serotypes were identified in 1965, and 24 in 1966. A total of 30 different serotypes appeared, but only 12 were found in both years. The serotypes isolated in only 1 year were usually from single sources.

The source of contamination of the 318 positive samples out of the total 2,081 samples is shown in table 2. The rendering plants had the highest overall level of contamination—48.4 percent. Feed and feed ingredients had the second highest level—44.2 percent.

The overall excretion rate by the birds was 18.2 percent. *Salmonella eimsbuettel* occurred most often in both years (45.9 and 38.8 percent respectively). The hatcheries had the lowest rate of *Salmonella* contamination. Only one sample of the 117 was positive, and this sample contained a *Salmonella braenderup*. Only 12 cultures from the processing plant yielded salmonellae. The samples came from the defeathering machines, an early handling stage.

Discussion

Only 12 of the 3,742 *Salmonella* isolations made from domestic fowl reported during 1965 in the United States were *S. eimsbuettel* (*2*). However, this serotype was the most frequently isolated in both poultry meal and fecal droppings during this 2-year study. Consideration of this unique circumstance might provide insight into the sources and vehicles of contamination at this poultry plant.

Since *S. eimsbuettel* was consistently isolated from fish meal, a feed ingredient, introduction of other serotypes by feeds seems logical. This is what apparently happened, since approximately half of the serotypes isolated from birds were ones most commonly found in feeds and feed ingredients. Furthermore, almost all the serotypes isolated from birds were also isolated from feed and feed ingredients at one time or other. One striking example was that in a group of 1-week-old chicks 25 of 50 of their fecal droppings were positive for *Salmonella montevideo*. Feed in the farm storage bin for this group also contained this serotype.

Over the three surveys, the excretion rate of 1-week-old birds ranged from 28 to 39 percent, that of 7-week-old birds, 0 to 14 percent, and that of breeding hens, 0 percent (table 3). These data imply that very young birds are more susceptable to *Salmonella* infection than older ones; this is supported by experimental studies showing low oral infection doses for the day-old chicks (*3*) and by the observation that resistance to *Salmonella* infection increases with age (*4*). Also it seems to imply that the birds recover spontaneously from *Salmonella* infection with age. Even though it appears that feed given to the breeding hens is less contaminated than feed given to the younger birds, both feeds contain similarly contaminated ingredients.

Although the 9- to 10-week-old birds have lower *Salmonella* frequency rates in their fecal droppings, birds slaughtered at this age could contaminate the rendering plant by offal and grossly contaminated feathers. However, mere

Table 3. Distribution of *Salmonella* isolations by age of bird and feed

Date of survey and age of bird	Isolation from feces		Isolation from feed [1]	
	Number samples tested	Number positive	Number samples tested	Number positive
July 1965				
1 week	100	39	9	4
2 weeks	50	23	4	0
3 weeks	100	10	10	5
5 weeks	50	1	5	0
6 weeks	50	5	5	2
7 weeks	50	7	5	0
Adult	100	0	10	0
June 1966 [2]				
1 week	100	29	10	1
2 weeks	100	8	10	0
6 weeks	100	0	10	0
7 weeks	50	0	5	0
8 weeks	50	1	5	0
Adult	100	0	10	0
December 1966				
1 week	100	28	10	5
2 weeks	50	3	5	2
3 weeks	50	3	5	2
5 weeks	100	7	10	3
8 weeks	50	0	[3] 5	0
9 weeks	50	0	[3] 5	0

[1] Feed samples collected from storage bins on the same farm or farms.

[2] Feed contained fishmeal but no poultry or feather meal.

[3] Pelleted feed (ring type die pelletor).

Table 4. *Salmonella* contamination of feed and feed ingredients

Feed or ingredients	1965		1966		Total	
	Number of samples	Percent positive	Number of samples	Percent positive	Number of samples	Percent positive
Finished feed	65	18.5	105	14.3	170	15.9
Poultry meal	26	96.2	22	90.9	48	94.0
Feather meal	20	10.0	12	50.0	32	25.0
Fishmeal	9	55.6	25	40.0	34	44.1
Vegetable protein concentrates	14	0	9	0	23	0
Shelled corn	4	0	3	0	7	0
Vitamins	2	0	1	0	3	0
Animal fat	5	0	1	0	6	0
Vegetable oil	0	----------	1	0	1	0

seeding of the rendering plant does not explain why the rendering plant had the highest overall level of contamination in the 2-year period. It would appear that the cookers could be actually incubating salmonellae instead of destroying them. Serotypes introduced in purchased feed ingredients were perpetuated subsequently in some manner in the rendering plant and recycled to the birds in their feeds. Proper cooking, improved environmental sanitation, and separation of raw and finished products should break the chain of recycling.

The feed ingredients and the rendering plant appeared to be equally contaminated, but this was not actually what happened. A sampling bias favored ingredients most likely contaminated; poultry meal, for example, with 94 percent overall contamination accounted for a third of the samples taken (table 4), but it composed only a seventh of the feed mixture. Therefore, 18.9 percent rather than 94 percent of the feed was contaminated. This compares closely with the actual 15.9 percent contamination of feeds at farms, and the overall 18.2 percent contamination found in birds.

Although one isolation of *S. braenderup* was obtained from shells from one of the hatcheries, the hatchery was not a source of *Salmonella* contamination. Another pool of shells collected from the same hatcher at the same time was negative, indicating that this serotype was not circulating freely in the air of the hatcher. The organism was probably an external shell contaminant. If so, the value of the continuous process of formaldehyde fumigation carried on during the hatching period is questionable. It was only mildly irritating to the noses and eyes of the investigators and had no apparent effect on the chicks.

The frequency of *Salmonella* isolations from the old processing plant's processing lines and end products was very low. This was no surprise and is expected in plants of its quality (*5*). Although the new plant was not surveyed, it also had excellent sanitation and should not offer any substantial *Salmonella* contamination.

Disregarding an epiornithic of *S. montevideo* in June 1966 among 1-week-old birds, the overall excretion rate of the 2-week-old and younger birds was 8 percent. The rate for older birds dropped to 2 percent or less. This is in contrast to the high excretion rates in the other two surveys. The one major difference during this survey was that fish meal was the only source of animal protein in the feeds. Rations during the other two surveys contained poultry meal and feather meal along with the fish meal. Table 4 shows that the fish meal was contaminated at only half the rate of the poultry meal (44.1 to 94 percent), which implies that reduction in feed contamination by using fish meal as the source of animal protein reduced *Salmonella* excretion rates. Unfortunately, quantitative counts of salmonellae per gram sample were not done.

Our data support the concept that feed contamination is an important link in the cycling of salmonellae in domestic poultry. They also show how in a vertically integrated operation investigators can evaluate various means of *Salmonella* control under field conditions. Since all aspects of the operation can be monitored, effects on the excretion rates of the broilers by a reduction in feed contamination can be measured. If salmonellosis can be eradicated in such

an operation by using *Salmonella*-free feed, it can be eradicated from the industry as a whole if all feeds can be made *Salmonella*-free.

Summary

Salmonella culture surveys of a vertically integrated broiler operation were conducted at intervals over a 2-year period. Salmonellae were isolated from 18.2 percent of 1,400 fecal samples and from 29.3 percent of 324 samples of feed and feed ingredients. A self-perpetuating cycle of infection was suggested by the isolation of the same serotypes from the live birds, their feed, and the poultry meal incorporated into the feed.

The poultry meal, which was rendered offal from the company's own processing plant, served as the major source of feed contamination. Salmonellae were isolated from 94 percent of 48 poultry meal samples examined. Another major source was the fishmeal added to the feed. Isolations were made from 44 percent of the 34 fishmeal samples. The hatcheries appeared to be unimportant in the perpetuation of infection in the operation.

REFERENCES

(*1*) Edwards, P. R., and Ewing, W. H.: Identification of Enterobacteriaciae. Ed. 2. Burgess Publishing Co., Minneapolis, Minn., 1962.

(*2*) U.S. National Communicable Disease Center: *Salmonella* surveillance report. Annual summary 1965. Table IX. *Salmonella* serotypes isolated from nonhuman specimens in the United States during 1965 by source. Atlanta, Ga., June 28, 1966.

(*3*) Henderson, W., Ostendorf, J., and Morehouse, G. L.: The relative pathogenicity of some *Salmonella* serotypes for chicks. Avian Dis 4:103–109, May 1960.

(*4*) Milner, K. C., and Shaffer, M. F.: Bacteriologic studies of experimental *Salmonella* infections in chicks. J Infect Dis 90: 80–96, January–February 1952.

(*5*) Glezen, W. P., et al.: *Salmonella* in two poultry processing plants. J Amer Vet Med Assoc 148: 550–552, Mar. 1, 1966.

Continue Search of Indian Plants for Cancer Inhibitors

The Indian Government's Central Drug Research Institute at Lucknow has renewed its agreement with the National Cancer Institute, Public Health Service, to study native Indian plants as a source of potential anticancer drugs. A Public Health Service contract, using blocked dollars available under Public Law 480, is providing $136,000 (1,020,400 rupees) to finance the next 3 years of investigation.

Under the program, the Indian research center collects indigenous plants and prepares extracts from them for study by the National Cancer Institute. The Natural Products Section of the Cancer Institute has the extracts tested for their ability to inhibit animal cancers. Any extracts that show effectiveness are re-collected by Indian botanists, reextracted, and fractionated by Indian chemists, and individual fractions are screened against animal cancers to identify active agents.

Of more than 600 plant materials studied by the Indian laboratory during 4 previous years of contract-supported research with U.S. scientists, 14 plants were identified with confirmed activity against animal cancer. Ten are still under study, but they have not yet been evaluated clinically. Plant extracts are considered a promising source of possible anticancer drugs, since one of the best-known agents, Vincristine, was derived from an extract of the periwinkle plant. Indian plants have already yielded medically useful extracts for treating other diseases, for instance, rauwolfia compounds for treatment of hypertension.

In addition to research with plants, the Central Drug Research Institute is also preparing to synthesize chemical compounds for testing in the United States-India program. It is anticipated that Indian chemists may produce new and varied materials for screening in animals and for possible use in human cancer.