Salmonella in meat and poultry

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INTRODUCTION

The *Salmonella* group of bacteria has long been recognized as being very important in causing food poisoning in man. Taylor (1964) has recorded that salmonellosis has been known to be a problem in Great Britain since the beginning of the present century. For a considerable time it was the human carrier who received most of the blame, but during World War II a collaborative study of salmonellae in imported dried egg was carried out in a number of laboratories in different parts of the United Kingdom, and serotypes previously unknown there were isolated from the imported dried egg and from human infection. This drew attention to the effect of imported contaminated human food on human salmonellosis. There are many known reservoirs and sources of infection. Reservoirs include domestic and wild animals, including pets such as turtles and chicks; also man, patients and convalescent carriers, especially mild and unrecognized cases. Sources of infection are faeces of animals and infected persons; whole eggs, particularly duck eggs, and egg products (frozen and dried), meat and meat products, poultry, animal feeds and fertilizers prepared from meat, fish meals and bones (Gordon, 1965). The number of serological types that may be involved in food poisoning now exceeds 1,000.

Meat was first implicated as a source of human salmonella infection by Gaertner in 1888. Since then there have been many improvements in the handling, inspection and hygiene of meat, but even today salmonella infection is often traced to infected or contaminated meat or meat products. Report (1964) examined the incidence of salmonellae in abattoirs, butchers' shops and homeproduced meat, and their relation to human infection in Great Britain. Thirtytwo abattoirs were studied. Salmonellae were isolated from 930 (21 %) of 4,496 swabs of abattoir drains, 218 (1.92 %) of 11,347 tissue specimens, 73 (6.5 %) of 1,117 drain swabs but only 0.8 % of 4,127 samples of meat and meat products yielded salmonellae. *Salmonella typhimurium* was the serotype most frequently isolated from all sources and it was often shown that the same serotypes or phage-types were occurring in abattoirs and in human cases in an area at the same time. Many other workers have

drawn attention to the role of meat or meat products in food infections due to salmonellae e.g. Burns, Mair and Hooper (1965) reported an outbreak of *S. brandenburg* infection caused by infected pork products; Dixon and Peacock (1965) examined 898 samples of imported Dutch chilled meat and offal, and 67 (7.5 %) yielded salmonellae. Van Schothorst and Kampelmacher (1967) also found a high percentage of salmonella contaminated samples from imported meat from South America.

Poultry meat and poultry products have long been recognized as sources of salmonella infection in men. Morris and Ayres (1960) recorded an incidence of up to 9 % of *Salmonella* in samples obtained in turkey processing operations and up to 14 % of samples obtained in a chicken processing plant. Dixon and Pooley (1961) isolated salmonellae from 75 (13.8 %) of 544 specimens at a factory processing broiler chickens; 9.9 % of the eviscerated carcases and edible viscera harboured salmonellae. Bryan, Ayres and Kraft (1968) examined the contributory sources of salmonellae in turkey products. They found that dissemination of salmonellae starts on the farm, the bacteria are brought to the plant by incoming turkeys and transferred to equipment and turkey meat during processing. Recently Tucker and Gordon (1968) surveyed nine poultry packing and one turkey packing station and found only four of 17.000 samples yielded salmonellae. They attributed the marked reduction from that recorded by Dixon and Pooley (1961) to success in eliminating salmonellosis from the parent flocks, and a high standard of hygiene in the processing plant preventing contamination of carcases. They felt however that the situation in duck packing plants required further investigation.

From this limited review it is clear that there is a *Salmonella* problem associated with meat and poultry and their products. The extent of this is difficult to assess without adequate data on the incidence of the organisms in the various foods, how contamination has occurred, and from what source. One possible source of infection in the live animal is the feedingstuff being fed. Many workers have suggested this possibility (Newell, McClarin, Murdoch, McDonald and Hutchinson, 1959); review of Pomeroy, Siddiqui and Grady (1964); Report (1965). Salmonellae have often been found in animal feedingstuffs (Report 1961, Dawkins and Robertson 1967) particularly those protein constituents derived from animals, such as meat and bone meal, blood meal, feather meal, fish-meal. Methods have been suggested whereby such risk of infection can be reduced (Report, 1965), Timoney (1968). The animal as a source of salmonellae both in the packing plant and rendering plant has been demonstrated by numerous workers.

When the extent of the problem has been determined, it is sometimes possible to devise measures to control or prevent it becoming more serious. With this in mind work has been carried out to determine the incidence of

Salmonella in (a) Poultry and poultry products viz. processed broilers and uneviscerated poultry, effluents from poultry packing plants, cooling waters, processed turkeys and ducks and cooked chicken-meat; (b) Meat and meat products; (c) Animal feedingstuffs.

There is a need to have more rapid methods to determine the presence of salmonellae in food for humans and animal feedingstuffs. Experience in the use of the Fluorescent Antibody (F.A.) microscopy technique which promises to give useful results is discussed briefly.

EXPERIMENTAL METHODS

Salmonellae in processed broilers and on New York dressed poultry

Two surveys were made, the first in 1961 and the second in 1966. The first was confined to one large processing plant producing eviscerated frozen carcases. Many poultry reach the consumer also as uneviscerated (New York dressed), or as chilled eviscerated carcases. In addition some of the larger plants had begun the practice of in-plant chlorination since 1961, which might have reduced the number of salmonellae present. For the second survey therefore, four plants were included, ranging from the large plant of the first survey to a small plant producing only uneviscerated carcases. The experimental procedures have been described by Stewart (1965) and Patterson (1967). Samples were taken from the eviscerated broilers as they passed along the processing line on overhead shackles and from the unevisceratedas they hung on racks before entering the cooling rooms, by means of a sterile wooden applicator 15 cm long with a 4 cm cotton gauze tip. This was rubbed over the cloacal area and in the case of the eviscerated birds, also over the lower part of the body cavity. In the laboratory each swab was broken into 8 ml of 0.1 % peptone water (Oxoid), incubated for 3 hr, then 5 ml added to 5 ml double strength selenite F broth (Oxoid) containing 2.0 mg cystine per 100 ml of broth. After 20—24 hr these broth cultures were streaked on to desoxycholate citrate agar (Oxoid) containing an additional 1 % of sucrose, and the plates incubated for 18—24 hr and examined for typical suspect salmonella colonies. This procedure was repeated from selenite broth after 48 and 72 hr incubation (at 37° in all cases). Suspect colonies were picked off into a multiple sugar medium (Stewart, 1962), incubated overnight and those isolates giving the typical salmonella reaction were tested with »Wellcome» *Salmonella* agglutinating sera. The results of these investigations are given in Table 1.

Table 1. *Salmonellae in broilers, turkeys and ducks*

No. of carcases examined	*Processing Plant*	*No. of Sampling Days*	*Type of Carcase*	*No. Positive*	*Serotypes Present*	*% Positive*
735*	A	15	Eviscerated, broiler	3	*S. typhimurium* var. *copenhagen* *S.livingstone*	0.4
531**	A, B C	23	Eviscerated, broiler	—	—	—
272**	C, D	15	New York dressed broiler and hen	—	—	—
84	E	3	Eviscerated turkey	—	—	—
444***	B	18	Eviscerated duck	39	*S. typhimurium*	9.0

* Data obtained in 1961 (Stewart, 1965).
** Data obtained in 1966 (Patterson, 1967), effluent data in Table 2.
*** More complete data in Table 5.

Salmonellae in ducks and turkeys

A similar swab technique was adopted, the ducks being swabbed after they had received the final wash, but before going into the spin-chiller; some carcases were examined after spin-chilling. The turkeys were examined after evisceration, or after overnight chilling in tanks of water with added ice. The gauze swabs were transported to the laboratory, 10 ml of selenite F broth (with mannitol substituted for lactose) added and incubated at 37° C. After 24 and 48 hr incubation subcultures were made from the selenite F enrichment broth on to the modified Wilson & Blair medium of McCoy (1962). The plates were incubated at 37° C for 24 hr and examined for typical *Salmonella* colonies. Results of these investigations are given in Tables 1 and 5.

Salmonellae in poultry plant effluents and in cooling waters

To see if large numbers of salmonellae were being shed into the cooling water being used to cool the carcases, swabs were on some occasions placed in the outlets of the spin-chillers in the broiler and duck processing plants, and in the outlet of the tank used to cool the turkey carcases. In addition swabs were also placed in the outlet sewer of two of the plants while broilers (and in one plant ducks) were being processed. The method adopted (McCoy, pers. comm.) was to suspend a sanitary towel in the outlet sewer for 3 days, or the outlet of the spin-chiller or tank for a shorter period (generally no longer than 1 hr). Afterwards the swab was placed in a plastic bag, the corner

cut from this and the absorbed fluid squeezed out and collected in a sterile container containing sodium thiosulphate pentahydrate to neutralize residual chlorine. The expressed fluid was examined for the presence of salmonellae by pipetting 10 × 1 ml, and 10 × 1/10 ml volumes into 10 ml selenite F broth, which were then incubated at 37° C for 48 hr. Subcultures were made on to selective media after 24 and 48 hr; to avoid overgrowth by large numbers of other Gram-negative bacteria on some occasions fresh selenite F broths were inoculated from the original broths after 24 hr incubation. Results obtained are given in Tables 2, 3, and 4.

Table 2. *Salmonellae in poultry processing plant sewer swabs Swabs in place 3 days*

Processing Plant	*No. of swabs Examined*	*No. Positive*	*% Positive*	*Serotypes isolated*
A	6	4	67	*dublin* 1; *pullorum* 1; *typhimurium* = 2a, 1; = 4, 1; = unt., 1
B*	6	4	67	*typhimurium* = 2a, 1; = 4, 2; = unt., 1

* Ducks were processed on one day weekly

Table 3. *Salmonellae in cooling water, hens and turkeys*

Processing Plant	*No. of swabs Examined*	*No. Positive*	*% Positive*	*M.P.N./100 ml*	*Swabs in place*
A, hens	5	—	—	<1	1—6 hours while tank drained
E, turkeys	1	—	—	<1	

Table 4. *Salmonellae in cooling water, ducks. Plant B*

Processing Plant	*No. of swabs Examined*	*No. Positive*	*% Positive*	*Serotypes isolated*
B	22	9	41	*typhimurium* = 2a, 2; = 23, 4; = unt., 2
Salmonellae	M.P.N./100ml.	10—5 samples;	23—1 sample;	1200 — 1 sample; 2300 — 1 sample; >2300 — 1 sample.

Table 5. *Salmonellae isolations from duck carcases*

Carcases Examined	*No. Examined*	*No. Positive*	*% Positive*
Before chilling	304	23	7.6
After chilling	140	16	11.4

Salmonellae in meat and meat products, and animal feedingstuffs

The method used is that suggested by McCoy (pers. comm.). Four 25 g samples from the material under examination were transferred into 150 ml quantities of selenite F broth (with mannitol substituted for lactose). In many cases, where other contaminants are not present in large numbers, sterile quarter-strength Ringer's solution, or sterile water can be used instead of selenite. After incubation for 24 and 48 hr at 37° C, plates of modified Wilson and Blair medium were streaked from these enrichment cultures. These plates were examined for typical *Salmonella* colonies after 24 hr at 37° C, and these were picked off on to slopes of Hartley's digest agar for detailed biochemical tests and serology. If positive results are obtained from one or more jars then a further examination was carried out to obtain a most probable number of salmonellae present (10 × 10 g and 10 × 1 g quantities). The results of such examinations on samples of various meat and meat products are given in Table 6, and of those from animal feedingstuffs in Table 7.

RESULTS AND DISCUSSION

The results shown in Table 1 indicate a low incidence of *Salmonella* in broiler chicks in 1961, and an even lower incidence in 1966. Although no salmonellae were found on 803 carcases in four processing plants in 1966, it was possible to obtain isolations from the drains of two plants, mainly *S. typhimurium* when swabs were left for several days (Table 2). This low incidence agrees with the findings of Tucker & Gordon (1968) already discussed. The presence of even one infected bird is of course undesirable since Stewart (1965) showed by means of a marker organism (*Serratia marcescens*) how such infection can be quickly spread to many other carcases and parts of the plant by the normal handling operations. This is particularly undesirable if further processing is being carried out elsewhere in the plant, and strict precautions to prevent cross-contamination are necessary. No salmonellae were found in spin-chiller or giblet washing water of plant A, nor in turkeys in plant E, either on the carcases or in the cooling water (Tables 1 & 3).

Table 6. *Salmonellae in various meat and poultry products*

Product	*No. of samples tested*	*No. of producers*	*No. of samples positive*	*Salmonellae M.P.N./100 g*	*Identification*	*Phage Type*
Beef sausages	19	3	0	—	—	—
Pork »	38	5	2	161	*S. typhimurium*	Untypable
				<5	»	23
Steakburgers, hamburgers, etc.	68	4	0	—	—	—
Frozen boneless beef	125	3	1	<5	*S. dublin*	—
» » pork	14	1	0	—	—	—
Vac. packaged meat	20	1	0	—	—	—
Semi-cooked or cooked products	98	6	0	—	—	—
Bacon and bacon joints	18	2	0	—	—	—
Spices, butter	2	1	0	—	—	—
Cooked, vacuum packaged chicken	49	1				
Cooked, boned-out poultry meat	29	3	3	<5	*S. typhimurium*	23
				16	»	23
				<5	»	23
Total	480	9	6	—	—	—

The position with regard to duck processing is however much more serious since 39 out of 444 carcases examined (9 %) were found to be contaminated with *S. typhimurium* (Table 5), though some of these could have been cross-contaminated in the spin-chiller. Salmonellae were found on 10 out of 18 visits made to plant B, when ducks were being processed. The source of the salmonellae has not yet been established but indications are that hygiene in the hatchery, breeding and rearing methods are all of importance in the spread of infection. Once infected carcases reach the plant it is difficult to have effective control measures. In-plant chlorination is some help, since Dixon & Pooley (1961) found that treatment of carcases with 200 p/m of chlorine for 10 min. usually prevented the subsequent recovery of salmonellae when <1,000 organisms had been inoculated into the carcases. Good results have also been claimed by Nilsson and Regner (1963) using 20 p/m in the chill tanks for 60 min. The data in Table 5 show that salmonellae were isolated from 23 out of 304 carcases before chilling (7.6 %) and from 16 out of 140 (11.4 %) after chilling, suggesting that the spin-chilling method used may have transferred salmonellae from infected to non infected carcases.

Table 7. *Salmonellae in animal feedingstuffs*

Feedingstuff	*No. of Samples*	*No. positive*	*Salmonellae M.P.N./100g.*	*Serotype isolated*	*Phage Type*
Protein meals of animal origin					
Fishmeals	24	—	—	—	—
Meat and bone meal	112	8	2	*eimsbuttel*	—
			1	*infantis*	—
			0.5, 7	*stormont*	—
			0.5	*senftenberg*	—
			2	*typhimurium*	23
			0.5	*raus*	—
			2	*brancaster*	—
Feathermeal	5	1	5	*heidelberg*	—
Bloodmeal	22	1	1	*give, senftenberg*	—
Milk powder	73	—	—	—	—
Vegetable protein meals	10	—	—	—	—
Cereals	5	—	—	—	—
Animal fat	1	—	—	—	—
Compound meals					
Duck, poultry, pig, dairy pellets	16	—	—	—	—
Poultry meal, dairy meal	29	—	—	—	—
Pig meal	35	3	4	*typhimurium*	1a
			0.5	*raus*	—
			0.5	*infantis*	—
Totals	332	13	—	9	—

Our own laboratory experiments using duck skin artificially contaminated with salmonellae have shown that even quite high levels of chlorine (of the order of 200 p/m) though reducing numbers considerably do not free the skin from salmonellae completely.

The incidence of salmonellae found in various meat and poultry products (Table 6) was not high, only 6 out of 480 samples from 9 processors having these organisms present, generally in small numbers. Two isolations of *S. typhimurium* were made from pork sausages, one isolation (*S. dublin*) from frozen boneless beef, and three (*S. typhimurium*) from cooked poultry meat. The source of the infection was not established for any of these isolations; conditions however at the poultry-processing plant were not good and cross-contamination from infected raw carcases was possible. Stricter hygienic precautions taken since these isolations were made appear to have reduced the extent of this particular infection.

Table 7 gives the results of examinations of 332 samples of animal feedingstuffs. No salmonellae were found in fishmeals (imported from South

Africa, Peru, Norway and Iceland), vegetable protein meals or cereals. However of 112 samples of meat and bone meal 8 (7 %) were found to contain 7 different salmonella serotypes, generally in small numbers. Of these *S. stormont* has recently been found in pigs in Ireland. One sample of imported feather meal and one of locally produced blood-meal also gave positive isolations. Of some significance too is the fact that of 35 samples of pig compound meal examined 3 (9 %) had salmonellae present though no isolations were made from any of the other compound meals. This shows one possible source of infection in the animal, with the subsequent possibility of contaminated meat or meat product. From the epidemiological point of view, many of the recent isolations of *S. typhimurium* have been of phage-type 23 (from ducks, poultry meat, pork sausages, meat and bone meal). This suggests a chain of infection which should be broken. One way is to eliminate contamination in the feedingstuff by a suitable extraction process and careful attention to hygiene in the rendering plants particularly the percolator area (Timoney, 1968), and by reduction of cross-contamination in the feed-mills by better dust control, use of disposable sacks and better machine cleaning (Dawkins and Robertson, 1967). If all raw materials were sterilized by heat or irradiation then the salmonella risk from this source could be eliminated. Pelleting of compound feeds greatly reduces the risk, but adds cost to the product.

Salmonellae are never easy to isolate, and many different methods have been used by different workers. Each type of material being examined may require a different type of approach (Hobbs, 1962) and methods are difficult to standardise and are always slow and tedious. Attempts have been made (Galton, Morris and Martin, 1968) to recommend procedures, but there is a great need for a method which would permit rapid screening of a large number of samples. A method holding great promise is the Fluorescent Antibody technique. Haglund *et al* (1964) described the technique for use in detecting salmonellae in egg products; and Georgala and Boothroyd (1964, 1965) for use with meat and meat products. Our experience has been that when commercially available sera are used, both the polyvalent and the conjugated sera have to be absorbed with a number of cross-reacting organisms. Work is continuing to determine the sensitivity and specificity of this indirect F. A. technique using absorbed sera in parallel with conventional plating and slide agglutination techniques.

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