THE ISOLATION OF *SALMONELLA THOMPSON* FROM OUTBREAKS OF DISEASE IN CHICKS

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Salmonella thompson was first isolated by Scott (1926) from an outbreak of human food poisoning in Yorkshire, thought to be caused by the consumption of rabbit and beef pie. Since then it has been shown in the *Annual Report* of the Chief Medical Officer, Ministry of Health (1938) to be the second commonest cause of *Salmonella* food poisoning in Great Britain. Scott (1939–40) also records the isolation of *S. thompson* from the mesenteric glands of healthy pigs at slaughter. *Salmonella* organisms, other than *S. pullorum* and *S. gallinarum*, are common in poultry, and in a discussion on the occurrence and distribution of *Salmonella* types in the United States, Edwards & Bruner (1943) enumerate forty-two strains derived from poultry. Out of a total of 3090 cultures examined by Edwards & Bruner, *S. thompson* was isolated only once in poultry and four times in man. The rarity of *S. thompson* is commented on, since it was responsible for only 0·13 % of the total outbreaks studied and 0·61 % of the outbreaks in man. In Germany, Boecker (1935) found *S. thompson* in 7·6 % of 119 outbreaks in man, and in Great Britain it is shown in the *Annual Report* of the Chief Medical Officer, Ministry of Health (1938), to have been isolated from approximately 16 % of the types identified. Apparently *S. thompson* is not so widespread in the United States as in Europe.

In Great Britain there are few references to the infection of poultry with members of the *Salmonella* group, and according to Garside & Gordon (1940), and Gordon & Garside (1944) the only types recorded in this country, apart from *S. pullorum* and *S. gallinarum*, are *S. typhi-murium* and *S. enteritidis*.

In a detailed search of the literature no mention could be found of the isolation of *S. thompson* from poultry apart from the single case reported by Edwards & Bruner (1943).

During the last two years an increase has been noted in the number of outbreaks of Salmonellosis in chicks examined at Weybridge, and a detailed survey of the incidence of the various strains isolated is now in progress. Previous to 1943 the only species encountered were *S. typhi-murium* and *S. enteritidis*; but in 1943, out of a total of twenty-eight confirmed outbreaks of Salmonellosis ten were found to be due to *S. thompson*. During the first 6 months of the present year seventy-nine isolations of *Salmonella* organisms other than *S. pullorum* and *S. gallinarum* have been made from fifty-nine different outbreaks. Of the organisms recovered, the commonest has been *S. thompson*, which was isolated from twenty-three outbreaks or approximately 40 % of the total incidence as shown in Table 1.

Table 1

Type	1942		1943		1944 first 6 months		Total	
	Cultures	Outbreaks	Cultures	Outbreaks	Cultures	Outbreaks	Cultures	Outbreaks
S. typhi-murium	17	16	19	16	13	7	49	39
S. enteritidis	6	5	3	2	1	1	10	8
S. thompson	—	—	14	10	34	23	48	33
*Other species of *Salmonella*	—	—	—	—	31	28	31	28
Total	23	21	36	28	79	59	138	108

* Typing now in progress.

FIELD OBSERVATIONS

In general the outbreaks of *S. thompson* have shown few distinctive features. Thirty-one of the outbreaks have occurred in chicks, and two in ducklings. The organism has also been isolated on two occasions from adult fowls. The mortality in chicks has varied from 20 % to as high as 80 %, and in one hatch a mortality of 100 % was reported.

Losses usually occurred when chicks were approximately a week old, but the organism has been isolated from chicks varying from 4 to 12 days of age.

As in most chick diseases, symptoms were inconclusive, and indistinguishable from those of *pullorum* disease, or other *Salmonella* infections. No characteristic lesions were found although some congestion

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of the lungs was commonly present and less frequently, congestion of the liver and retention of the yolk sac.

Two of the outbreaks have been investigated in detail.

Outbreak no. 1 (*strain* 2768)

This outbreak occurred in a large hatchery in south-west England, and infection with *S. thompson* was confirmed in twenty groups of chicks received at the laboratory from fourteen different owners in this area. In all cases the chicks were hatched at the central hatchery and the outbreaks occurred in chicks purchased from the hatchery or in chicks from supplying farms custom-hatched at the hatchery. The outbreak extended over ten different hatches from February to May 1943. (Custom-hatching refers to farms which supply eggs to a central hatchery and receive back a number of their own chicks for stock replacement.)

A further outbreak occurred simultaneously in an adjacent flock (farm A), and although eggs from this owner were incubated at the hatchery only on one occasion, the farm appears to have played an important part in the outbreak. This owner brought day-old chicks to the hatchery weekly for sexing, the operation being carried out in the incubator room.

The hygiene on farm A was of a low standard and there was evidence of mice having access to both the incubator room and brooder house. Survivors of previous outbreaks were found in the brooder house and there was contact between diseased and healthy chicks. Dirty litter and old excreta could be seen everywhere. In addition, there was a large refuse dump approximately 5 ft. high and 10 ft. in diameter, consisting of the carcasses of partially cremated chicks and adult birds, incubator refuse, broken egg shells and dirty litter. There was clear evidence of the presence of vermin in the dump.

Apart from the obvious link between this outbreak and the hatchery, due to sexing of chicks there, the owner of farm A also had a consignment of eggs in the hatchery incubators at the time of the first outbreak, and these eggs actually hatched out with the chicks in the second infected hatch a week later. This does not necessarily suggest egg transmission, for it may be that the contamination of the hatchery incubators originated from infection on the outside of the egg shells from farm A, a reasonable assumption in view of the unhygienic conditions existing on this farm. It is of importance to note in searching for the origin that this was the only known outbreak in the area apart from those directly traceable to the hatchery. A number of the farms supplying the hatchery with eggs were visited and no outbreak of Salmonellosis could be found in chicks hatched and reared by the suppliers on their own premises. All recently hatched stock on these premises appeared healthy, and as far as is known no complaints were received from purchasers of chicks hatched by the suppliers themselves. The only exception to this was in the case of a supplier (farm B) who had Salmonellosis confirmed in chicks sent to the laboratory from his own premises but custom-hatched at the hatchery.

A detailed study was made of the hatchery records showing the suppliers of eggs to the various affected hatches. Some twenty different owners supplied eggs over the ten affected hatches. Most of the purchased chicks in which the infection was confirmed were bought from the hatchery as 'mixed chicks' (i.e. from more than one owner's eggs). It was only possible, therefore, in a few cases to trace the eggs from which affected chicks were derived, and there was insufficient evidence to incriminate any one owner or to suggest egg transmission.

If egg transmission did occur the most likely source was farm B. This owner had eggs at the hatchery at the time of the initial outbreak, and from this hatch losses (not confirmed as *S. thompson*) were experienced by a purchaser of White Wyandotte chicks of which farm B was the only supplier in that hatch. The same farm owner also supplied eggs to the second hatch when infection was confirmed in his own custom-hatched chicks, and he later also supplied eggs to subsequent hatches. At a later date the hatchery manager incubated eggs from farm B and from another owner in a separate machine not previously used during that season. The chicks from the latter owner were healthy, but there were losses from those from farm B and *S. thompson* was isolated. It must be repeated, however, that farm B experienced no losses in chicks hatched and reared on his own premises.

The hatchery premises, themselves, were clean, and no direct evidence could be found that infection originated there except from the sexing of chicks from farm A. The incubators were regularly cleaned between hatches, and formalin fumigation of both the incubating and hatching compartments was carried out in the approved manner between each hatch. Vermin were present but not in large numbers and did not appear to have access to the incubators. Clean new boxes with fresh wood shavings as litter were used to convey chicks from the incubator for sexing and the chicks were dispatched to the purchasers in these new boxes. One bad feature to which previous reference has been made was the carrying out of sexing in the incubator room.

Samples of fluff, debris, etc., from various parts of the incubators were examined and *S. thompson* was isolated from the hatching compartment of the incubator which had contained the eggs from farm B, from the outlet ventilator of the main

incubator in which most of the infected chicks had been hatched, and from the outside of the shell of one of two goose eggs present in the incubator. It is difficult to assess these findings, and they do not assist in indicating the origin. The main incubator was fairly certain to be contaminated by this time, and the recovery of the organism from the ventilator would suggest the danger of infection passing from the hatching to the incubating chamber on air currents. The infection of the subsidiary incubator strengthens the suspicion against farm B, although contamination may have occurred from general infection of the incubator room. The goose egg shell may have been contaminated either before collection or in the incubator, and is not of much significance regarding this outbreak since goose eggs had not been placed in the incubators until after the last hatch was completed.

The origin of this outbreak must remain obscure, but the weight of the evidence would point to infection spreading from farm A at sexing.

Outbreak no. 2 (*strain* 3845)

Outbreak no. 2 was confirmed during February 1944, and occurred in a fairly large breeding farm in the Midlands together with a number of subsidiary outbreaks in chicks purchased from this owner. *S. thompson* was isolated from the main outbreak and from the purchased chicks.

Disease had appeared in each batch of chicks hatched since December 1943—the average mortality being about 20 %. The chicks were hatched in a 6000 egg cabinet incubator and then transferred to a battery brooder with wire floors until the ninth day when they were moved to a brooder house.

As in outbreak no. 1, most of the evidence again pointed to infection having occurred subsequent to hatching. The incubator and incubator rooms were presumably heavily contaminated by the time this investigation was made, and infection may have occurred at hatching time or even before by penetration of the shell. A possibility in this respect is that before setting the fertile eggs were stored in a mice-infested cupboard in the incubator room. Furthermore, in all infected hatches losses did not start before the fourth day, reaching a peak by the ninth day, while by the twelfth day mortality had usually ceased. If the disease had been egg-borne, losses would have been expected before the fourth day with a peak at the fifth to sixth day as in *pullorum* disease. Again, higher losses would have been expected if infection had occurred via the egg. It will also have been noted that when chicks were reared on wire floors losses were only approximately 20 %, whereas losses amongst purchased chicks kept on solid floor brooders were consistently higher (75–100 %). There was some further evidence that infection had occurred subsequent to hatching in that fumigation of the incubators reduced the mortality in subsequent hatches, while the carrying out of agglutination tests of the breeding flock and the removal of the reactors was still followed by an outbreak in the next hatch. It is difficult to suggest an origin, but the owner admitted having had similar losses in chicks, with identical symptoms, during the previous year.

This opens up the possibility of carriers persisting in the breeding flock from the previous year and disseminating infection either by egg transmission, faecal contamination of egg shells, or mechanical transmission of infection by attendants or vermin.

BACTERIOLOGICAL EXAMINATION

Routine method. In all cases during the bacteriological examination of chicks, primary cultures from the livers and hearts were sown on McConkey agar plates and into 10 c.c. peptone broth. After incubation for 18 hr. at 37°C., pale colonies were picked off the McConkey plates and inoculated into lactose, maltose, dulcite and on an agar slant. Organisms which failed to ferment lactose but fermented maltose and dulcite with gas production were tested by the rapid microscopic agglutination method against both polyvalent and non-specific *Salmonella* sera. Cultures agglutinated by either or both of these sera were retained for further typing.

Biochemical tests. Strain 2768 was isolated from the liver of a chick, received from farm A, during outbreak no. 1, while strain 3845 was isolated from the liver of a chick received from the breeding farm of outbreak no. 2. Both strains were found to be Gram-negative, motile cocco-bacilli, growing on McConkey's agar as relatively large greyish white opaque discrete colonies with a diameter of 2–3 mm. In peptone broth, a rapid dense growth with uniform turbidity was produced throughout the medium. Both strains produced acid and gas in the following carbohydrates: maltose, dulcite, mannite, glucose, galactose, rhamnose, arabinose, sorbite, xylose, laevulose and inosite. No fermentation occurred in lactose, saccharose, inulin, dextrin, raffinose, adonite and salacin. Litmus milk became slightly alkaline after 72 hr. incubation. Tests for H_2S production were positive. Indol was not produced.

Serological tests. When first examined, both cultures were in the non-specific phase and each strain was subcultured six times in beef infusion broth to which *S. cholerae suis* (Kunzendorf) serum had been added. Although the serum had the corresponding agglutinin to the somatic agglutinogen of the culture, this treatment did not produce any detectable roughness in the strains under examination. This method was found successful in altering the diphasic

flagella antigens into the specific phase sufficiently to obtain agglutinations to titre with *S. thompson* H-specific serum. Agglutinin-absorption tests were carried out using the double absorption technique of Edwards & Bruner (1942). In order to obtain a heavy suspension of antigen, one drop of an actively motile 6 hr. broth culture was spread on to each of a number of agar plates and incubated at 37° C. for 36–48 hr. The growth from each plate was washed off with 1·0 ml. normal saline containing 0·4% phenol, and these suspensions were used fresh for the absorption of agglutinins. Specific serum for each strain was produced in rabbits by the intravenous inoculation of formalinized broth cultures in three doses of 0·3, 0·5 and 1·0 ml. at intervals of 7 days and collected 7 days after the final inoculation.

To complete the identification of these strains, each culture in the non-specific phase was agglutinated at a dilution of 1/20 against absorbed serum containing the single non-specific factor 5. The results of these serological tests are summarized in Table 2.

From Table 2, it is evident that strains 2768 and 3845 removed all agglutinins capable of reacting with *S. thompson*, both from their own sera and from the serum prepared from the type strain. Reciprocally, the type strain removed all agglutinins from the sera prepared from strains 2768 and 3845.

In addition, both cultures in the non-specific phase agglutinated an absorbed serum containing the single factor 5.

The results of the serological examination of strains 2768 and 3845 demonstrated that their antigenic structures were VI, VII : *k* : 1, 5 ..., which is identical with that given for *S. thompson* in the Kauffmann-White schema. In addition, the biochemical properties were in agreement with those of *S. thompson*, as described by Scott (1926) and Lovell (1932).

DISCUSSION

It is difficult to explain the sudden fairly widespread occurrence of *S. thompson* infection in poultry in this country. The organism has, however, been known in man since 1926 and is now recognized to be the second commonest cause of *Salmonella* food poisoning in Great Britain. *S. thompson* has also been isolated from the mesenteric glands of healthy pigs. It is possible, therefore, that the appearance of this organism since 1943 may be associated with the feeding of kitchen waste, canteen and camp swill, or other infected war-time poultry foods. Conversely, it is possible that poultry may be the reservoir of *S. thompson* infection in man. The public health aspect of the problem is most important, and it should be noted that in a survey of the incidence of *Salmonella* organisms in poultry during 1944, the authors have isolated the following additional types not previously reported in poultry in this country: *S. bareilly*, *S. california*, *S. montevideo* and *S. anatum*. This work is not yet completed and will be reported later. Edwards (1939) states that poultry constitute

Table 2

Serum	Absorbed by	Antigen	Result
	Strain 2768		
**S. paratyphosum* C (0)	Unabsorbed	Strain 2768	+++ 1/250
**S. thompson* (Hsp.)	,,	,,	+++ 1/250
†*S. thompson* (Hsp.)	,,	,,	+++ 1/12,000
Strain 2768	,,	†*S. thompson*	+++ 1/12,000
Strain 2768	Strain 2768	,,	−ve 1/25
†*S. thompson* (Hsp.)	,,	,,	−ve 1/25
Strain 2768	†*S. thompson*	Strain 2768	−ve 1/25
‡Pure 5 (absorbed serum)	—	Strain 2768 (phase 2)	+++ 1/20
	Strain 3845		
**S. paratyphosum* C (0)	Unabsorbed	Strain 3845	+++ 1/250
**S. thompson* (Hsp.)	,,	,,	+++ 1/250
†*S. thompson* (Hsp.)	,,	,,	+++ 1/12,000
Strain 3845	,,	†*S. thompson*	+++ 1/12,000
Strain 3845	Strain 3845	,,	−ve 1/25
†*S. thompson* (Hsp.)	,,	,,	−ve 1/25
Strain 3845	†*S. thompson*	Strain 3845	−ve 1/25
‡Pure 5 (absorbed serum)	—	Strain 3845 (phase 2)	+++ 1/20

* Supplied by Standards Laboratory, Oxford (titre 1/250).
† Type strain, serum prepared at Weybridge.
‡ Supplied by Emergency Public Health Service, Oxford.
+++ = complete agglutination. −ve = no agglutination.

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the greatest reservoir of paratyphoid infection in the United States of America, and mentions that most of the types found in fowls are capable of producing disease in man. There are many references in this country and Europe to outbreaks of bacterial food poisoning from the consumption of duck eggs infected with *Salmonella* organisms, notably *S. typhi-murium*, and in the *Annual Report* of the Chief Medical Officer of Health (1938), attention is drawn to the risk of severe or fatal gastro-enteritis involved in eating insufficiently cooked duck eggs. Although a number of *Salmonella* organisms have been isolated from duck eggs (Warrack & Dalling, 1932; 1933; Beller & Reinhardt, 1934), no reference can be found to the isolation of *Salmonella* organisms other than *S. pullorum* and *S. gallinarum* from hen eggs. This is of importance not only to public health but in the epidemiology of *S. thompson* infections in fowls. A group of survivors from an affected hatch have been purchased, and it is intended to study this point by the cultural examination of eggs laid by them.

The transmission of *S. thompson* infection in poultry is not clear. It will be noted that the organism has been isolated from the intestinal tracts of two adult fowls, one of which was the survivor of an affected hatch. It is probable that survivors or carriers excrete infection in their faeces, and in this way infection may be either mechanically transmitted to chicks by attendants, or introduced into the incubator by faecal contamination of the egg shell. It has been shown by Schalm (1937) that *Salmonella* organisms can penetrate the shell of the egg, and so infect the embryo and give rise to an incubator infection. In the present study *S. thompson* was isolated from the outside of the shell of a goose egg and from incubator debris. It has been clearly shown by Hinshaw, Upp & Moore (1926) and by Hinshaw, Scott & Payne (1928) that dissemination of infected fluff and debris by incubator air currents is one of the commonest methods by which *pullorum* disease is spread. In the two outbreaks investigated this would appear to have been the most likely method by which infection was disseminated, and there was little evidence to suggest direct egg transmission from the infected ovum as in *pullorum* disease. The part played by vermin is unknown, since the incidence of *S. thompson* in rodents does not appear to have been investigated, but *S. thompson* was isolated by the authors from two mice from the farm concerned in outbreak no. 2.

A limited amount of agglutination testing has also been carried out in an attempt to control infection by the elimination of carriers in the breeding stock. The results so far have been inconclusive, and the investigation is being continued this hatching season on the farm concerned in outbreak no. 2.

SUMMARY

During the years 1943 and 1944 *S. thompson*, not hitherto reported in poultry in this country, has been isolated on forty-four occasions from thirty-one outbreaks in chicks, and two outbreaks in ducklings. Two extensive outbreaks are described in detail, and the epidemiology of the disease and its possible importance to public health are discussed.

We wish to record our appreciation of the help given by the late Dr R. B. Haines and by Miss E. M. L. Elliot, Ministry of Food, Department of Pathology, Cambridge, in the identification of some of the strains isolated from outbreak no. 1, and for the gift of a stock strain of *S. thompson* which was used in the typing of strains 2768 and 3845.

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