

THE EPIDEMIOLOGY AND CONTROL OF *SALMONELLA* *THOMPSON* INFECTION OF FOWLS

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(With 2 Figures in the Text)

INTRODUCTION

Since *Salmonella thompson* was first identified by Scott (1926) it has become one of the commonest causes of human food poisoning in this country. In Topley & Wilson (1946) it is stated that *S. thompson* was the fourth commonest cause of salmonella food poisoning in man in England and Wales, and that it was identified in 142 out of a total of 1506 outbreaks studied during the years 1923-44. In Europe, *S. thompson* infection has been reported by Boecker (1935) in only a few cases of human food poisoning, and he emphasized the rarity of the organism as a human pathogen. Knorr (1931) isolated *S. thompson* from preparations of Chinese eggs imported into Germany. In the U.S.A., Edwards & Bruner (1943) succeeded in isolating *S. thompson* only once from poultry and four times from man, out of a total of 3090 cultures examined.

In Great Britain, the incidence of salmonella infections among poultry, excluding *S. pullorum* and *S. gallinarum*, has increased during the period 1942-5, and although a greater variety of salmonella types has been isolated from outbreaks of disease during that time, *S. thompson*, which was first recognized as a pathogen of poultry in 1943 (Gordon & Buxton, 1945*a*), was the most commonly encountered salmonella type. In their survey of avian salmonellosis, Gordon & Buxton (1946) showed that out of eighty-nine outbreaks recorded during the 2 years 1943-4, *S. thompson* was isolated on seventy-nine occasions from fifty separate outbreaks, and over the whole 12-year period reviewed in their survey, this organism was the second commonest encountered. In view of this increase an investigation was carried out into the pathogenesis of the organism in relation to its possible control and eradication in poultry flocks.

Part 1 of the paper deals with the periodic blood testing and bacteriological examination of eggs, cloacal swabs, random faecal samples and autopsy findings on the two groups of birds. The first group consisted of chicks which had survived the outbreak of *S. thompson* infection described by Gordon & Buxton (1945*a*), and the second group were adult birds from the breeding stock of the same farm, and which had reacted to a routine agglutination test with *S. thompson* (O and H) antigens.

Part 2 of the paper gives the results of an investigation into the methods of transmission of the disease via the egg and the control of infection in incubators and hatcheries.

TECHNIQUES

(1) *Antigens*

A stock strain of *S. thompson* supplied by the late Dr R. B. Haines, Cambridge, was employed in the preparation of all antigens. The somatic antigen was made by seeding the stock culture into peptone broth, incubating at 37° C. for 18 hr. and heating in a water-bath at 70-80° C. for 45 min. A sterility test was then carried out and the antigen standardized to Brown's opacity tube no. 2, preserved by the addition of 0.5% formalin (40% formaldehyde) and stored at 1° C. Each batch of antigen was discarded after 1 month and replaced by a fresh brew.

A specific flagella antigen was prepared by growing the organism (specific phase) in peptone broth for 18 hr. and then adding 1% formalin (40% formaldehyde). The standardization and storage conditions were similar to those employed for the somatic antigen.

(2) *Agglutinations*

The drop method (Dreyer) was employed for all agglutination tests, using serum dilutions of 1/25, 1/50, 1/125 and 1/250, with adequate controls. The notations used for recording the agglutinations were as follows:

- +++ = complete agglutination.
- ++ = well-marked agglutination without sedimentation.
- + = trace visible to the naked eye.

In order to obtain constant values for all readings, the figures suggested by the Standards Laboratory (M.R.C.) for evaluating the relative quantities of serum involved were employed in the following order:

- +++ × 1.5, ++ × 1.0, + × 0.7.

Somatic agglutinations were incubated in a water-bath at 52° C. for 18 hr. and flagella agglutinations at the same temperature for 2 hr.

(3) Cloacal swabs

Sterile swabs of cotton-wool on the end of 6 in. applicators were used. After being withdrawn from the cloaca, each swab was placed into a test-tube containing 10 ml. tetrathionate broth and the culture tube was then rapidly rotated so that the faeces became dislodged from the swab. The cultures were incubated at 37° C. for 18 hr. and a loopful plated on MacConkey's agar. The methods used for the identification of salmonella types were those reported by Gordon & Buxton (1946).

(4) Examination of eggs

Separate cultures were made from the shell swab, yolk, white, remainder of the yolk plus white and from the shell plus shell membrane, using the technique of Gordon & Buxton (1945b).

(5) Faecal samples

Fresh samples of faeces were collected and transferred to the laboratory in sterile Petri dishes. Sterile physiological saline was added to each sample of faeces at the rate of 6 ml./1 g. faeces. The faeces were washed out of the Petri dish into a sterile mortar, ground into a thick suspension, and 2 ml. of this suspension were inoculated into flasks containing 100 ml. tetrathionate broth, and examined by the same method as the cloacal swabs.

PART I

A. The examination of surviving chicks from an outbreak of *Salmonella thompson* infection

In December 1943 an outbreak of *S. thompson* occurred among some week-old chicks on a farm in the Midlands (Gordon & Buxton, 1945a). A mortality rate of approximately 20% persisted throughout six lots of 120 chicks each, hatched during the period December 1943 to June 1944. Fifty survivor chicks from the last infected hatch were purchased and kept at the laboratory for the following 20 months. Five of the chicks died in transit and *S. thompson* was isolated from all five. During the first 3-4 weeks after arrival, a severe outbreak of caecal and duodenal coccidiosis occurred from which only twenty-four chicks survived. *S. thompson* was recovered from the intestine of one of these dead chicks.

Blood tests and cloacal swabs

At the age of 8 months when these birds had reached maturity and were coming into production, the flock was examined for agglutinins to *S. thompson* (blood tested) and cloacal swabs were examined bacteriologically. These examinations were repeated at monthly intervals for the next 10 months, and the results are shown in Table 1. At the beginning

of the 9th month, six pullets of approximately the same age and from the laboratory healthy stock, were added to the flock as controls. During the 15th month, three cockerels were introduced for breeding purposes. The results of the tests carried out on these birds are included in Table 1.

This table shows a number of interesting features. Out of the total of twenty-four survivors, seven (29%) failed to show any evidence of infection as judged by the results of blood testing and cloacal swabbing. Out of a total of 208 blood samples, twenty-nine (13.9%) were positive to the H antigen and only six (2.8%) to the O antigen.

It will be seen that the isolation of *S. thompson* from cloacal swabs had no real correlation with agglutinin production above the normal flock titre, since of the eight birds which excreted *S. thompson* and which yielded thirteen infected swabs (23.2%), only five showed agglutinins in their sera. In these birds H agglutinins were only observed on twelve occasions (21.4% of tests) and O agglutinins on three occasions (5.3% of tests). Three of the birds which gave infected cloacal swabs failed to react to any of the blood tests. Although the cloacal swabs from the six control pullets were all negative, three birds developed agglutinins to the H antigen and on one occasion to the O antigen. Nine birds, which reacted on at least one occasion to the agglutination tests, gave consistently negative cloacal swabs.

Of the twenty-four survivors, five gave infected cloacal swabs and showed agglutinin production some time during the period of the experiment, three gave infected cloacal swabs and no agglutinin response, and nine produced agglutinins but *S. thompson* was not recovered from their cloacal swabs. Seven birds failed to show any evidence of infection.

It may be concluded, therefore, that in this group of surviving chicks, eight showed definite evidence of infection when mature (33% carrier-rate) while a total of seventeen may be considered as potential carriers, giving a total probable carrier-rate of 70.8%.

Faecal samples

During most of the time that the flock was under observation, six random samples of faeces were collected from the floor of the poultry house each week and examined for *S. thompson* infection. Care was taken to select fresh samples, and preference was given to those having a mucoid surface. The results of these examinations are recorded in Table 2 which clearly shows that the frequency of infection during the early part of the experiment was high, and that it slowly declined until after the 14th month no further infection of the faecal samples could be demonstrated.

Cultural examination of eggs

During the early part of the laying season there was some delay in obtaining trap-nests with the result that the eggs were laid on the floor of the house and the shells were frequently covered with faeces. When the trap-nests were employed, eggs were produced under cleaner conditions and it was only occasionally that the shells of these trapped eggs were grossly contaminated with faeces. The bacteriological examination of both groups showed that of the 166 untrapped eggs nine (5.4%) were infected (shells only), and of the 608 trapped eggs, one (0.18%) was infected (shell only). It will be

Table 2. *Showing the results of the bacteriological examination of random faecal samples taken from a flock of pullets which survived an outbreak of Salmonella thompson infection when chicks*

No. of month	No. of week	No. of samples examined	No. positive	Organism recovered
10	1	6	6	All <i>S. thompson</i>
	2	6	3	All <i>S. thompson</i>
11	3	6	1	<i>S. thompson</i>
	4	6	5	All <i>S. thompson</i>
	5	6	3	All <i>S. thompson</i>
	6	6	1	<i>S. thompson</i>
12	7	6	2	Both <i>S. thompson</i>
	8	6	—	—
	9	6	—	—
13	10	6	—	—
	11	6	1	<i>S. thompson</i>
	12	6	2	Both <i>S. thompson</i>
	13	6	—	—
14	14	6	5	Both <i>S. thompson</i>
	15	6	—	—
	16	6	1	<i>S. thompson</i>
	17	6	—	—
15	18	6	—	—
	19	6	—	—
	20	6	—	—
16	21	6	—	—
	22	6	—	—

seen from Table 1 that the single infected egg which was trapped had been laid by bird no. 3000 which had given consistently negative cloacal swabs and which did not develop diagnostic agglutinins. It is probable that the egg shell came in contact with infected faeces in the nest box after it had been laid. It is important to note, however, that in every case where *S. thompson* was recovered from eggs it was only from the egg shell, and that out of a total of 774 eggs examined in no case was there infection of the egg white or egg yolk.

Breeding experiment

After the examination of a sufficient number of eggs had shown that infection of the egg shell was not uncommon and infection of egg meat did not

occur, it was decided to carry out a breeding experiment to observe the danger of the spread of infection from naturally contaminated egg shells to newly hatched chicks. At the time when the eggs were being collected for hatching (i.e. during the 16th–18th months) the epidemiology of this disease in poultry was not fully understood, and it did not become evident until later that from the 15th month onwards the flock was no longer infected. The results of the two breeding experiments (Table 3) have been included in this paper, since they give additional proof that from the 16th month onwards infection had either died out or at most had become of no practical significance.

In Exp. I (Table 3) a total of forty-eight eggs were collected. At the 15th month, three birds (3307, 3334 and 3393) showed agglutinins to the H antigen and 3393 was again positive at the 16th month, while 3301 gave the last positive cloacal swab during the 14th month. It was unfortunate that of these four birds, only one (3393) was in lay at the time the eggs were collected for hatching. Five eggs were collected from this bird of which two were infertile. The remaining three hatched healthy chicks.

In Exp. II a further 102 eggs were collected and set with similar results.

Table 3. *Breeding experiment with eggs from survivors of an outbreak of Salmonella thompson*

All infertile eggs, dead-in-shells and chicks which died were examined bacteriologically with negative results.

	Eggs set	Infer- tile	Dead in shell	Chicks hatched	Chicks died	Chicks reared
Exp. I	48	13	13	22	6	16
Exp. II	102	17	22	63	3	60
Totals	150	30	35	85	9	76

Throughout both experiments there was no indication that infection had been transmitted from the parent stock to the chicks, and the seventy-six chicks which were reared failed to develop agglutinins to *S. thompson* at 8 months of age. The high rate of infertility and of incubator culls is explained by the fact that only young poor-quality cockerels could be obtained for the breeding experiments. The low hatching results were in no way caused by either the presence or result of infection with *S. thompson* in the parent stock. In each experiment a group of control eggs from healthy stock was incubated in the same incubator as the experimental eggs, and a comparison of the percentage fertility, hatchability and viability of all groups of eggs is given in Table 4.

In this table the eggs set from the experimental flock have been divided into two groups, those laid

by birds which had been positive on at least one occasion to either the blood test or to cloacal swab examinations, and those from birds which had been consistently negative to both examinations. The percentage fertility of eggs laid by reactor birds, negative birds and control birds showed no significant variation. The results are similar regarding the fertile eggs which hatched from each group. There was a very slight variation in the viability of the various groups. In the case of eggs laid by the positive and negative birds it was 89.4%, while in the case of the two groups of control eggs it was 94.9%, a difference of only 5.5%. It is justifiable

of this organism, it may be harboured for many months in the gall bladder. It is probable that this condition is similar to that which often pertains in cases of human typhoid infection (Pratt, 1901; Scott, 1915; and others). It is also probable that the gall bladder becomes infected from the liver in the early stages of the disease, when it is common for chicks to suffer from a generalized septicaemia (Nichols, 1916; and others).

The reasons for the deaths of a number of birds in the flock before the conclusion of the investigation have not been entered in Table 1, as they bore no reference to the experiment, but a post-mortem and

Table 4. Summary of the two breeding experiments in Table 3

The eggs have been divided into those derived from birds reacting positively to the blood tests or giving positive rectal swabs, and those from birds which were negative to all tests. The fertility, hatchability and viability of the groups are compared.

Origin of eggs set	No. of eggs set	No. of infertile eggs	No. of fertile eggs	% fertility	No. of dead-in-shells	No. hatched	% fertile eggs hatched	No. of chicks reared	% viability
Eggs from birds reacting positively to blood tests or giving positive rectal swabs	123	26	97	78.9	28	69	71.1	76	89.4
Eggs from birds negative to all tests	27	4	23	85.2	7	16	69.8		
Total	150	30	120	82.0	35	85	70.5	76	89.4
Eggs from healthy flock incubated in same machine at same time:									
Control group I	60	10	50	83.3	10	40	80.0	37	92.5
Control group II	35	8	27	77.1	8	19	70.3	19	100.0
Total	95	18	77	80.2	18	59	75.1	56	96.2

to conclude, therefore, that there was no significant reduction in the fertility, hatchability or viability of eggs laid by the experimental flock.

Autopsy findings

At the conclusion of the experiment the flock was destroyed and a post-mortem and bacteriological examination was carried out on each bird, cultures being made from the liver, heart, gall bladder, spleen, ovary, and from the duodenum, small intestine, caecum and large intestine. *S. thompson* was recovered from only two of the twenty-four birds in the flock, once from the caecum (3336) and once from the gall bladder (3301). In neither case were any symptoms of disease observed before the birds were killed, nor were there any post-mortem lesions. It is interesting to note that bird 3301 gave five infected cloacal swabs during the experiment; the highest number given by any bird in the flock. The fact that *S. thompson* was finally isolated from the gall bladder of the bird is of significance, and it is evident that when poultry become chronic carriers

a bacteriological examination was carried out in each case.

A summary of the results of the blood tests and the examination of cloacal swabs and random faecal samples is given in Fig. 1, which shows more clearly than Table 1 a number of interesting features. The H agglutinins in the experimental birds could always be detected in higher dilutions than the O agglutinins, and the number of birds at any one test possessing H agglutinins was always greater than those showing O agglutinins. These two observations are of common occurrence in man and animals infected with motile salmonella bacilli. It is important that this fact should be recognized in connexion with poultry, since it is of much significance in the interpretation of routine blood-testing results. In attempting to assess the results of the bacteriological examination of faecal samples and cloacal swabs it must be remembered that although many samples failed to show the presence of *S. thompson*, it would be incorrect to assume that they were not, in fact, infected. Nevertheless, it is

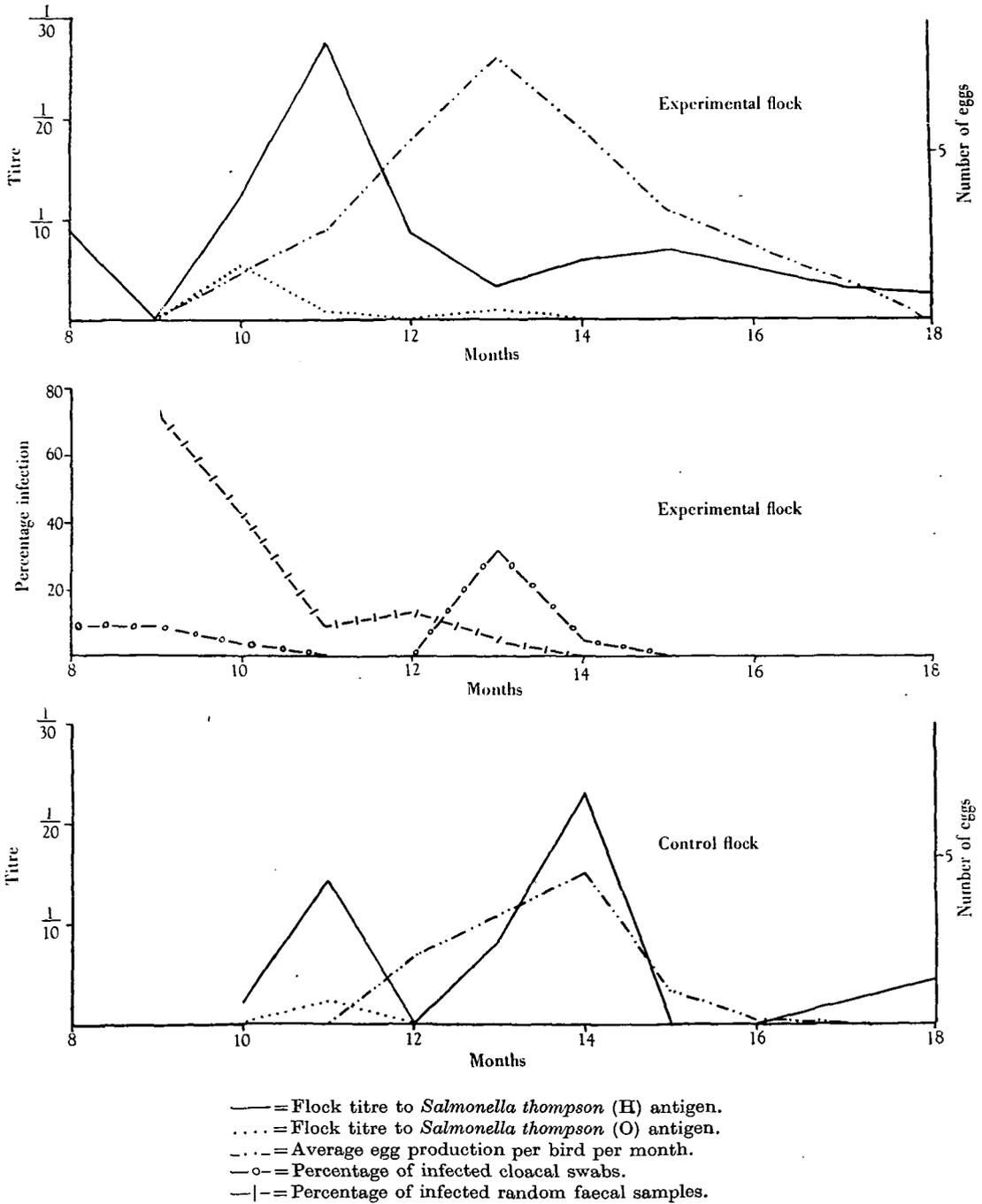


Fig. 1. Showing the agglutinin production, egg production and percentage infection of cloacal swabs and random faecal samples, in a flock of survivors from an outbreak of *Salmonella thompson* infection in chicks.

clear from Fig. 1 that the frequency with which the bacilli were being excreted in the faeces was high during the 9th and 10th months (75 and 42% infection respectively), and that the percentage isolation rapidly declined after the end of the 14th month when no further infected samples were detected. During the 15th to 18th months, however, the flock titre to the H antigen showed a fairly constant level, ranging from 1/7 to 1/2.8. This was maintained by four birds (nos. 3307, 3334, 3393 and 3303) having individual titres of 1/25–1/75. *S. thompson* was not isolated from any of these birds.

The six control birds developed H agglutinins soon after their introduction into the flock and to almost the same extent as the experimental birds. They showed an even greater variation in titre from month to month. In one case (3023) a titre of 1/17.5 to the O antigen was demonstrated. In view of the fact that during the 9th and 10th months, in particular, the rate of excretion of viable bacilli by the experimental birds was high, it is probable that the control birds rapidly became infected soon after their introduction into the flock, from the ingestion of contaminated food, water, etc.

It has been noticed by earlier workers (Gordon & Garside, 1944, Warrack & Dalling, 1932) that there was a slight correlation between the rise and fall of the titre of a flock infected with salmonella bacilli and the rate of egg production by the flock. In Fig. 1 the rate of egg production has been plotted for both the experimental and control birds so that a comparison can be made between ovarian activity and the flock titres. There is a similarity between the two, especially in the case of the control birds where the maximum egg production rate and the maximum flock titre coincide at the 14th month. The possible reasons for this, together with the general results of the various examinations carried out on this flock, will be discussed more fully with the results obtained from the adult flock.

B. The examination of a flock of adult birds, suspected of being carriers of *Salmonella thompson*

Observations were made on a flock of twenty-nine adult birds during their second laying season. All birds were suspected of being carriers of *S. thompson* since they had shown titres of 1/20 and 1/50 to a routine agglutination test carried out on the farm premises before purchase. They represented sections of two breeding flocks whose progeny suffered from outbreaks of *S. thompson* infection during the first 10 days after hatching, and some of which constituted the flock described in the first section of this paper. Monthly blood samples and cloacal swabs, together with random faecal samples, were examined from these adult birds in the same manner as in the previous experiment, and all eggs were examined bacteriologically. These investigations were carried

out during 6-monthly periods and again at the 10th month, when the flock was killed and all the birds examined bacteriologically.

A summary of the results is given in Table 5. Out of a total of 165 blood samples tested, eighty-one (50%) showed the presence of H agglutinins and only four (2.4%) showed any O agglutinins. In contrast to these results *S. thompson* was not isolated from any of the 165 cloacal swabs examined. During the course of the experiment 409 eggs were examined bacteriologically, of which sixty-three were untrapped. *S. thompson* was not recovered from any of these eggs. From the 4th to the 8th month, a total of 102 random faecal samples were collected from the floor of the hen house and examined. *S. thompson* was recovered from only one sample towards the end of the 4th month.

The agglutinin production and egg yield are shown graphically in Fig. 2. In studying this graph it is well to remember that the period of time which had elapsed between the probable initial infection of the birds and the 1st month represented in this experiment may have been as much as 1 year.

The flock titre is very similar to that of the pullet flock (Fig. 1). Once again H agglutinins were more frequent and reached a higher level than the O agglutinins. The two control cockerels introduced for breeding purposes showed a rapid production of H agglutinins during the 1st month after their introduction into the flock, reaching a maximum titre of 1/375 in one bird, and then declining to approximately 1/30 at the end of the 10th month.

The egg production of the whole flock was low throughout the 10 months of the experiment, and it bears little relationship to the flock titre (H). The production of O agglutinins was slight and intermittent. In the 3rd, 4th and 10th months the average O titre for the whole flock was 1/0.7, 1/2.2 and 1/1.5 respectively, the highest O titre shown by an individual bird at any one test being only 1/25.

A breeding experiment from the eggs of this flock was carried out after the introduction of the two cockerels. A total of 214 eggs was collected and incubated. The hatching results were similar to those from the pullet-breeding experiment, and there was no indication of *S. thompson* infection either in the eggs or the chicks.

At the conclusion of the experiment, post-mortem and bacteriological examinations were carried out on all the adult birds, with negative results.

PART 2

A. A comparison of the efficiency of heat-killed and alcohol-killed O antigens

Soon after the examination of both flocks had commenced it was realized that an alcohol-killed O antigen prepared from *S. thompson* might be

Table 5. Results of agglutination tests, cloacal swabs, cultural examination of eggs and bacteriological findings at autopsy of birds from breeding flock of infected farm

Bird no.	1		2		3		4		5		6		10		Bacteriological examination of cloacal swabs	No. of tests blood (H or O)	No. of tests blood (H or O) examined of eggs autopsy	Bac-teri-logical find-ings at autopsy			
	H	O	H	O	H	O	H	O	H	O	H	O	H	O					No. Result	Titre	Titre
2970	1:25	0	1:75	0	1:17.5	0	1:37.5	0	1:17.5	0	1:87.5	0	1:17.5	0	7 Neg.	7	23 Neg.	Neg.			
3716	1:25	0	0	0	1:75	0	1:75	0	0	0	1:37.5	0	0	0	7 Neg.	7	46 Neg.	Neg.			
2914	1:25	0	0	0	1:35	0	1:75	0	1:25	0	1:37.5	0	0	0	7 Neg.	7	7 Neg.	Neg.			
9645	1:25	0	1:50	0	1:75	0	1:75	0	1:25	0	1:35	0	1:75	1:25	7 Neg.	7	19 Neg.	Neg.			
7923	0	0	0	0	0	0	0	0	1:17.5	0	1:17.5	0	0	0	7 Neg.	7	218 Neg.	Neg.			
167	1:17.5	0	1:75	0	1:187.5	0	1:87.5	0	1:25	0	1:17.5	0	0	0	7 Neg.	7	5 Neg.	Neg.			
169	1:35	0	1:250	0	1:250	1:17.5	1:87.5	0	1:87.5	0	1:50	0	1:37.5	0	7 Neg.	7	9 Neg.	Neg.			
7840	0	0	1:25	0	1:25	0	0	0	1:37.5	0	1:250	0	0	0	7 Neg.	7	422 Neg.	Neg.			
7849	0	0	1:50	0	1:75	0	1:50	0	1:75	0	1:75	0	1:37.5	0	7 Neg.	7	21 Neg.	Neg.			
2002	0	0	1:37.5	0	1:187.5	0	1:75	0	1:50	0	1:17.5	0	1:75	0	7 Neg.	7	16 Neg.	Neg.			
2997	0	0	1:50	0	1:37.5	0	1:37.5	1:25	1:37.5	0	1:75	0	1:50	0	7 Neg.	7	644 Neg.	Neg.			
7867	0	0	0	0	1:75	0	0	0	0	0	0	0	0	0	7 Neg.	7	16 Neg.	Neg.			
188	0	0	0	0	0	0	1:25	0	1:50	0	0	0	0	0	7 Neg.	7	20 Neg.	Neg.			
7958	0	0	0	0	1:75	0	0	0	0	0	0	0	0	0	6 Neg.	6	125 Neg.	Neg.			
2189	1:250	0	1:75	0	1:75	0	1:125	0	1:35	0	D.	—	—	—	5 Neg.	5	0 Neg.	Neg.			
7996	0	0	0	0	1:37.5	0	1:87.5	0	D.	—	—	—	—	—	4 Neg.	4	21 Neg.	Neg.			
7850	0	0	1:87.5	0	1:250	0	1:125	0	D.	—	—	—	—	—	4 Neg.	4	31 Neg.	Neg.			
3129	0	0	0	0	0	0	1:37.5	1:25	D.	—	—	—	—	—	4 Neg.	4	10 Neg.	Neg.			
7888	1:87.5	0	1:75	0	1:37.5	0	D.	—	—	—	—	—	—	—	3 Neg.	3	0 Neg.	Neg.			
7817	1:35	0	1:50	0	D.	—	—	—	—	—	—	—	—	—	2 Neg.	2	4 Neg.	Neg.			
2069	1:35	0	0	0	D.	—	—	—	—	—	—	—	—	—	2 Neg.	2	10 Neg.	Neg.			
3627	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7 Neg.	7	1 Neg.	Neg.			
3794	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7 Neg.	7	2 Neg.	Neg.			
1843	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7 Neg.	7	9 Neg.	Neg.			
7868	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7 Neg.	7	7 Neg.	Neg.			
2979	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6 Neg.	6	9 Neg.	Neg.			
3134	0	0	0	0	0	0	D.	—	—	—	—	—	—	—	4 Neg.	4	14 Neg.	Neg.			
3738	0	0	0	0	0	0	D.	—	—	—	—	—	—	—	4 Neg.	4	7 Neg.	Neg.			
3637	0	0	0	0	D.	—	—	—	—	—	—	—	—	—	2 Neg.	2	0 Neg.	Neg.			
Total																165	165	81	346	Neg.	
Total																Untrapped eggs		63	409		
Control cockerels																6	Neg.	6	5	—	Neg.
1650	Not tested	0	0	1:375	0	1:75	0	1:75	0	1:17.5	0	1:37.5	0	0	K.	6	Neg.	6	5	—	Neg.
1954	Not tested	0	0	1:75	0	1:250	0	1:250	0	1:125	0	1:75	0	1:25	K.	6	Neg.	6	5	—	Neg.
Total																12	Neg.	12	10		

D. = Died. K. = Killed. Figures in titre column = End-point.

more sensitive than a heat-killed suspension, especially in the detection of birds with low titres. In order to maintain a uniformity throughout both experiments it was decided that the use of a heat-killed broth O antigen should be continued, and that a separate experiment should be carried out to compare the accuracy of the two antigens. The alcohol-treated antigen was prepared as follows:

Smooth colonies of *S. thompson* were subcultured on agar slopes, and after 18 hr. incubation the growth was washed off with sufficient sterile saline (0.8%) to give a density equal to that of tube 5, Brown's scale. Roux flasks of agar were inoculated with 2.0 ml. each of this suspension and rocked until the inoculum was evenly spread over the surface

suspension of *S. thompson* containing approximately 500 million organisms per ml. Four birds were infected by intravenous inoculation, four by subcutaneous inoculation and four were infected *per os*. In each group of four birds, two were given a dose of 1 ml. each and two 0.2 ml. each. The birds were kept isolated in individual coops and were blood tested at intervals during the 147 days after infection, using both heat-treated and alcohol-treated antigens. Two healthy birds from the same source as the other twelve were kept as controls. The results of this experiment are summarized in Table 6.

Eleven of the twelve birds developed diagnostic titres sometime during the experiment. In no case

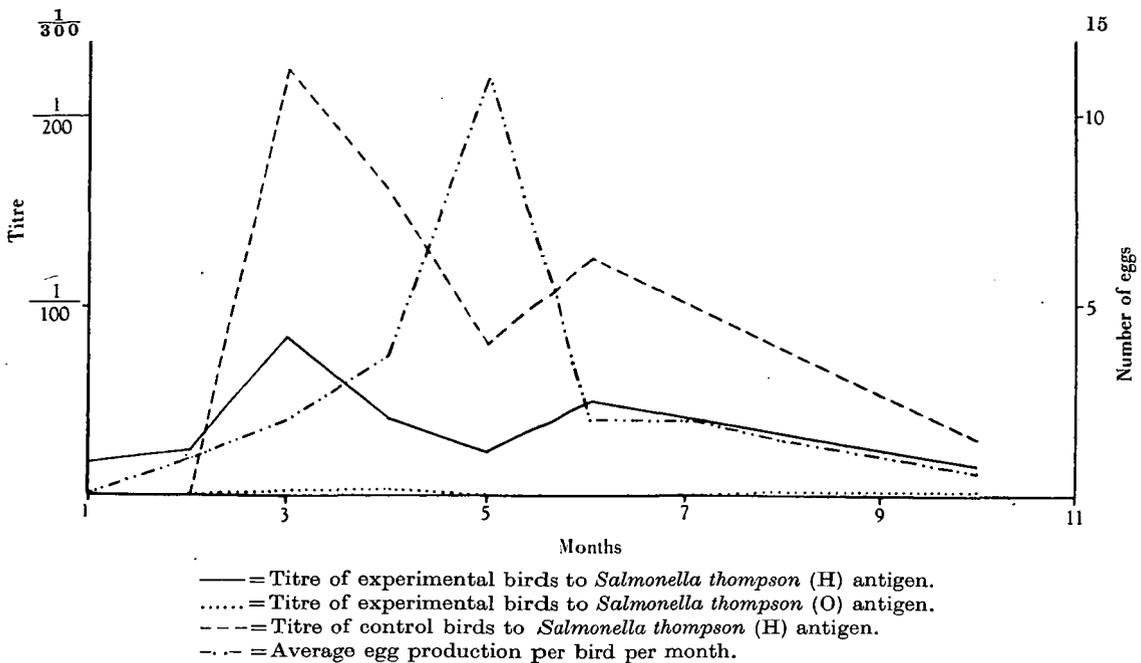


Fig. 2. Showing the agglutinin production and egg production of a breeding flock suspected of being carriers of *Salmonella thompson*.

of the medium. The flasks were incubated for 48 hr. at 37° C. After the growth from each flask had been washed off with 10 ml. sterile saline and sterile glass beads, the washings were filtered through glass-wool and pooled. To every 100 ml. of washings, 170 ml. absolute alcohol were added, mixed and allowed to stand in the cold room. After 48 hr. the organisms had settled to the bottom of the flask and the antigen was stored in this manner. Small quantities of antigen for agglutination tests were made up as required by suspending a quantity of the sediment in saline (0.8%) and standardizing to Brown's opacity tube no. 2.

Twelve healthy adult birds in their second laying season were artificially infected with a live saline

were agglutinins developed before the 9th day, and in the case of bird 8067, which received approximately 100 million live organisms by subcutaneous inoculation, no agglutinins were demonstrated before the 40th day. The two control birds remained consistently negative.

Although the experiment was not large enough to show the exact accuracy of agglutinin production as an indication of infection, some important observations can be made. Omitting the two control birds, a total of eighty-four bloods were examined, and of these thirty-seven were positive to the alcohol-treated antigen and only eleven to the heat-treated antigen. If the test carried out on the 9th day is disregarded—as it was negative in all

Table 6. Comparing the sensitivity of alcohol-treated and heat-treated *O. antigens* of *Salmonella thompson* on twelve artificially infected birds

Bird no.	Dose (ml.)	Route	No. of days after infection													
			9		12		14		19		27		40		147	
			A.	H.	A.	H.	A.	H.	A.	H.	A.	H.	A.	H.	A.	H.
2725	1.0	I.V.	0	0	87.5	0	187.5	0	175	125	75	0	0	0	0	0
1699	1.0	I.V.	0	0	125	0	175	0	50	0	175	0	35	0	0	0
1523	0.2	I.V.	0	0	0	0	87.5	0	75	0	0	0	0	0	0	0
1574	0.2	I.V.	0	0	175	0	187.5	0	375	375	250	250	0	0	0	0
8024	1.0	S.C.	0	0	35	0	35	0	87.5	0	17.5	0	0	0	0	0
2747	1.0	S.C.	0	0	17.5	50	0	0	125	0	0	0	0	0	0	0
8067	0.2	S.C.	0	0	0	0	0	0	0	0	75	0	0	0	0	0
1742	0.2	S.C.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1548	1.0	Oral	0	0	0	50	0	35	0	87.5	35	0	35	0	35	0
1542	1.0	Oral	0	0	25	50	0	35	0	25	0	0	0	175	17.5	0
1561	0.2	Oral	0	0	0	50	0	0	0	0	0	0	0	175	0	0
1535	0.2	Oral	0	0	0	0	25	0	25	25	175	35	0	0	0	0
8068		Controls	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8069		Controls	0	0	0	0	0	0	0	0	0	0	0	0	0	0

A. = alcohol-treated antigen; H. = heat-treated antigen.
 I.V. = intravenous inoculation; S.C. = subcutaneous inoculation.
 The figures in the table refer to the end-points.

cases to both antigens—out of a total of seventy-two tests, 51.4% were positive to the alcohol antigen and 15.3% to the heat-treated antigen. Of the eight birds which were infected by either intravenous or subcutaneous inoculation, only one bird (2747 on the 12th day) showed a higher titre to the heat-treated than to the alcohol-treated antigen. On the other hand, each of the four birds which were infected *per os* gave the same or a higher titre to the heat-treated antigen on at least one occasion. The variation in the titres of individual birds in all three groups was considerable and especially among the four birds which were infected orally. This is of special importance, as there is no doubt that the commonest method of *S. thompson* infection among adult poultry is by ingestion.

In conclusion, it may be said that under experimental conditions, where poultry are given one large dose of *S. thompson* and where reinfection can only occur from the contamination of food and water by the same birds, the production of agglutinins is erratic. It is also clear that an alcohol-treated antigen is more sensitive than a heat-treated broth antigen, and that under experimental conditions the former is only agglutinated at diagnostic titres by 50% of sera from known infected birds, and the heat-killed antigen by only 15% of sera.

B. *The penetration of Salmonella thompson through the shell of the hen's egg*

It has been shown in Part 1 of this paper that the infection of the ovary of adult birds with *S. thompson* either does not occur or is not the commonest method by which eggs become infected. Similarly, from the bacteriological examination of eggs laid by the survivors of an outbreak of *S. thompson*, the organism was isolated from only one egg shell out of a total of 608 trapped eggs examined. As has been previously pointed out, nine egg shells were infected in a total of 166 eggs which were untrapped and frequently coated with faeces from the floor of the poultry house. Most of the naturally occurring outbreaks of *S. thompson* in chicks which have been investigated have shown very similar characteristics to outbreaks of *S. pullorum* infection. In particular, deaths which sometimes involved the entire hatch have all occurred during the first 10 days after hatching, and survivors were shown to harbour the organism in their intestines for periods of at least 18 months after the outbreak had occurred. In the majority of cases where dead chicks and dead-in-shells have been examined from these outbreaks, *S. thompson* was isolated in pure culture from the yolk sacs and livers. It was tentatively assumed from these data that under certain conditions *S. thompson* was capable of penetrating the egg shell and infecting the yolk, either while the eggs were

being stored before incubation or during the incubation period. It has already been shown by Scott (1933), Kathé & Lerche (1936), Lerche (1936) and Jansen (1937) that salmonella organisms can penetrate the shells of duck eggs. The work of Haines & Moran (1940), in particular, has shown that the structure of the egg shell is such that under certain conditions bacteria can penetrate from the outside of the shell through to the egg meat. The shell is composed of four layers, an outer cuticle (mucin fibres), a spongy layer (crystals of calcite), a mammillary layer (crystals of calcite) and an inner shell membrane. The pores in the shell pass from the outer cuticle through to the mammillary layer only and do not pass straight through the entire structure of the shell. The average diameter of the pores varies from 6 to 13 μ , which means that if conditions are suitable, *S. thompson* would be capable of passing through these pores.

In view of the foregoing evidence, two small experiments were carried out to observe the conditions required for the infection of egg yolk by painting egg shells with cultures of *S. thompson*.

A batch of thirty infertile clean eggs from known healthy stock was divided into three groups of ten eggs each. The shell of each egg was painted with an 18 hr. broth culture of *S. thompson*. Each group of eggs was then placed in a separate glass jar and stored at 37° C. in a dry-air incubator. The first group of eggs was cultured after 7 days' incubation, the second after 14 days' and the third after 21 days' incubation.

A similar experiment involving the same number of eggs was carried out storing the eggs at room temperature for 9, 14 and 21 days.

The technique of the bacteriological examination of the eggs was the same as that employed by Gordon & Buxton (1945b). The results are given in Table 7. The first group, incubated at 37° C. for 7 days, consisted of only nine eggs, since one egg was accidentally transposed to the third group.

After incubation for 21 days at 37° C., seven out of eleven yolks were infected, while at the end of 14 and 7 days' incubation *S. thompson* was recovered from one yolk and four yolks respectively. After the storage of ten eggs at room temperature for 21 days only one yolk and one white (from separate eggs) were infected. None of the yolks of the ten eggs stored for 7 days at room temperature and only one yolk out of the ten stored for 14 days at room temperature became infected. Although the scale of the experiment was too small for any definite conclusions to be drawn, it has been included in this report as an indication that *S. thompson* will frequently penetrate egg shells and infect the yolks of eggs during incubation, but that it is less common for such infection to occur in eggs which are stored at room temperature. At the same time, the bacilli

remain viable on the outside of the shell after storage at room temperature for 17–21 days, and in a subsequent experiment it was shown that they penetrated the shell and infected the yolk when the eggs were finally incubated. In assessing these results, however, it must be borne in mind that, first, the eggs used for the experiment were not fertile, and, secondly, the incubator was of the ‘still-air’ variety.

A study of the conditions which promote the rapid multiplication and penetration of *S. thompson* bacilli through the egg shell and subsequent infection of the yolk would not be complete without a reference to the bacteriolytic action of lysozyme, of

C. *The spread of Salmonella thompson infection in the incubator*

It has been shown in Part 1 of this paper that the most common method of egg infection with *S. thompson* is from the contact of egg shells with the contaminated faeces of carrier birds. It has also been shown that *S. thompson* can remain viable on the outside of egg shells for at least 21 days during egg storage, and following incubation can penetrate to the yolk. In view of these facts, two small experiments were carried out to demonstrate the danger of the spread of infection by shell contact in the incubator and by the contact of clean egg shells with an infected incubator drawer.

Table 7. *Showing the penetration of Salmonella thompson through the egg shell*

	No. of samples infected after 7 days' storage	No. of samples infected after 14 days' storage	No. of samples infected after 21 days' storage
Eggs stored at incubator temperature			
No. of eggs comprising each group ...	9	10	11
Samples examined:			
Shell swab	6	9	10
Shell plus shell membrane	9	4	10
Pure yolk	4	1*	7
Pure white	3	1*	5
Remainder of yolk plus white	6	2	8
No. of eggs infected in each group	9	10	11
Eggs stored at room temperature			
No. of eggs comprising each group ...	10	10	10
Samples examined:			
Shell swab	10	2	7
Shell plus shell membrane	5	8	8
Pure yolk	0	1*	1†
Pure white	0	1*	1†
Remainder of yolk plus white	1	2	2
No. of eggs infected in each group	10	10	10

* Both cultures from the same egg.

† Cultures from different eggs.

which egg white is one of the richest sources. Fleming & Allison (1922) showed that some bacteria were dissolved by the lysozyme in egg white, and that with large numbers of bacteria this bacteriolytic property was enhanced. From the results of the experiments which demonstrated that *S. thompson* could penetrate the egg shell and infect the yolk of a hen's egg, it was assumed that *S. thompson* was resistant to the bacteriolytic action of lysozyme. This was later confirmed in a series of experiments in which *S. thompson* was successfully grown in dilutions of 1/10,000, 1/1000, 1/100, 1/10 and also in undiluted egg white. The experiment was repeated using the ‘cup method’ of Fleming (1932) and similar results were obtained.

A total of ninety fertile eggs from healthy laboratory stock were used for the first experiment. The shells of thirty of these eggs were artificially infected by painting them with an 18 hr. broth culture of *S. thompson*. These infected eggs were mixed with the remaining sixty eggs hatched in a ‘still-air’ incubator. Following hatching all chicks were killed and examined bacteriologically together with the unhatched eggs. *S. thompson* was recovered from 80% of the chicks and unhatched eggs derived from the group of thirty infected eggs, and from 21.7% of chicks and eggs derived from the group of sixty in-contact eggs.

In assessing these results it must be remembered that a small ‘still-air’ incubator was employed, and

the eggs were turned twice daily by hand which facilitated the spread of infection. Although this daily handling of eggs is done mechanically on many poultry farms where cabinet incubators are employed, in this experiment the eggs were hatched in muslin bags which decreased the danger of the ingestion and inhalation of infected incubator debris by the chicks at hatching time. Furthermore, the dissemination of infection among the chicks from the contamination of food and water with infected faeces, although a potential danger under more natural conditions of chick rearing, did not occur in this experiment since the chicks were killed on the day after they hatched.

Immediately after this experiment the incubator drawer was cleaned by brushing the perforated zinc and wooden surround with hot water, without the addition of any disinfectant. The drawer was then replaced in the incubator and a further thirty fertile eggs from healthy stock were incubated. On the 22nd day all chicks and unhatched eggs were examined bacteriologically, and *S. thompson* was recovered from a total of nine chicks and eggs.

Although these two experiments have been carried out only on a limited scale, they demonstrated that in a 'still-air' incubator the spread of infection by shell contact did occur, and that infection was transmitted from one hatch to another by the use of a clean incubator which had not been disinfected.

D. The susceptibility of chicks to experimental infection with *Salmonella thompson*

Attempts to estimate the susceptibility of chicks to *S. pullorum* infection under experimental conditions have given variable results. For example, Mulsow (1919*a, b*) reported that chicks were not susceptible to visible infection when dosed orally. Rettger & Plaistrige (1932) have commented on the different degrees of virulence which the same strain or different strains of *S. pullorum* possess for chicks. These findings are in accordance with earlier experimental results obtained by these authors. It is now known that the results of such pathogenicity tests are dependent upon a number of variable factors, including the age of the culture employed and the media on which it has been grown, the age and general health of the chicks and certain hereditary factors (Card & Roberts, 1931) which vary in different groups of chicks.

The experimental infection with *S. thompson* showed considerable variation in the susceptibility of different groups of chicks to one strain of organism. In view of these results only a summary of six experiments which have been carried out are reported.

A total of sixty-six chicks were infected with a saline suspension of a recently isolated strain of

S. thompson. Each infecting dose consisted of approximately 4500 million organisms, which was mixed in the food or water and fed to groups of day-old chicks. Three groups of chicks each received only one supply of infected food or water, and three groups received either infected food or water every day for the first 7 days of the experiment. There was no significant difference in the mortality rates between the groups. Out of a total of sixty-six chicks which received infected food or water on at least one occasion, twenty-nine (44%) died from *S. thompson* infection. The thirty-seven survivors were killed when 21 days old and *S. thompson* was recovered from the intestines of twenty-six (a carrier rate of 70.3% among the survivors) and from no other organ.

Groups of control chicks from the same source as the experimental chicks were kept isolated in the same brooder house and under identical conditions. There was no indication of *S. thompson* infection among these chicks, all of which were successfully reared during the experiment.

Although it was not possible to simulate under these conditions the high mortality rate often encountered in natural outbreaks, the experimental results showed that *S. thompson* did cause a severe mortality among young chicks when given in the food or water, and that the carrier rate among surviving chicks was high. The danger of survivors of an outbreak spreading infection is again stressed by the fact that *S. thompson* was recovered from samples of faeces from each experimental group of chicks during the 21 days of the experiment.

E. The formaldehyde fumigation of eggs artificially infected with *Salmonella thompson*

From the results of the preliminary experiment on the conditions necessary for the penetration of *S. thompson* through the shell of the hen's egg, it is clear that the optimum time for the disinfection of eggs would be either during the first 7 days of storage or during the 1st or 2nd day of incubation, that is, before the bacteria have left the outer surface of the shell. The dipping of eggs in solutions of disinfectants is not a practical method for controlling incubator infection on most farms, unless special equipment is employed. Moreover, a number of disinfectants have been used experimentally, and although sodium hypochlorite (20%) gave the most satisfactory results, it only reduced artificial shell infection from 100 to 20%. Until such time as a suitable disinfectant is found, the use of formaldehyde vapour is to be recommended for hatchery disinfection. The details are given below of the formaldehyde fumigation of artificially infected eggs carried out in a humid, still-air incubator, using the proportions of 1½ oz. formalin plus 1 oz. potassium permanganate per 100 cu.ft. of incubator space. It

is hoped to repeat this work using a mammoth forced-draught incubator where the conditions of humidity and ventilation are those more commonly in use in the modern hatchery.

Three groups of ten eggs each were infected by painting the shells with an 18 hr. broth culture of *S. thompson*, and eggs were then kept at room temperature. The first group was fumigated after storage for 24 hr., the second after 7 days, and the third after 14 days' storage at room temperature. With each group of eggs there were, in addition, five control eggs which were infected and stored for varying periods under the same conditions but which were not fumigated.

It is clear that under the conditions described, formaldehyde fumigation of infected eggs is of great assistance in reducing the danger of hatchery infections with *S. thompson*, provided that the conditions under which the eggs have been stored

S. thompson may become attenuated and fail to regain its original virulence until it has been passaged through several chicks.

DISCUSSION

The increasing importance of *S. thompson* as a cause of economic loss among poultry flocks has already been stressed. During 1943-4, a total of fifty separate outbreaks of disease in young poultry were diagnosed, and during 1945 and the first 7 months of 1946, a further fifty-two outbreaks have been identified. With the exception of *S. pullorum*, *S. thompson* has become the most frequent cause of mortality in chicks during this latter period. The importance, therefore, of understanding the pathogenesis of *S. thompson* in poultry and of using this knowledge to develop reliable methods of controlling the disease, is becoming increasingly urgent.

Table 8. Showing the bacteriological results of fumigating eggs in formaldehyde gas at various intervals, after the shells had been painted with *Salmonella thompson* culture and stored at room temperature

	Fumigated 24 hr. after infection	Controls not fumigated	Fumigated 7 days after infection	Controls not fumigated	Fumigated 14 days after infection	Controls not fumigated
No. of eggs comprising each group ...	10	5	10	5	10	5
Samples examined:						
Shell swab	3	5	1	1	0	5
Shell plus shell membrane	0	4	0	4	0	5
Pure yolk	0	0	0	0	0	0
Pure white	0	0	0	0	0	0
Remainder of yolk plus white	0	0	0	0	0	0
No. of eggs infected in each group	3	5	1	5	0	5

prior to fumigation were not suitable for the rapid multiplication and penetration of the bacilli through the egg shell. The fumigation of hatcheries infected with *S. pullorum* has been widely employed in both the U.S.A. and this country with successful results (Graham & Michael, 1932). During the last 12 months it has been applied in this country in connexion with *S. thompson* infection, and under field conditions it has not proved to be completely effective. It has been found that when an incubator was fumigated after an infected hatch had been withdrawn, and refumigated the day after it was replenished with fresh eggs from healthy stock, no mortality attributable to *S. thompson* infection occurred. In later hatches, however, it was not uncommon to find a mortality of up to 10% of the newly hatched chicks caused by a septicaemic infection with *S. thompson*. It is probable that in certain parts of an incubator the concentration of formaldehyde vapour is insufficient to have a lethal effect on the bacilli. Under such conditions,

Pathogenesis

From the experiments recorded in this communication it was possible to trace the pathogenesis of *S. thompson* infection in fowls. Many of the twenty-four chicks which survived an epidemic and which formed the flock of pullets (Part 1 A) became carriers and the bacilli were excreted intermittently in their faeces. During the 9th and 10th months of the experiment, *S. thompson* was isolated from 75 and 42%, respectively, of random faecal samples. This rate of infection steadily decreased until only one positive sample was identified in the 14th month and none during the 15th and 16th months of the experiment. When the birds were 18 months old and had completed their first laying season the flock was destroyed, and bacteriological examination showed that only two birds still remained carriers. In no case was *S. thompson* isolated from the reproductive organs. No symptoms of disease were ever shown by any of the birds

during the experiment. The flock of adult fowls (Part 1B), some of which were the parents of the pullet flock, showed no symptoms of disease, and although agglutinins to the H-specific antigen of *S. thompson* could be demonstrated in most samples of serum during the 10 months of the experiment, *S. thompson* was isolated from only one random faecal sample taken during the latter half of the 4th month. The results obtained from these two flocks showed that infection of fowls with *S. thompson* slowly decreased as the birds became older, and that during the latter part of their first laying season and the whole of the second laying season there was very little danger of these birds infecting their progeny provided that they were kept under hygienic conditions.

A total of 1183 eggs were examined bacteriologically during the investigation, and of 409 eggs derived from the adult flock none was infected. Of the remaining 774 eggs laid by the pullets, 166 were untrapped and frequently the shells were coated with faeces. While 5.4% of these untrapped eggs were infected with *S. thompson* on the shell, only one (0.18%) of the 608 trapped eggs were similarly infected. On no occasion in either of the two groups was *S. thompson* recovered from the yolk of an egg. It was concluded, therefore, that the most common method of transmitting infection from adult breeding fowls to chicks was from infected faeces which contaminated the outside of the egg shell, and that if egg production was carried out under hygienic conditions when the incidence of faecal contamination of the shell was reduced, the frequency of shell infection with *S. thompson* also decreased.

In most severe outbreaks of this disease in hatcheries, the general hygiene on the farm has been of a high standard. This fact, together with the low incidence of shell contamination, suggested that the greatest spread of infection occurred either during incubation or at hatching time. Under conditions of egg storage there was very little penetration of *S. thompson* through the egg shell, although the bacilli remained viable on the outside of the shell for at least 21 days. When eggs with infected shells were placed in an incubator the warm humid atmosphere promoted rapid multiplication of the bacilli which penetrated the shell and infected approximately 50% of yolks by the 7th day of incubation.

It has been shown that the spread of infection in an incubator occurred from the handling of eggs, from shell contact and from the ingestion and inhalation of incubator debris at hatching time. In the experiment recorded in this communication (Part 2C) over 20% infection occurred among healthy eggs which were in contact with artificially infected shells.

Blood testing control

The testing of poultry for the presence of agglutinins to salmonella organisms has been employed for many years, especially in the control of bacillary white diarrhoea (*S. pullorum*), and is used as the official method of eradication. The application of this test for the diagnosis of poultry infected with other (motile) salmonella organisms has not proved so reliable. There are a number of possible reasons for the failure of the test as a means of detecting carriers. It has already been pointed out that *S. thompson* in the adult bird was most commonly situated in the intestines and that it was frequently excreted and reingested. Unlike pullorum disease the ovary did not appear to be a common site of infection, and there was no indication of ovarian infection in the experimental flocks. These conclusions are in accordance with those of Wanner (1937), who stated that in adult fowls salmonella infections (other than *S. pullorum* and *S. gallinarum*) were intestinal in nature. In this respect a comparison may be made between *S. thompson* infection in adult fowls and bacillary dysentery in man, where the presence or absence of agglutinins is not a reliable indication of infection.

A further problem concerning the inefficiency of the agglutination test in the diagnosis of *S. thompson* infection was the constant variation in the agglutinin content of the fowl sera. These variations have been frequently noticed by those who have extensively employed the agglutination test in the eradication of *S. pullorum* infections. For example, Winters (1929) observed that chicks which had survived an outbreak of pullorum disease possessed specific agglutinins in their sera from the 50th to 60th day onwards, but he stressed the important point that a number of known infected chicks never produced agglutinins, and in some chicks which did produce agglutinins at the 50th–60th day, their sera became intermittently positive and negative to subsequent tests throughout the rest of their lives. As the result of further investigations, Winters concluded that 'the fluctuations from positive to negative and from high to extremely low titre when the same antigen is employed evidently indicates some serological change which is yet to be satisfactorily explained'. The results from the blood testing of the two flocks in this experiment clearly show the same variability of agglutinin production in respect of both *S. thompson* O and H antigens.

An examination of the many reports on the variation of *S. pullorum* agglutinins in fowls would lead to the assumption that provided there are no non-specific stimuli, the serum titres of chronic 'carrier' birds would be more constant and probably only of the order of 1/25. A great deal of work has been done on the effects of non-specific stimuli on the serum titres of immunized mammals, such as

extensive bleeding (Friedberger & Dorner, 1905; Hektoen & Carlson, 1910; Hinshaw & McNeil, 1940), and Conradi & Bieling (1916) have shown that an anamnestic rise in the titres of rabbits immunized against *S. typhosum* occurs, following the inoculation of *B. coli* organisms. Under the present conditions of poultry farming, where the ingestion of *B. coli* in food and water contaminated with avian faeces cannot be completely avoided, it is not improbable that the findings of Conradi & Bieling apply equally well in the case of avian carriers of *S. pullorum* and *S. thompson*.

A specific stimulus which must be considered in connexion with poultry is egg production. The protein metabolism of a bird in full lay is much higher than that of a bird not in production. Work carried out on the physiology of the hen's egg (see Needham, 1931) has shown the high-protein content of the egg, especially the yolk. Jukes & Kay (1932) showed that the livetin fraction forms 20% of yolk protein, and that it is very closely related to, if not identical with, serum globulin. In connexion with protein metabolism of mammals Bjerneboe (1939, 1941) and Boyd & Bernard (1937) have shown that the increase in serum globulin in rabbits immunized with pneumococci closely parallels the increase in antibody production. In the examination of the two flocks recorded in this communication, there was a slight relationship between flock titre to *S. thompson* and egg production. It is probable, therefore, that this direct relationship is dependent upon the globulin (or livetin) metabolism of the birds, especially in view of the fact that antibodies have been demonstrated in the sera and the yolks of eggs laid by birds which had been inoculated with the corresponding antigens (Frank & Edgington, 1937; Ramon, 1928).

The blood-testing results of both flocks described in this paper show that if the presence of infection is judged by the examination of random faecal samples, cloacal swabs and autopsy findings, then agglutinins are still demonstrable long after infection has disappeared. The production of agglutinins by the control birds, especially to the H antigen of *S. thompson*, without any positive indication of infection, is a further problem which must be considered. Pijper & Dau (1930) and Greenwood, Topley & Wilson (1931) reported that the oral administration of killed suspensions of the flagellated bacilli of the typhoid-paratyphoid group resulted in the production of O agglutinins, while the H agglutinins were generally not demonstrable or only present in very low titres. In considering the titres of the control birds, it must be concluded that either they became infected with live organisms which were not identified in the cloacal swabs, or that in the case of fowls, H agglutinins only are produced following the oral administration of dead bacilli.

It has been shown that an alcoholized O antigen is more sensitive than a heat-killed antigen in the detection of low-titred sera, and that this increased sensitivity is more marked in the case of birds artificially infected by intravenous and subcutaneous inoculations than it is in birds which were infected *per os*. The greater sensitivity of the alcoholized antigen increased the number of positive tests to the heat-treated antigen from 15 to 50% of all tests carried out on known infected birds. In view of the specific and non-specific factors which may be responsible for the variations in flock titre, it has to be considered whether the use of a more sensitive alcoholized antigen is to be recommended in place of a heat-killed antigen. For the most accurate use of any antigen as a diagnostic agent, it is necessary to be well acquainted with the normal flock titre. During the past two years some hundreds of birds have been tested from different farms, and the results showed that in most flocks there was a slight response to the agglutination test at non-diagnostic titres to *S. thompson* O antigen (alcoholized). In the original blood test which was carried out on the parent flock of birds from which the adult flock of this experiment was composed (Gordon & Buxton, 1945*a*), a serum titre of 1/20 or more was accepted as an indication of infection. From the subsequent experiment it is clear that most of the birds, although not heavily infected, were sensitized to *S. thompson*, and that this sensitization lasted throughout the second laying season. As infection in the pullet flock diminished until it had almost completely disappeared by the time the birds were 18 months old, it may be assumed that the adult flock probably was infected at a date previous to the time of the first blood test.

Although it is known that the blood test is not a completely reliable indication of infection, in the light of present knowledge of *S. thompson* infection in fowls, and bearing in mind the low economic value of the individual fowl and the economic loss resulting from an epidemic among young chicks, it is advisable to consider any adult bird which shows agglutinins at a titre of 1/20 or more to an alcoholized antigen of *S. thompson*, as a potential carrier.

Hatchery control

The preliminary experiments reported in this communication showed that very little penetration of the shell by *S. thompson* occurred under egg-storage conditions, but that in the atmosphere of the incubator, penetration was far more rapid. It was therefore necessary, in order to obtain maximum disinfection, to fumigate the egg shell either before incubation or within 24–48 hr. of the eggs being placed in the incubator. The formaldehyde fumigation of incubators in the control of pullorum disease is extensively employed as the most practical and

satisfactory method of egg disinfection. Bushnell & Payne (1931) and others have shown that optimum results were obtained when fumigation was carried out under the atmospheric conditions of incubation.

It has been recommended that for the control of *S. thompson* infection in hatcheries, only clean eggs should be used, and that dirty egg shells should be cleaned by scraping and not by washing with a damp cloth, as this will increase the rate of penetration of organisms through the shell (Wilson, 1945). Prior to incubation eggs should be stored in a cool atmosphere (max. temp. 22° C.), free from contact with vermin, and should be fumigated in the incubator 24 hr. after they have been set, as recommended for the control of pullorum disease in the *Rules for Accredited Hatcheries* (1946).

Public health

During the last 5–10 years the importance of poultry as a reservoir of salmonella infections for man and animals has become increasingly apparent (Hinshaw, McNeil & Taylor, 1944; Edwards & Bruner, 1939), and the danger to man from the consumption of infected eggs has been repeatedly stressed (Scott, 1930; Weber, 1937; De Koning, 1936; Hedström, 1941; Muller, 1941; Gordon & Buxton, 1945*b*; Gillespie, 1946). In most cases, however, it has been the duck's egg which has been incriminated rather than the hen's egg. The infection of the duck's ovary and hence the yolk of the egg (Gordon & Garside, 1944; Gordon & Buxton, 1945*b*) is a feature which has not been frequently observed in fowls infected with salmonella organisms other than *S. pullorum* and *S. gallinarum*.

From a study of the incidence of the salmonella types infecting man and animals in different countries, it is significant that *S. thompson* is most frequently isolated from both man and fowls in Great Britain. Such a condition is unlikely to be a coincidence, although the true relationship between them is not clear. There are two possible methods by which infection may spread from poultry to the human population. In the first place, there is a potential danger to the poultry farmer of becoming infected in the course of his daily work from the infected faeces of carrier birds. This point has already been stressed by Hinshaw, McNeil & Taylor (1944) in connexion with other salmonella types in the U.S.A. In the second place there may be a potential danger to the consumer from eggs laid by reactor birds if the eggs have been laid or stored under unhygienic conditions. The advisability of storing eggs for human consumption in a cool, dry atmosphere is important, as such conditions will not favour the penetration of the bacilli through the shell and subsequent infection of the yolk. The possibility of poultry becoming infected from human sources has been discussed by Gordon & Buxton

(1946) when they drew attention to the increased incidence of avian salmonellosis generally and of *S. thompson* in particular. This increase coincided with a similar rise in the number of outbreaks among the human population, and was thought to have some relationship to wartime conditions (e.g. movement of populations from urban to rural districts and the feeding of canteen and camp swill and household refuse).

The discussion of this subject would not be complete without a reference to the possibility of vermin as a reservoir and a disseminator of infection to both man and poultry. The frequency with which mice may be carriers of *S. typhi-murium* is well established, but whether a similar state of affairs exists in connexion with *S. thompson* is not known. Khalil (1938) examined 750 wild rats trapped in Liverpool and isolated *S. thompson* from one. In the case of an outbreak of *S. thompson* infection in a hatchery, a number of mice were trapped in the incubator room and *S. thompson* was isolated from two of them. As the outbreak had been in existence for some weeks before the mice were trapped, it is impossible to decide whether the mice had been carriers of *S. thompson* before the outbreak, or whether they had become infected from the poultry. Similar isolations of *S. thompson* from mice have been reported by Wilson (1945). The fact that mice can carry *S. thompson* without showing any indication of infection is an important point which warrants further investigation from both the agricultural and public health points of view.

SUMMARY

The details are given of the epidemiology of *S. thompson* infection in a flock of pullets which survived an outbreak of this disease when chicks, and also of a flock of adult birds suspected of being carriers of the organism. The results of blood testing and bacteriological examinations of cloacal swabs, random faecal samples and eggs from both flocks indicated that:

(1) Many chicks which survived an outbreak of *S. thompson* continued to carry the organism for some months without showing any symptoms. In most cases the organism was harboured in the intestines and was excreted intermittently in the faeces. On one occasion *S. thompson* infected the gall bladder, and the bacilli were excreted in the faeces for at least 18 months after the outbreak had occurred.

(2) The common method of egg infection was by the contamination of the shell with infected faeces. Under conditions of incubation the bacilli penetrated the egg shell and infected the yolk. Although there was little penetration of the organism under storage conditions, the bacilli on infected shells remained viable for at least 21 days.

(3) The common methods of spreading infection in a hatchery were from:

- (a) The contact of infected and non-infected egg shells.
- (b) The handling of eggs before and during incubation.
- (c) The contact of egg shells with infected incubators.
- (d) The ingestion and inhalation of infected fluff and incubator debris at hatching time.
- (e) The ingestion of food and water contaminated with infected faeces from survivor chicks.

For the control of *S. thompson* infection in poultry, the following procedures have been recommended:

(1) The production of agglutinins by carrier birds was not a reliable indication of infection. In known infected flocks, however, the detection of carriers by blood testing and by the examination of cloacal

swabs is of value. For such a test an alcoholized antigen is preferable to a heat-treated broth antigen, and a titre of 1/20 or more should be regarded as an indication that a bird is infected.

(2) Only clean eggs should be used for hatching. Dirty egg shells should be cleaned by scraping or brushing and not by wiping with a damp cloth.

(3) Fertile eggs should be stored in a cool, dry atmosphere for as short a period as possible before incubation.

(4) Eggs should be fumigated in the incubator with formaldehyde vapour, not later than 24 hr. after they have been set.

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