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Salmonella typhimurium contamination of processed broiler chickens after a subclinical infection

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SUMMARY

A subclinical infection of *Salmonella typhimurium* in a broiler flock was investigated and attempts were made to eradicate the infection by treatment with furazolidone. One-quarter of the chickens were still infected after they had been through the processing plant. Washing in heavily chlorinated water reduced the number of contaminated carcasses. Infected chickens were also found in four other companion flocks on the same farm.

INTRODUCTION

Now that *S. pullorum* and *S. gallinarum* infections of poultry in Britain have declined, *S. typhimurium* remains the chief cause of poultry salmonellosis (Sojka & Field, 1970; Stevens, 1971). These infections are variable, but even when they are mild clinically the pathogen may persist in the infected carcass and cause food poisoning. Anderson (1969) has shown a relationship between human food poisoning and salmonellosis in poultry by using bacteriophage typing. Phage type 14 was the strain of *S. typhimurium* most commonly isolated from poultry (74 %) and also from humans (17·5%), but this strain was not found in cattle. Phage type 20a, the next commonest strain in humans (9·15 %), was not found in poultry but did occur in cattle. A salmonella infection in a breeding flock may spread successively to the broiler flock, the processing plant and thence to the consumer, who may not appreciate the danger of handling uncooked carcasses or the importance of adequate thawing and cooking (Anon, 1969; Pennington, Brooksbank, Poole & Seymour, 1968; Semple, Turner & Lowry, 1968).

Whilst some flocks of broilers had a high incidence of salmonella infection (Griffith, 1969; Timoney, Kelly, Hannen & Reeves, 1970) other flocks had little or none (Tucker & Gordon, 1968; Patterson, 1967, 1969). *S. typhimurium* was the commonest serotype found in some poultry packing stations (Galton *et al.* 1955; Sadler, Yamamoto, Adler & Stewart, 1961; Dixon & Pooley, 1961*a*; Timoney, 1969) and also on poultry in shops (Felsenfeld, Young & Yoshimura, 1950; Galbraith, Taylor, Patton & Hagan, 1964).

Dixon & Pooley (1961*b*) recommended treatment of the spin-chiller water with 200 p.p.m. available chlorine to reduce cross-contamination. However, Barnes

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(1965) pointed out that although such treatment might destroy all the bacteria washed off the carcass, it would not destroy those present in the hair follicles or in the cavity. Chlorination is of some benefit and has been shown to prolong the storage life of the chicken by reducing the number of organisms present (Nilsson & Regnér, 1963; Ranken, Clewlow, Shrimpton & Stevens, 1965; Thomson, Banwart, Sanders & Mercuri, 1967; Patterson, 1968; Wabeck *et al.* 1968).

This paper describes an outbreak of *S. typhimurium* infection in a broiler flock and the difficulty of eradicating the pathogen with furazolidone therapy and careful attention to hygiene during processing.

MATERIALS AND METHODS

Chicks

Day-old chicks were supplied by a commercial hatchery. Each intake consisted of 2000 pullets and 8000 'as hatched' chicks.

Housing

The chicks were housed in broiler houses with fresh deep litter of wood shavings, and were supplied with food and water *ad lib.* The feed was varied from starter crumbs, raising crumbs and finisher pellets as appropriate. Furazolidone was administered in the drinking water at specified times.

Broiler processing plant

The plant handled 1200 birds/hr. After evisceration the carcasses passed through two spin-chillers containing cold chlorinated water, and then were drained and packed. Vent swabs were taken at $\frac{1}{2}$ hr. intervals from ten birds as they left the spin-chillers. Some carcasses were tagged and swabbed after evisceration and again after passing through the spin-chillers. The concentration of free chlorine in the spin-chillers was monitored during the day using potassium iodide and a Lovibond comparator. Fresh hypochlorite was added to maintain the concentration of 200–250 p.p.m. free chlorine.

Bacteriological examination

Samples. Livers were washed and macerated as described by Knivett & Stevens (1971) and the homogenized tissue was added to 50 ml. selenite broth. One ml. of this mixture was further diluted with 10 ml. selenite broth. Caeca were removed aseptically and small pieces were added to 50 ml. selenite broth. One ml. of this mixture was diluted with 10 ml. selenite broth. A few samples of the duodenum were taken and treated similarly.

Soiled litter was taken from several parts of the broiler house and 10 g. samples added to 100 ml. selenite broth. Dust was taken from crevices round the walls and 1 g. samples added to 10 ml. selenite broth. Samples of feed were taken from storage bins outside the broiler house and 50 g. added to 100 ml. selenite broth.

Cloacal, vent and equipment swabs were placed in 10 ml. selenite broth.

Enrichments. Selenite mannitol broth was freshly prepared and adjusted to pH 6·8. Cultures were incubated overnight at 43° C. and streaked on selective agar (Knivett & Stevens, 1971). Second enrichments were made by transferring 0·04 ml. of culture to 10 ml. of fresh selenite broth and streaking usually after 8 hr. incubation. Plates were incubated overnight at 37° C. and non-fermenting sulphide-producing colonies were checked for the absence of urease and tested with salmonella agglutinating sera.

RESULTS

An outbreak of Salmonella typhimurium *infection in a broiler house*

The events following an outbreak of *S. typhimurium* infection in a broilerhouse are described in Table 1. As soon as the Veterinary Investigation Centre diagnosed the infection, the pullets were destroyed. The remaining 'as hatched' chicks,

Table 1. *Calendar of events in house no.* 1

Age (days)	
1	Arrival of 2000 pullets and 8000 'as hatched' chicks. These were penned separately in the same house.
3	Pullets showed abnormally high mortalities and clinical symptoms of disease. Aureomycin was administered to these chicks for 5 days. Veterinary Investigation Centre subsequently diagnosed *S. typhimurium* phage type 1a.
8	Two thousand pullets destroyed and the litter removed and burnt. Furazolidone (0·04 %) administered to the remaining 'as hatched' chicks for 8 days.
18	Dead birds examined – 0/2 positive.
20	Dead birds examined – 3/4 positive.
24	Dead birds examined – positive.
32	Dead birds examined – 2/3 positive. Ten chickens examined – 6 caeca positive, all livers negative. Feed – negative. Litter and dust – positive.
47	Dead birds examined – 1/3 caeca and duodena positive, all livers negative.
66	Fifteen chickens examined – 10/15 caeca positive, 7/15 livers positive. Thirty-five cloacal swabs examined – 11 positive. Furazolidone (0·04 %) administered for 4 days.
69	Fifteen chickens examined – 9/15 caeca positive, all livers negative. Cloacal swabs examined – 4/35 positive.
74	All chickens processed. After evisceration, 10/10 vent swabs positive. After washing, 3/10 vent swabs positive. Total samples, 24/90 vent swabs positive.

separated from the pullets by a wire fence, appeared quite healthy, with no abnormal mortalities or symptoms of disease. They were kept under observation in case they might also have become infected and treated with 0·04 % furazolidone for 8 days as a precautionary measure. Subsequently samples of litter, dust, feed, dead chickens and some live chickens were taken for bacteriological examination, and showed that in spite of furazolidone treatment, some of the chickens were infected. A second treatment with 0·04 % furazolidone for 4 days was given 8 days before they went to the packing station. To assess the efficacy of furazolidone treatment, cloacal swabs were taken at random from 35 of the chickens, which were then tagged and penned separately (Table 2). Another 15 chickens were taken to the laboratory for examination. After the furazolidone treatment was over, those

chickens which had positive cloacal swabs were identified and taken to the laboratory for examination. No salmonellas were isolated from the livers after furazolidone treatment. There were fewer positive cloacal swabs after treatment than before and the infection still remained in many caeca.

Table 2. *Isolation of* Salmonella typhimurium *from chickens before and after furazolidone treatment*

Samples	Before treatment	After treatment
Randomly selected chickens		
Cloacal swabs	11/35* positive	4/35 positive
Livers	7/15 positive	0/5 positive
Caeca	10/15 positive	1/5 positive
Infected chickens†		
Cloacal swabs	10/10 positive	Caeca 8/10 positive Livers 0/10 positive

* These chickens were tagged and penned separately from the others.

† Chickens whose cloacal swabs were positive before treatment were examined in the laboratory after treatment for the presence of salmonellas in the liver and caeca.

As a precaution, the other flocks on the farm were treated with 0·02 % furazolidone for 8 days, although they had shown no clinical signs of disease, adverse weight gains or abnormal mortalities to suggest that they might be infected. A few tests were made to see whether infection had spread to these flocks (Table 3). Eventually the infection was detected in the litter from the five houses from which it was examined, and as a further precaution all flocks were given 0·04 % furazolidone treatment for 4 days, 8 days before they were due to go to the packing station.

Bacteriological tests at the packing station

Salmonella isolations from processed chickens. The equipment was thoroughly cleaned after the day's operations, and the next morning swabs were taken from several surfaces before the new intake was started. These were all negative on each occasion. Vent swabs were taken from ten successive chickens emerging from the second spin-chiller at ½ hr. intervals (Table 4). There was little evidence for any build-up of infection during the working day. Flocks II and III and the combined total had slightly more positive results during the second half of the day than during the first half, but this was not so in every case. The water in the first spin-chiller was changed at mid-day (except for flock V), but this had no significant effect on the number of isolations subsequently. All bacteriological samples taken from either spin-chiller were negative.

Effect of chlorination. Approximately 200–250 p.p.m. free chlorine was maintained in the spin-chillers. Considerably fewer positive results were obtained after chickens had been through the spin-chillers compared with the same samples examined before washing (Table 5). There was no evidence for cross-contamination in the spin-chillers, except for one sample which was negative before entering the bath and was positive on leaving.

Table 3. Salmonella typhimurium *infection in other flocks on the farm*

	Flock no. and date of birth					
Age (days)	I 13 Mar.	II 24 Mar.	III 31 Mar.	IV 7 Apr.	V 14 Apr.	VI 31 Mar.
3	M+	.	.	.	.	.
7	.	.	.	L− D−	.	0/2M+
14	.	.	L+ F+†	.	L− D− 0/5M+	.
18	0/2M+	.	.	.	.	.
20	3/4M+	.	.	.	.	.
21	.	L+	.	.	.	.
23	.	.	.	L− D− 0/5M+	.	.
32	L+ D+ F− 2/3M+ 6/10C+	.	0/2M+	.	.	.
35	.	0/4M+	.	.	.	.
46	.	.	.	.	L+ 1/5CS+ D+	.
47	1/3M+	.	.	.	.	.
53	.	.	.	D− 0/5CS+ L+	.	.
66	10/15C+ 11/35CS+	.	.	.	.	.
69	9/15C+ 4/35CS+	.	.	.	.	D−
Pro.*	24/90VS+	40/92VS+	17/90VS+	20/90VS+	21/80VS+	0/89VS+

C = chickens; CS = cloacal swabs; D = dust; F = feed; L = litter; M = dead chickens; VS = vent swabs.

Flock no. I received 0·04% furazolidone for 8 days at 8 days of age, and the other flocks received 0·02% furazolidone for 4 days. All flocks received 0·04% furazolidone for 4 days, 8 days before processing.

* Pro. = processed chickens. † Unidentified salmonella.

Possible spread of contamination from carcass to carcass. There were 122 positive results from 442 samples (flock VI omitted), i.e. about one in four positives. However, these were not randomly scattered, and several positives occurred in succession. There was one group of seven positives in succession, one of six, and one of five all from flock II during the latter part of the day. There was one group of five in succession, two groups of four, seven groups of three, and seven pairs together. The remaining 56 isolations were all singles. Thus twenty groups of between

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two and seven successive chickens made up over half of the total salmonella isolations. Not all of these chickens may have been positive when they entered the packing station.

Table 4. Salmonella typhimurium *contaminated carcasses after washing* (*no. of positive swabs/batch of* 10 *samples*)

	Flock no.						
Batch no.	I	II	III	VI	IV	V	Total
1	3	0	0	0	1	4	8
2	6	2	1	0	4	2	15
3	1	5	1	0	5	1	13
4	3	3	1	0	0	3	10
5	4	6	1	0	2	5	18
6	1	6	4	0	4	1	16
7	1	4	5	0	1	3	14
8	3	7	4	0	1	2	17
9	2	6	0	0*	2	—	10
10	—	1†	—	—	—	—	1
Total	24/90	40/92	17/90	0/89	20/90	21/80	122
%	26·7	43·5	18·9	0	22·2	26·2	

Combined total (omitting flock no. VI): 122/442 (27·6 %).

Batches of ten successive samples were taken at ½ hr. intervals (with a ½ hr. mid-day break with flocks nos. I, III and VI). The water in the first spin-chiller tank was changed at mid-day (except flock no. V).

* Nine samples. † Two samples.

Table 5. *Effect of washing* Salmonella typhimurium *contaminated carcasses in chlorinated water* (*no. of positive swabs*/10 *samples*)

	Flock no.				
	I	III	VI	IV	V
Before washing	10	10	0	9	10
After washing	3	4	0	4*	1

Vent swabs were taken from ten tagged carcasses after evisceration and before they entered the first spin-chiller. These carcasses were again swabbed after leaving the second spin-chiller.

* One swab was negative before washing and positive afterwards.

DISCUSSION

Experiments at the Houghton Poultry Research Station (Knivett & Tucker, 1971) have shown that 2 months after a challenge with *S. typhimurium*, half of the chickens still had infected alimentary tracts, irrespective of whether they had received furazolidone treatment or not. They also showed that the caeca were the most frequently infected portions of the alimentary tract (86 % of infected chickens), whereas liver and spleen were the most frequently infected tissues (about 25 %). Livers are of interest because they are often eaten without adequate cooking and may cause food poisoning.

They also showed that, except during the early stages of an infection, cloacal swabs were inefficient, and on average only 1 out of 5 infected chickens gave a positive cloacal swab and sometimes this proportion was as low as 1 in 12, or even 1 in 20. Brobst, Greenberg & Gezon (1958) swabbed 265 chickens entering a poultry-processing plant and later they swabbed the abdominal cavities of these chickens. Every cloacal swab was negative whilst 43 % of the cavity swabs were positive. Although persistent carriers may remain after a mild infection, it is difficult to discover which chickens remain carriers or how many might be present in the flock. The examination of litter samples indicated whether the flock was infected or not and this was later confirmed by tests on the processed chickens.

The infection spread from house to house, but did not reach the furthest one (no. VI), and no infected chickens were found in this flock. Thus some flocks may be heavily infected and others completely free.

The plant was thoroughly sterilized after each day's work and there was no carry over of infection from previous occasions. Test swabs taken from equipment before the day's work commenced all proved negative. Some workers (Galton *et al.* 1955; Glezen *et al.* 1966) allege that chickens were rarely infected when they entered a processing plant and a few contaminated chickens spread infection to many others. The possible extent of such cross-infection was shown in an interesting experiment by Stewart (1965), who deliberately infected a single bird with a tracer organism (a red pigmented *Serratia*) and followed the distribution of this organism to other birds and the operative's hands. The following 42 birds on the line were all heavily infected with the tracer organism and infection was still detectable on the 150th bird. The organisms were also detectable on the hands of all but one of the operatives on the eviscerating line. He estimated that perhaps one-third of contaminated birds had become infected during processing.

Washing in chlorinated water effectively reduced the number of contaminated chickens. Although almost all the freshly eviscerated chickens were contaminated, it was not possible to tell whether they had become contaminated during evisceration or whether they might have already been infected on the farm or both.

The farmer suffered a considerable economic less in house no. I through the abnormally high death-rate, poorer food conversion, increased cost of medication, as well as the loss of the condemned pullets. There would have been no indication that *S. typhimurium* was present in the other houses had not bacteriological samples been taken. The existing methods of combating salmonellosis in poultry have not been adequate.

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