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The virulence of salmonella strains for chickens: their excretion by infected chickens

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SUMMARY

Inoculated orally, 16 *Salmonella typhimurium* strains belonging to 12 phage types varied greatly in their ability to kill 1-day-old chickens; variation was noted even between strains of the same phage type. Fourteen strains belonging to 11 food poisoning serotypes other than *S. typhimurium* were practically non-lethal when examined in this manner. All of them were lethal by the intramuscular route but some were more so than others. Two were more lethal by this route than one of the *S. typhimurium* strains that was highly lethal when given orally. With age, chickens rapidly became resistant to fatal infection with the food poisoning strains; given orally, a *S. typhimurium* strain killed 79% of 1-day-old chickens but only 3% of 2-day-old chickens. Of 2 specific poultry pathogenic strains, one, of *S. gallinarum*, was lethal by oral inoculation to chickens of all ages but the other, of *S. pullorum*, was only lethal to very young ones.

Some salmonella strains, such as those of *S. infantis* and *S. menston*, were more efficient at infecting and colonizing the alimentary tract of chickens than were the more virulent *S. typhimurium* strains, the *S. gallinarum* and *S. pullorum* strains and a *S. cholerae-suis* strain.

INTRODUCTION

Recent studies in chickens on the effect of antibiotics on the faecal excretion of salmonella organisms (Smith & Tucker, 1975*a*, *b*, 1978) and on the effect of antibiotic resistance plasmids on virulence (Smith & Tucker, 1979) were performed with one strain of *Salmonella typhimurium* of phage type 14. Administered orally, it was highly lethal for chickens in their first day of life; chickens inoculated when 4 days old, however, remained well but they usually excreted the organisms in their faeces for several weeks afterwards. *Salmonella typhimurium* was used in these studies because it is the most common serotype that causes food poisoning in man. Because other serotypes that cause food poisoning infect poultry under natural conditions the behaviour of a selection of them in chickens was compared with that of the *S. typhimurium* phage type 14 strain; the results are reported in this paper. Included in this comparison was a strain of *Salmonella cholerae-suis*, a serotype not normally found in poultry, and one strain each of *Salmonella gallinarum* and *pullorum*, serotypes that are not involved in food poisoning but which are important natural pathogens of poultry. Also included were several different

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phage types of *S. typhimurium* to see how their behaviour compared with that of the phage type 14 strain.

MATERIALS AND METHODS

Salmonella strains

All were believed to be epidemiologically-unrelated; those belonging to the same serotype or phage type were distinguished from each other by being given strain numbers. The strain of *S. typhimurium* (1, 4, 5, 12: i: 1, 2) of phage type 14 that had been used in the earlier experiments, 8 strains of this serotype of phage type 1, 12a, 20 (strain 1), 40 (strain 1), 49 (strain 1 and 2), 104 (strain 1 and 2) and one strain each of *S. heidelberg* (1, 4, 5, 12: r: 1, 2), *S. agona* (4, 12: f, g, s: -), *S. montevideo* (6, 7: g, m, s: -), *S. menston* (6, 7: g, s, t: -), *S. oranienburg* (6, 7: m, t : -), *S. virchow* (6, 7: r: 1, 2), *S. gallinarum* (9, 12: -: -), *S. pullorum* (9, 12: -: -), *S. enteritidis*, strain 1 (1, 9, 12: g, m: -), *S. senftenberg* (1, 3, 19: g, s, t: -), and *S. anatum* (3, 10: e, h: 1, 6) had been isolated from poultry. Five strains of *S. typhimurium* of phage type 10, 20 (strain 2), 32, 40 (strain 2) and 56, three strains of *S. enteritidis* (strains 2, 3 and 4) and also one strain of *S. infantis* (6, 7: r: 1, 5) had been isolated from human beings. One strain of *S. typhimurium* of phage 56 was of bovine origin; the source of another of phage type 36 was unknown. The *S. cholerae-suis* strain (6, 7: c: 1, 5) had been isolated from a pig. Three of the salmonella strains possessed conjugative plasmids. Those of the *S. agona* strain determined tetracycline resistance and those of the *S. anatum* strain determined streptomycin and sulphonamide resistance; markers of the plasmids in the S. heidelberg strain were unidentifiable. In some experiments, the salmonella strains were used as spontaneous chromosomal mutants resistant to sodium nalidixate (*nal*^{r}). These had been obtained by inoculating cultures heavily onto plates of MacConkey's agar containing 20 μg/ml of sodium nalidixate. Cultures prepared from colonies that grew on the plates were purified by replating. All strains were maintained on Dorset's egg medium at 5 °C. For use, they were grown in 10 ml of nutrient broth (Oxoid, CM67) in a shaking water bath at 37 °C for 24 hr. These cultures contained approximately $1{\cdot}5 \times 10^9$ colony-forming organisms per ml.

Experimental animals

Chickens, whose sex was not determined, were from a salmonella-free Light Sussex flock maintained at this station. They were kept in groups on wire-mesh floors in identically constructed solid-walled pens in an animal house maintained at 21 °C; additional heating was provided, when necessary, by an infra-red lamp suspended above each pen. They were fed *ad libitum* on a diet of the following composition: wheat meal, 40%; maize meal, 40%; British white fish meal, 20% mineral and vitamin supplement, 0·25%.

Virulence tests

The virulence of orally administered salmonella strains was determined by placing 0·3 ml of a broth culture directly into the crops of groups of chickens by means of a Pasteur pipette – before they were given food or water in the case of

chickens in their first day of life. The virulence of intramuscularly-administered salmonella strains was assessed by inoculating groups of 13 chickens with 0·1 ml of falling ten-fold dilutions of broth cultures. Because batches of chickens, especially very young ones, may vary in their susceptibility to infection, each complete experiment was performed on the same batch. The livers of all dead chickens were examined bacteriologically to confirm that they had died of salmonella infection.

Tests for duration of faecal excretion and infectivity

For assessing duration of faecal excretion, groups of chickens were inoculated orally with 0·3 ml of a broth culture of one or other of the salmonella strains. At intervals afterwards, faecal swabs were taken from the cloacae of the chickens and inoculated in a uniformly reproducible manner onto half of the surface of plates of brilliant green agar (Oxoid CM263) containing 20 μg/ml of sodium nalidixate and 1 μg/ml of novobiocin; very few faecal bacteria grow on this medium and the colonies of those that do can easily be differentiated visually from those of salmonellas. After incubation at 37 °C for 24 h the amount of salmonella growth on the plates was recorded according to the following notation: + + + + = confluent; + + + = almost confluent; + + = partly confluent; + = numerous mainly discrete colonies; ± = numerous discrete colonies; 50 = approximately 50 colonies; 1 = one colony. After being used for inoculating the brilliant green agar plates, the swabs were incubated in selenite broth at 37 °C for 24 h and then subcultured on to brilliant green agar. Infectivity studies were performed by the same technique except that only five chickens in each group of 33 were experimentally infected. The faeces of all the chickens were examined at frequent intervals afterwards so that the rate at which the infection spread from these five to the remainder of the chickens in the same group could be assessed.

RESULTS

The mortality rate in groups of chickens inoculated with different salmonella strains

By the oral route

The results of inoculating groups of 1-day-old chickens orally with *nal*^{r} and *nal*^{s} forms of 12 salmonella strains of 11 serotypes that cause food poisoning and of one strain each of *S. gallinarum* and *S. pullorum* are summarized in Table 1; similar mortality rates were produced by both forms of each strain. None or very few deaths occurred in the groups inoculated with most of the food poisoning strains. The two *S. typhimurium* strains, though, were exceptional in that the phage type 36 strain produced a moderate mortality rate and the phage type 14 strain produced a high mortality rate. The most severe and acute disease occurred in the group inoculated with the *S. gallinarum* strain, all the chickens in this group having died by the fourth day after inoculation; the disease in the *S. pullorum* group was much milder. In an identically-planned experiment, the mortality rate in groups given strain 2, 3 or 4 of *S. enteritidis* or the *S. virchow* strain were 6, 6, 3 and 6%

Table 1. *The mortality rate in groups of* 33 *chickens inoculated orally when one day old with different salmonella strains*

Strain inoculated	% of chickens that had died by the following days after inoculation								
	3	4	5	6	7	8	9	10	15
S. typhimurium 14*	0	18	67	73	73	73	76	79	79 (77)
S. typhimurium 36*	3	9	9	9	15	15	21	21	21
S. enteritidis	0	0	0	0	3	3	3	6	6 (12)
S. senftenberg	3	6	6	6	6	6	6	6	6 (0)
S. agona	0	3	3	3	3	3	3	3	3 (0)
S. oranienburg	0	0	3	3	3	3	3	3	3 (0)
S. montevideo	0	0	0	0	3	3	3	3	3 (0)
S. heidelberg	0	0	0	0	0	0	0	0	0 (0)
S. infantis	0	0	0	0	0	0	0	0	0 (3)
S. menston	0	0	0	0	0	0	0	0	0 (3)
S. anatum	0	0	0	0	0	0	0	0	0 (0)
S. cholerae-suis	0	0	0	0	0	0	0	0	0 (6)
S. pullorum	0	0	0	0	24	36	55	55	55
S. gallinarum	67	100	100	100	100	100	100	100	100

* = Phage types 14 and 36.

The 14 strains were *nal*^{r} mutants; where applicable, the total mortality rates produced by their *nal*^{s} parent strains are shown in brackets.

Table 2. *The mortality rate in groups of* 30 *chickens inoculated orally when* 4 *or* 56 *days old with different salmonella strains*

Age of chickens (days)	Strain inoculated	% of chickens that had died by the following days after inoculation								
		4	5	6	7	8	9	10	11	15
4	*S. gallinarum*	0	17	60	83	86	90	93	93	93
	S. pullorum	0	0	0	3	3	3	3	3	3
	Other 12 strains in Table 1	0	0	0	0	0	0	0	0	0
56	*S. gallinarum*	0	0	0	0	17	43	53	77	77
	S. pullorum	0	0	0	0	0	0	0	0	0
	Other 12 strains in Table 1	0	0	0	0	0	0	0	0	0

respectively – no higher than that of *S. enteritidis* strain 1 in Table 1. No deaths occurred in groups of chickens inoculated when 4- or 56-days-old with the two *S. typhimurium* strains or with any of the other food poisoning strains (Table 2). A high mortality rate occurred in both age groups inoculated with the *S. gallinarum* strain, but not with the *S. pullorum* strain, the disease being severest in the 4 day-old group. The phage type 14 *S. typhimurium* strain was also tested in groups of 40 2-day-old and 3-day-old chickens; only one chicken, a member of the 2-day-old group, died.

No difference in mortality rate was noted between groups of 80 1-day-old chickens inoculated with the phage type 14 strain either before or after being given food or

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Table 3. *The mortality rate in groups of* 33 *chickens inoculated orally when one day old with strains of Salmonella typhimurium of different phage type*

Phage type of strain inoculated	% of chickens that had died by the following days after inoculation										
	3	4	5	6	7	8	9	10	12	14	15
14	3	22	59	78	81	84	84	84	88	97	97
40, strain 1	0	3	53	69	88	91	94	94	94	97	97
40, strain 2	0	0	16	16	19	28	31	44	47	50	53
49, strain 1	6	13	25	28	28	31	31	34	47	53	53
49, strain 2	3	6	6	6	6	6	9	9	9	19	19
104, strain 1	0	3	3	25	25	25	31	38	44	47	47
104, strain 2	3	6	13	13	13	13	16	25	38	41	44
20, strain 1	0	3	3	6	9	9	13	16	22	22	22
20, strain 2	6	13	16	16	16	16	16	16	19	19	19
44	3	6	19	31	34	38	41	44	44	50	56
12a	6	13	13	13	25	25	31	34	41	41	41
56	6	13	25	28	31	31	34	34	38	38	38
1	3	3	3	9	9	9	13	16	22	22	22
10	0	0	0	0	0	0	0	0	3	6	6
32	3	3	3	3	3	3	3	3	3	3	3

All the strains were *nal*^{s}; the phage type 14 strain is the one featured in Table 1.

between another two groups of this size, one of which had been fed normally and one which had not been given food until after they were inoculated when 2 days old.

The mortality rates observed in groups of 1-day-old chickens inoculated with 15 strains of *S. typhimurium* belonging to one or other of 11 different phage types varied from 97 % in the groups given the phage type 14 strain or a phage type 40 strain to 6 % in the group given a phage type 10 strain (Table 3). Considerable differences were found between groups given different strains of the same phage type, for example phage types 40 and 49 but not phage types 104 and 20.

By the intramuscular route

The results of inoculating groups of 1-day-old and 4-day-old chickens intramuscularly with 10-fold falling doses of the 14 salmonella strains featured in Table 1 is summarized in Table 4. All strains were lethal for these chickens but some were more so than others. Some, like the *S. agona* strain which had produced a much lower mortality rate than the phage type 14 *S. typhimurium* strain when given by the oral route, were lethal in numbers similar to or lower than those of phase type 14 *S. typhimurium* when given intramuscularly. All the strains were much less virulent for 4-day-old than for 1-day-old chickens. This was least noticeable in the case of the *S. gallinarum* strain which was the most virulent of all the strains tested.

The faecal excretion of different salmonella strains by chickens

The faeces of groups of chickens were examined at different times after they had been inoculated orally when 4 days old with 13 of the 14 salmonella strains

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Table 4. *The mortality rate in groups of* 13 *one-day-old and four day old chickens inoculated intramuscularly with different-sized doses of different salmonella strains*

Strain inoculated	% of chickens that died after being inoculated with the following number of organisms							
	10^8	10^7	10^6	10^5	10^4	10^3	10^2	10^1
S. typhimurium 14	100 (100)	92 (85)	86 (31)	69 (0)	46	38	0	0
S. typhimurium 36	100 (100)	100 (23)	100 (0)	100	92	69	31	8
S. enteritidis	100 (85)	92 (8)	46 (0)	15	8	0	...	...
S. senftenberg	100 (54)	85 (15)	0 (0)	0	...	...	...	...
S. agona	100 (92)	100 (77)	100 (8)	85 (0)	77	69	31	23
S. oranienburg	100 (69)	92 (0)	31	0	0	...	...	...
S. montevideo	100 (62)	100 (0)	54	15	8	0	...	...
S. heidelberg	100 (100)	100 (15)	100 (8)	69 (0)	31	0	0	0
S. infantis	100 (100)	100 (8)	69 (0)	38	23	0	...	...
S. menston	100 (77)	100 (8)	77 (0)	23	0	0	0	...
S. antum	100 (77)	77 (8)	0 (0)	0	0	...	...	...
S. cholerae-suis	100 (15)	62 (0)	23	15	0	...	...	...
S. gallinarum	...	...	...	...		100 (100)	90 (50)	31 (10)
S. pullorum	85 (77)	77 (8)	61 (0)	8	...	...	...	...

The figures in brackets are the results for the 4-day-old chickens. ... no observation; for other abbreviations and details see Table 1.

Table 5. *The isolation of organisms of different strains of salmonella from the faeces of groups of* 21 *chickens that had been inoculated orally with them when* 4 *days old*

Time* (days)	% of chickens from which salmonella organisms were isolated following the oral inoculation with										
	S. typhimurium † of phage type 14			*S. infantis*			*S. pullorum*			*S. cholerae-suis*	
	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D
3	24	67	81	71	100	100	0	7	7	0	0
8	24	62	81	76	100	100	4	7	11	0	0
15	0	48	67	52	100	100	4	48	52	0	0
22	14	52	67	10	52	67	0	11	11	0	0
29	14	29	48	0	43	52	0	7	7	0	0
36	0	24	29	5	5	24	0	0	4	0	0
43	0	5	14	0	19	29	0	0	0	0	0
50	0	0	0	0	5	14	0	0	0	0	0
56	0	0	0	0	5	5	0	0	0	0	0

* After the chickens were inoculated with 5×10^8 viable salmonella organisms.

† > 50 = > 50 salmonella colonies grew on the culture plate; D = salmonellas isolated by direct culture; T = salmonellas isolated by selenite enrichment or by direct culture.

featured in Table 1; *S. gallinarum* was not included in this experiment because of its high lethality. The results for the chickens inoculated with the phage type 14 *S. typhimurium* strain and the *S. infantis*, *S. pullorum* and *S. cholerae-suis* strains are summarized in Table 5. In general, the results for the phage type 36 *S. typhi-*

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Table 6. *The isolation of organisms of different strains of salmonella from the faeces of groups of* 30 *chickens that had been inoculated orally with them when* 56 *days old*

Time (days)	% of chickens from which salmonella organisms were isolated following oral inoculation with: *S. typhimurium* of phage type 14 > 50	D	T	*S. infantis* > 50	D	T	*S. cholerae-suis* > 50	D
3	27	77	80	40	80	100	0	27
8	3	60	77	23	67	67	0	0
15	7	17	33	23	47	63	0	0
22	3	10	10	20	73	83	0	0
29	0	7	7	23	57	57	0	0
36	0	7	10	10	24	27	0	0
43	3	3	7	24	52	55	0	0
50	0	7	7	10	38	45	0	0
56	0	3	7	10	31	34	0	0
71	0	3	3	17	28	28	0	0

For details and abbreviations see Table 5.

murium strain and the *S. enteritidis, S. senftenberg, S. oranienburg, S .montevideo* and *S. anatum* strains resembled those for the phage type 14 *S. typhimurium* strain in that most of the chickens excreted organisms of these strains for about 29 days whereas the results for *S. agona, S. menston* and *S. heidelberg* resembled *S. infantis* in that they produced a heavier initial intestinal infection and were excreted for a rather longer period of time. *S. cholerae-suis* was not found by direct culture in the faecal specimens from any of the chickens that had been inoculated with it; these specimens were not cultured in selenite broth because of its unsuitability for isolating organisms of this serotype (Smith, 1952). The excretion pattern of *S. pullorum* was different from that of all the other salmonella strains in that it was found much less commonly in the early period after inoculation than it was later. Similar results were obtained when this strain was re-examined.

The above experiment was repeated with all except the *S. pullorum* strain in groups of thirty 56 day-old chickens; the results for three of the strains are summarized in Table 6. The infections that developed in the chickens inoculated with the *S. typhimurium, S. enteritidis, S. senftenberg, S. oranienburg, S. montevideo* and *S. anatum* strains were lighter than those that had developed in the corresponding groups of 4-day-old chickens and they persisted for shorter periods of time, salmonella organisms being no longer detectable in the faeces of most of the chickens at 15 days after inoculation and subsequently. Compared with the 4-day-old chickens the groups inoculated with the *S. infantis, S. heidelberg* and *S. menston* strains developed a much heavier infection and they excreted the organisms for longer periods of time; about half the chickens in each of these 3 groups were still excreting salmonella organisms in their faeces 41 days after infection. The pattern of excretion by the *S. agona* group was intermediate between that of these 3 groups and the other groups; salmonella organisms were found in the faeces of

Table 7. *The isolation of salmonella organisms from the faeces of groups of* 28 *chickens in contact with five chickens experimentally infected with different salmonella strains*

	% of 28 chickens whose faeces had become infected with organisms of this strain by the following days after exposure								
Salmonella strain	3	7	9	15	22	29	36	43	50
S. typhimurium 14	4	4	10	11	11	16	19	20	20
S. typhimurium 36	8	23	32	38	43	46	52	54	54
S. agona	9	18	22	30	34	38	43	47	47
S. anatum	3	15	23	24	32	33	34	35	37
S. enteritidis strain 1	0	6	9	14	16	18	19	19	19
S. heidelberg	1	5	9	14	16	18	22	23	24
S. infantis	6	29	33	41	44	47	51	52	52
S. menston	1	20	41	58	65	67	67	70	70
S. montevideo	8	15	18	22	25	25	25	25	25
S. oranienburg	8	15	28	29	52	54	56	58	60
S. senftenberg	3	10	16	19	22	22	24	24	24
S. cholerae-suis	0	0	0	0	0	0	0	0	0
S. pullorum	0	0	0	0	0	0	0	0	0
S. gallinarum	0	0	0	0	0	0	0	0	0

The 5 chickens were infected orally when they were 4 days old and in contact with the 28 non-infected chickens.

The results for *S. pullorum* are those for one such experiment; those for each of the other strains are compiled from three separate experiments.

some of the chickens 3 days after they had been inoculated with *S. choleraesuis* but not at any of the subsequent examinations. The experiment was repeated with the phage type 14 *S. typhimurium* strain and the *S. infantis* strain. On this occasion, the infections that developed, especially in the *S. infantis* group, were lighter and of shorter duration than those that had developed in the previous experiment – only about half the chickens in each group were found to have become infected and at 20 days after inoculation only a few were still excreting salmonella organisms.

The difference between the phage type 14 strain and the *S. infantis* and *S. menston* strains noted in most of the experiments described above was also apparent in an experiment in which groups of fifty 500-day-old hens were inoculated with these strains. Most of the hens in each group became infected but whereas less than half of the phage type 14 group were still excreting salmonella organisms in their faeces at 15 days after inoculation, it was about 40 days before this position was reached in the case of the *S. infantis* and *S. menston* groups.

The spread of organisms of different salmonella strains from experimentally-infected chickens to chickens with which they were in contact

The spread of organisms of the 14 different salmonella strains featured in Table 1 amongst groups of 28 chickens kept in the same pen from hatching time as 5 chickens inoculated orally with one or other of these strains when 4 days old is illustrated in Table 7. Each of 13 of the strains were used in three separate experiments; *S. pullorum* was only used in one. No spread of infection was detected

in any of the experiments with *S. cholerae-suis*, *S. gallinarum* and *S. pullorum* but it was detected in all the experiments with the other 11 strains. Most of the infections that had been acquired were no longer detectable at the fiftieth day. Although variation was occasionally noted between the behaviour of the same strain in different experiments, it was clear that some strains, such as *S. menston*, were able to spread more extensively than others, such as the phage type 14 *S. typhimurium* strain.

The above experiment was repeated once using chickens that were 63 days old instead of 4 days old. Again, no spread of *S. choleraesuis*, *S. gallinarum* or *S. pullorum* were detected and differences in degree of spread was noted between the other 11 strains, the differences, in general, resembling those found in the 4-day-old chickens. The percentage of chickens that became infected in the groups exposed to infection with those 11 strains was, phage type 14 *S. typhimurium*, 16%; phage type 36 *S. typhimurium*, 32%; *S. agona*, 48%; *S. anatum*, 44%; *S. enteritidis*, 28%, *S. heidelberg*, 40%; *S, infantis*, 60%; *S. menston*, 68%; *S. montevideo*, 16%; *S. oranienburg*, 40% and *S. senftenberg*, 12%.

DISCUSSION

The results of the present study emphasize the difficulties of generalizing on the *in vivo* behaviour of salmonella strains. It was not unexpected that the *S. pullorum* and *S. gallinarum* strains, the two specific poultry pathogens, would differ from the food poisoning strains. Clinical evidence, too, is in keeping with the observation that the *S. typhimurium* strains, as a group, were more lethal for 1-day-old chickens by the oral route than were the other food poisoning strains. What was less expected was the difference in lethality not only between different phage types of *S. typhimurium* but between strains of the same phage type indicating that virulence is not even phage type-determined but strain-determined.

Although all the salmonella strains examined were lethal when given by the intramuscular route to 1 and 4-day-old chickens, their virulence varied considerably from strain to strain and it did not always reflect that of the orally-administered organisms. The *S. agona* strain, for example, judged to be almost completely avirulent for 1-day-old chickens by oral inoculation, was more virulent for similarly aged chickens by intra-muscular inoculation than the highly 'orally virulent' phage type 14 *S. typhimurium* strain. This infers that the reason for the great difference in lethality between these 2 strains administered orally is that the phage type 14 strain possesses an additional property possessed not at all or in low degree by the *S. agona* strain – the property of invasiveness.

The susceptibility of chickens to lethal infection with the food poisoning strains, especially when orally administered, was markedly age-dependent and did not appear to be associated with the functional state of the alimentary tract because chickens starved until they were inoculated when 2 days old were as resistant to lethal infection with orally-administered phage type 14 *S. typhimurium* organisms as were 2-day-old chickens that had been fed normally. The results of the infectivity and faecal excretion studies, though, were probably to some extent, a reflexion of

the differing ability of the different strains to tolerate the activity of bacterial competitors and/or antagonists in the alimentary tract. Virulence did not appear to be involved because, for example, the highly virulent phage type 14 *S. typhimurium* strain was less efficient at surviving in the alimentary tract and spreading from chicken to chicken than the lowly virulent *S. infantis* and *S. menston* strains. The variation occasionally found in these studies between the results for the same strain in different experiments, too, might have been the consequence of differences in the bacterial flora of the chickens used in these experiments. Unlike the other salmonella strains, the swine pathogen, *S. cholerae-suis* and the two specific poultry pathogens, *S. pullorum* and *S. gallinarum*, were not found to spread from one chicken to another under the conditions of our infectivity experiments. Neither were they excreted in large amounts or for long periods of time by infected chickens. In both experiments in which groups of chickens were inoculated orally with the *S. pullorum* strain, the infecting organisms were only isolated from the faeces of a few of the chickens in the immediate post-infection period and from a greater proportion later. This was completely different from the behaviour of the food poisoning types and suggests that *S. pullorum* is unable to colonize the alimentary tract, its presence in the faeces being the consequence of tissue infection. This has already been shown to be so for *S. gallinarum* (Smith, 1955).

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