Res. Microbiol.
1991, 142, 541-549

The activity in the chicken alimentary tract of bacteriophages lytic for *Salmonella typhimurium*

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SUMMARY

Bacteriophages lytic for *Salmonella typhimurium* were isolated in considerable numbers from chickens experimentally infected with *S. typhimurium*, and in much lower numbers from the chicken feed. Lytic phages were also regularly isolated from human sewerage systems. One of these was used to inoculate *S. typhimurium* – infected two day-old chickens orally and *via* the feed. The phage took longer to establish in the caeca than did the *Salmonella* and it disappeared when the caecal *S. typhimurium* counts fell to 10^6 CFU/ml. No neutralizing antibodies to the phage were detected in the serum of these chickens. In a second experiment, five of 30 chickens similarly infected with *S. typhimurium* were inoculated with the phage. Within 3 days, the phage was isolated from 72 % of the "in-contact" birds. A second phage, isolated from sewage, when inoculated into newly-hatched chickens simultaneously with any of 3 strains of *S. typhimurium*, produced a considerable reduction in mortality in the birds. This effect was only produced by inoculation of high concentrations of phage ($> 10^{10}$ PFU/ml). The phage produced reductions in the viable numbers of *S. typhimurium* in the crop, small intestine and caeca for up to 12 h after inoculation, with smaller reductions in bacterial numbers in the liver at 24 and 48 h after infection.

Key-words: Phage therapy, *Salmonella typhimurium*, Bacteriophage, Alimentation; Poultry, Gut, Foodpoisoning.

INTRODUCTION

Poultry are an important source of *Salmonella* serotypes associated with cases of human food-poisoning in which a source of infection can be found. In addition to disease-free intestinal carriage of *Salmonella* serotypes by chickens, infection of chicks within a few hours of hatching with a variety of serotypes can result in considerable morbidity and mortality depending on the strain involved (Williams, 1972; O'Brien, 1988). *S. typhimurium* is one of the serotypes most frequently isolated from poultry and, as has been demonstrated experimentally (Smith and Tucker, 1980) can be particularly virulent for young chicks. This high virulence is explained by the relative immunological immaturity displayed by macrophages in young chicks (Karthigasu *et al.*, 1965), but also by the absence of a complex intestinal microflora

Submitted November 13, 1990, accepted January 31, 1991.

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(Coloe *et al.*, 1984) which allows massive bacterial multiplication from small inocula. Reducing the numbers of *Salmonella* in the gut might reduce not only the associated risk of food-poisoning, but also mortality in the chickens.

Despite a continued interest in biological measures, such as competitive exclusion and vaccination, for controlling *Salmonella* infections in poultry (Barrow, 1989), there is little evidence that research in these areas has produced any lasting reduction in the level of infection in commercial flocks. Consequently, novel methods of control are being sought, one of them being the use of virulent bacteriophages (Report, 1988). The potential for the use of virulent bacteriophages in the treatment of enteric diseases was recognized early in the twentieth century (Wilson and Miles, 1975). Virulent phages have been used recently to treat successfully experimental *Escherichia coli* infections in mice (Smith and Huggins, 1983), and in calves, pigs and lambs (Smith and Huggins, 1983; Smith *et al.*, 1987). There is also some recent evidence that this approach might be used to treat *Pseudomonas aeruginosa* and suppurative bacterial infections (Slopek *et al.*, 1983; Soothill *et al.*, 1988). The aim of the present study is to assess, in a preliminary way, whether bacteriophages may by used to reduce the effects of salmonellosis in chickens. They might provide the poultry industry with a means for *Salmonella* control.

Bacteriophages lytic for *S. typhimurium* can occasionally be isolated from the alimentary tract of chickens infected experimentally with this organism, being detected as individual plaques on areas of confluent bacterial growth (Barrow *et al.*, 1987b). Some of these originate from the chicken feed (Barrow *et al.*, 1987b). Because it was initially unclear whether phages would multiply on a propagating strain in the chicken alimentary tract, the activity of phage in the chicken gut was studied, both to clarify this point and to investigate the response of the bacterial infection to the phage activity. The results are presented here. Since phages lytic for a variety of *Salmonella* serotypes can be isolated from human sewage (Barrow, 1986) a preliminary investigation into the efficacy of such phages in reducing the mortality produced by strains of *S. typhimurium* in newly hatched chickens was also carried out.

MATERIALS AND METHODS

Bacterial strains

S. typhimurium strains F98 (phage type 14), Beauville (phage type 40) and 1116 (phage type 141) were isolated from chickens and were highly virulent for newly hatched chicks under experimental conditions (Smith and Tucker, 1986; Barrow *et al.*, 1987a). In several experiments, F98 was used as a nalidixic acid-resistant mutant; this was as virulent as the antibiotic-sensitive parent strain (Smith and Tucker, 1980). Unless otherwise indicated, broth cultures were made in 10 ml nutrient broth (Oxoid, Basingstoke, United Kingdom, CM67) incubated at 37°C for 24 h in a shaking water bath (100 strokes per min). These usually contained approximately 10^9 CFU/ml (colony-forming unit).

Isolation and propagation of phages

Bacteriophages lytic for *S. typhimurium* were isolated from raw human sewage by an adaption of the methods of Smith and Huggins (1982) and Barrow (1986). Samples of sewage in 5-l volumes were mixed with 75 g of nutrient broth powder, and 1 ml of a broth culture of F98 was added. After static incubation overnight at 37°C, 10 ml were withdrawn and centrifuged at 1500 *g* for 30 min. The supernatant was decanted and heated at 58°C for 30 min and one drop spot-inoculated onto the surface of a plate of nutrient agar that had previously been inoculated with a lawn of *S. typhimurium* F98. Any lysis in the area inoculated was picked and mixed with a small volume of broth culture of F98 on a plate of nutrient agar and the plate incubated until discrete plaques were visible. Single plaques of different morphological types were picked and replated with F98 to plaque-purify each type. Purified plaques were picked with a small amount of the surrounding bacterial growth and cultured overnight in nutrient broth. These were centrifuged and the supernatants heat-treated at 58°C for 30 min before being stored at 4°C.

Phage was propagated in one of two ways to produce either a low titre or a high titre lysate: low titre lysates were prepared from the supernatant described above. To this was added 0.2 ml of a broth culture; the mixture was reincubated at 37°C in a shaking water bath until further lysis had occurred. This procedure was repeated and the final preparation was heat-treated and the number of lytic phage particles counted. High titre lysates were prepared

from 10^{10} host bacterial cells from a broth culture mixed together with 10^8 phage particles from a low titre lysate at 37°C for 30 min with intermittent shaking. This mixture was added to 100 ml of nutrient broth and shaken overnight at 37°C. Additional lysis was promoted by the addition of 2 ml of chloroform followed by further shaking at 37°C for 30 min. After cooling to room temperature, NaCl was added to a concentration of 1 M and dissolved and the preparation centrifuged at 11000 *g* for 10 min at 4°C. Polythene glycol 6000 was added to the supernatant to a concentration of 10 %. After leaving for 1 h at 0°C, the preparation was again centrifuged at 11000 *g*, the pellet resuspended in 2 ml of nutrient broth and a phage count was made. Both types of phage preparation were held at 4°C until used.

Experimental animals

Unsexed Rhode Island Red chickens was obtained from a specified pathogen-free flock at this laboratory. Their management and diet have been described previously (Smith and Tucker, 1975). All chickens were kept on wire floors.

Experimental plan

A group of fifty two-day old chickens were inoculated orally with 0.1 ml of a broth culture of *S. typhimurium* F98 Nal^r followed by 0.1 ml of a dilution of a low titre preparation containing 10^5 PFU/ml (plaque-forming unit) of a phage designated ΦAB2 which was isolated from sewage. The chickens were reared on a diet containing 10^3 PFU/g of ΦAB2 for the first week followed by normal food for the rest of the experiment. The diet containing phage was prepared by grinding the required dilution of phage plus a small amount of food with a pestle and mortar prior to mixing with a large quantity of food. At intervals after infection, groups of five chickens were killed and counts of F98 Nal^r and phage were made from the caecal contents.

In a second experiment, a group of thirty two-day old chickens was inoculated orally with 0.1 ml of F98 Nal^r, but only five of the birds were inoculated with phage, this time with 0.1 ml of a lysate containing 10^{10} PFU/ml. At weekly intervals, two cloacal swabs were taken from each of the 25 chickens not inoculated with phage. The swabs were used for semi-quantitative assessment of the numbers of F98 Nal^r and phage excreted in the faeces. At 34 days post-infection, the chickens were reinfected with 0.3 ml of a broth culture of F98 Nal^r.

Several phages isolated at different times from different sewage treatment plants were tested for their ability to reduce mortality from salmonellosis. Groups of 30-32 chickens, less than 24-h old, were inoculated orally with 10^6 F98 organisms in 0.1 ml followed within 10 min by 0.1 ml of a high titre lysate preparation of the phages containing 10^{12} PFU/ml. The mortality in these and in a control group of chickens inoculated with F98 only was recorded over a three-week period. One phage (Φ2.2) which produced the greatest reduction in mortality was tested in the same type of experiment using two different *S. typhimurium* strains.

In a subsequent experiment, Φ2.2 was tested for its ability to reduce mortality induced by F98 by inoculation with different dilutions of phage preparation.

In a final experiment, two groups of twenty chickens less than 24-h old were inoculated orally with 10^6 F98 Nal^r organisms in 0.1 ml and one group was inoculated with Φ2.2 as described above. At intervals after inoculation, groups of five chickens from both groups were killed. The outsides of the carcasses were soaked in industrial alcohol and organs removed aseptically in the order: liver, small intestine, crop and caeca. Viable counts of F98 Nal^r were made on a homogenate of the liver and on the contents of the gut samples.

Enumeration of bacteria and phages

Quantitative assessments of F98 Nal^r and phage in samples were made with a modification of the method of Miles, Misra and Irwin (1938) using nutrient broth as a diluent. F98 Nal^r was counted on brilliant green agar (Oxoid, CM263) containing 20 μg/ml of sodium nalidixate and 1 μg/ml of novobiocin. Dilutions of the samples for phage counts were heated to 58°C for 30 min prior to plating onto lawns of F98 Nal^r on tryptose agar (Difco) containing 20 μg/ml of sodium nalidixate.

Semi-quantitative assessments of the numbers of *Salmonella* organisms excreted were made using the method of Smith and Tucker (1980). In brief, individual swabs were eluted in 2 ml of selenite broth (Oxoid, CM395) before being plated out in a standard manner onto the selective brilliant green agar described above. The swabs were then incubated overnight in the selenite broth before being replated. This has been found to give reproducible results (Smith and Tucker, 1980). An adaption of this method was used to estimate the numbers of phage excreted. A second swab taken from each chicken was eluted into 2 ml of nutrient broth. This was heated at 58°C for 30 min and a 0.03-ml aliquot was plated onto a lawn of F98 Nal^r. The same volume of a 10^3 dilution of a broth culture of F98 was then added to the swab eluate and reincubated overnight to increase any small numbers of phage not detectable

by initial counting. This enriched culture was again heated before replating.

RESULTS

Infectivity and persistence of phage in the alimentary tract

Bacteriophages lytic for *S. typhimurium* were isolated from all of 20 sewage samples examined over several months. One of these, ΦAB2, was assessed for its persistence in the alimentary tract of *S. typhimurium* F98 Nal^r-infected chickens. The counts of F98 Nal^r and ΦAB2 made from caecal contents at various times after simultaneous inoculation of two-day old chickens are shown in table I. High viable counts of F98 Nal^r were initially found in the caecal contents and these decreased with time, although detectable numbers were still present when the experiment was terminated 32 days after inoculation. The presence of phage did not produce any dramatic reductions in the numbers of F98 Nal^r. High phage counts were not found in every chicken soon after infection although the median count was very high by 4 days post-infection, indicating that phage multiplication had taken place. The phage counts fell more quickly than those of the *Salmonella* until none was detected when the $\log_{10}$ median F98 count fell below 6.1.

At 2, 4, 7 and 10 days post-infection, 50 colonies of F98 Nal^r were picked from the brilliant green agar plates. Lawns were made from this growth and loopfuls of phage ΦAB2 was spotted onto them to ascertain whether F98 Nal^r remained predominantly sensitive to phage or whether resistance developed with time. The number of phage-resistant colonies detected on the above days post-infection was 17, 27, 41 and 21, respectively. All of these agglutinated with acriflavine, indicating that they were rough and had not become resistant by lysogeny.

The chickens killed at 32 days post-infection were bled and their serum compared with that from uninfected adult chickens and from uninfected day-old chickens for anti-phage antibodies. The sera were titrated and mixed with a dilution of phage, 0.03 ml of which would just produce confluent lysis. The phase-serum dilution mixtures were incubated at 37°C for 1 h and were plated onto a lawn of F98 Nal^r. None of the sera reduced the numbers of phage detected.

The spread of phage infection from inoculated chickens to "in-contact" chickens inoculated with F98 Nal^r only is shown in table II. The incidence of infection with F98 Nal^r remained high for several weeks and was boosted by reinfection at 34 days. Within a few days of being placed in the same pen as five chickens ex-

Table I. Counts of *S. typhimurium* F98 Nal^r and bacteriophage ΦAB2 in the caecal contents of experimentally infected chickens.

Time (days) after inoculation[1]	$\log_{10}$ numbers of organisms or phage particles per g of caecal contents	
	S. typhimurium F98 Nal^r	ΦAB2
2	8.1 (6.9-8.6)[2]	N (N-8.2)
4	8.8 (7.8-8.8)	9.4 (N-9.9)
7	7.4 (6.1-7.6)	7.3 (3.1-8.7)
10	6.9 (6.7-8.4)	6.6 (N-6.9)
11	7.2 (6.7-8.3)	4.0 (4.0-8.4)
13	6.1 (4.3-7.0)	N (N-7.3)
18	3.0 (2.5-4.0)	N (N)
25	2.5 (2.0-4.7)	N (N)
32	2.8 (N-4.7)	N (N)

[1]Two-day-old chickens inoculated orally simultaneously with *S. typhimurium* F98 Nal^r (10^8 CFU in 0.1 ml) and ΦAB2 (10^5 PFU in 0.1 ml). Birds fed for 7 days on food containing 10^5 PFU/g.

[2]Median value from 5 chickens with range in parenthesis.

N = < 2.0.

Table II. Faecal excretion of *S. typhimurium* F98 Nal^r and phage ΦAB by chickens inoculated orally with the *Salmonella* and placed in contact with chickens inoculated with the *Salmonella* and phage[1].

Time (days) after inoculation of phage	The percentage[2] of "in-contact" chickens excreting: *S. typhimurium* F98 Nal^r			ΦAB		
	50[3]	D[3]	T[3]	C[4]	D[4]	T[4]
3	56	92	100	28	72	72
6	52	80	92	4	32	48
10	60	96	100	4	44	44
13	48	80	100	0	20	26
20	12	60	96	0	20	15
27	20	52	84	0	0	7
34[5]	12	36	56	0	0	2
35	32	76	100	0	0	4
36	24	80	96	0	0	4
37	24	60	96	0	0	0

[1]Two-day old chickens (group of 30) inoculated orally with *S. typhimurium* F98 Nal^r (10^5 CFU in 0.1 ml). Five of these inoculated orally simultaneously with ΦAB (10^{10} PFU in 0.1 ml).

[2]The percentage values are for the 25 "in-contact" chickens.

[3]$\geqslant 50$ = $\geqslant 50$ colonies of F98 Nal^r grew on the culture plate; D = F98 Nal^r isolated by direct culture; T = F98 Nal^r isolated by selenite enrichment or by direct culture.

[4]C = confluent lysis obtained from 0.03 ml drop on a lawn of F98; D = $\geqslant 1$ plaque obtained on a lawn; T = ΦAB isolated by enrichment with F98 broth culture or by direct culture on a lawn.

[5]All chickens reinoculated orally with 3×10^8 CFU of F98 Nal^r in 0.3 ml.

creting phage ΦAB2, a very high percentage of the "in-contact" chickens were excreting phage in their faeces. This decreased with time and at a faster rate than the reduction in frequency of isolation of F98 Nal^r. Reinoculation of all the chickens with F98 had little effect on the isolation of the phage.

In both of these experiments, no phage was isolated from the chicken feed and the phage isolated from the swabs or caecal contents produced plaques identical to those produced by preparations of ΦAB2, namely 1.5 mm in diameter with an uneven edge, a small number of colonies of secondary growth and no halo.

The effect of phages on mortality produced in young chickens by *S. typhimurium*

The effect of nine phages isolated from different sewage samples on the mortality produced in newly-hatched chicks by *S. typhimurium* F98 is shown in table III. In the absence of phage, F98 produced 53% mortality. Several of the phages produced no effect on mortality, while a smaller number produced statistically significant reductions. One of the phages, Φ2.2, also produced considerable reductions in the mortality produced by two other virulent strains of *S. typhimurium* (table III).

It was apparent that preparations containing high numbers ($> 10^{10}$ PFU/ml) of phage particles were required to produce statistically significant reductions in mortality (table IV). Dilutions of phage containing this number or less were ineffective.

The effect of phage Φ2.2 on the viable numbers of *S. typhimurium* in the alimentary tract and liver of the chicken

Phage Φ2.2 produced reductions in the viable numbers of F98 Nal^r in the gut of chickens compared with those not given the phage (table V). Reductions of more than one $\log_{10}$ were seen in the crop and small intestine soon after infection and throughout the alimentary tract at

Table III. The mortality rate in chickens inoculated orally when one-day-old with three different *S. typhimurium* strains followed by different bacteriophages.

S. typhimurium strain (phage type)	No. of chicks in group	Treated with phage	Phage used	% of chickens that had died by the following days after inoculation																				χ^2 when compared with appropriate control	*P*
				4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
F98 (Nal^r (14)	31	–	–	6	6	16	26	29	29	35	35	39	45	45	45	48	53	53	53	53	53	53	53	53	–	
	32	+	Φ4.2	0	13	19	25	28	31	34	38	41	50	53	53	53	53	53	53	56	56	56	56	56	ND	
	32	+	Φ5.1	6	6	9	19	22	31	31	41	41	44	44	47	50	50	50	53	53	53	53	53	53	ND	
	32	+	Φ3.1	0	0	9	22	25	38	38	47	47	47	53	53	53	53	53	53	53	53	53	53	53	ND	
	31	+	ΦAB4	0	10	16	19	23	23	23	26	32	35	39	39	39	39	42	42	42	45	45	45	48	0.07	0.8
	32	+	Φ4.1	0	9	13	16	19	25	28	34	38	38	38	38	44	44	44	44	44	44	44	47	47	0.13	0.75
	32	+	Φ4.3	0	0	6	6	6	9	13	16	19	19	22	22	25	28	31	31	31	34	34	34	34	1.9	0.15
	32	+	Φ1.1	3	6	6	22	22	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	4	0.04
	32	+	ΦAB1	0	6	9	9	13	13	13	19	22	22	22	22	22	22	22	22	22	22	22	22	22	6.0	0.02
	32	+	Φ2.2	0	0	0	0	6	6	6	9	9	9	9	9	9	16	16	16	16	16	16	16	16	9.3	< 0.01
Beauville (40)	30	–	–	0	7	13	17	23	23	27	27	33	33	33	33	33	37	37	37	37	40	40	40	40	–	
	30	+	Φ2.2	0	0	0	0	3	7	7	7	10	10	10	10	10	13	13	17	17	17	17	17	17	4.0	0.04
1116 (40)	30	–	–	0	7	17	27	27	30	40	43	53	57	57	57	57	57	57	57	60	60	60	60	60	–	
	30	+	Φ2.2	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	24.3	< 0.01

– = not appropriate. ND = not done.

Chicks less than 24-h old inoculated orally with 10^6 *Salmonella* organisms in 0.1 ml followed within 10 min by 0.1 ml of phage preparation.

Table IV. The mortality rate in chickens inoculated orally when one-day old with *S. typhimurium* strain F98 followed by different dilutions of bacteriophage Φ2.2.

Dilution tested	% of chickens that had died by the following days after inoculation																			χ^2 when compared with phage-free control	*P*
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
No phage	0	0	12	16	20	28	36	40	44	48	48	48	48	48	52	56	56	56	56	–	–
10^{-4}	0	0	8	8	8	20	24	24	24	28	32	32	32	32	32	44	44	44	44	0.72	0.4
10^{-2}	0	0	4	4	12	12	12	16	20	28	32	32	32	32	36	36	36	36	36	2.01	0.15
10^{0} (neat)	0	0	0	4	4	4	4	4	8	12	16	16	16	20	20	20	20	20	20	6.88	<0.01

Groups of 25 chicks less than 24-h old were inoculated orally with 10^8 *Salmonella* organisms in 0.1 ml followed within 10 min by 0.1 ml of phage preparation. The neat phage preparation contained 10^{12} PFU/ml.

Table V. Viable numbers of *S. typhimurium* F98 Nal^r in the alimentary tract and liver of chickens treated or not-treated with phage Φ2.2.

Time (h) after inoculation	Treated with phage	Log_{10} median[1] viable counts of *S. typhimurium* F98 Nal^r per g of sample from the following organs: crop	small intestine	caecum
1/2	+	3.8	3.0	3.7	N
	–	6.2	4.4	3.4	N
3	+	4.8	3.4	4.3	N
	–	5.9	4.5	6.6	N
6	+	5.3	3.3	5.6	N
	–	5.6	3.5	8.0	N
24	+	6.5	4.3	8.7	2.0
	–	5.7	2.0	9.1	2.9

[1]Median of five birds.
N = < 2.0.
Chicks less than 24-h old inoculated orally with 10^6 F98 Nal^r organisms in 0.1 ml followed within 10 min by phage Φ2.2.

3 days post-inoculation. Some of the reductions in the crop and caeca were greater than two logs. These changes had disappeared by 24 h post-inoculation. However, at this time, a small reduction in the *Salmonella* numbers recovered from the liver was seen in the phage-treated chickens.

DISCUSSION

It was clear from the first experiment that the phage used (ΦAB2) multiplied in the alimentary tract, resulting in high numbers of active phage particles in the caeca. Whether this was the result of multiplication in the caeca or in more proximal regions of the alimentary tract is unclear. It was surprising that phage counts were initially low in several of the chickens and the phage was not found in some of the birds thereafter. This is not easy to explain, but was related to the number of *S. typhimurium* organisms present in the individual birds (data not presented). It may suggest that in these birds, the phage infection had arisen in a sporadic way and probably primarily from the infected feed rather than from the single oral inoculation of phage which should have infected every bird.

The *S. typhimurium* organisms persisted longer than the phage in both experiments. It also appeared that a caecal viable count of F98 of less than approximately 10^6 CFU/ml was insufficient to sustain multiplication of this particular phage. The low counts and frequencies of isolation in the later stages of the experiments suggested that environmental contamination did not affect the numbers present in the caeca or cloaca and that on wire flooring, environmental re-infection did not occur even when the chickens were re-inoculated with bacteria. Although phage AB2 multiplied in the gut, it did not affect the number of *Salmonella* organisms present in the caeca. It also did not select for an increase the number of phage-resistant organisms; those that were present were rough rather than lysogenic. No detectable anti-phage antibodies were present in serum. This suggests that, although *S. typhimurium* is invasive for young chicks (Barrow *et al.*, 1987a), phage was not taken into the tissues during the process of invasion. This might be an advantage should phage therapy against intestinal salmonellosis be feasible, since the absence of a buildup of antibodies would be an advantage.

A relatively small number of phages were examined in order to find one which reduced mortality caused by *S. typhimurium*. It was therefore not surprising that we did not find one which would eliminate mortality completely. More effective phages might be found from a more extensive search. The best phage (Φ2.2) was effective against two other strains of *S. typhimurium*, but appeared to be effective only when large numbers of phage were administered and soon after infection with the *Salmonella*. Since the phage initially outnumbered the bacteria, it is quite likely that in this case, the reductions in bacterial numbers observed in the gut were the result of a single infection cycle, and phage multiplication of Φ2.2 was not involved. It is also possible that non-specific lysis, whereby many phages attach to single bacterial cells and cause cytoplasmic leakage which results in lysis, was involved. The numbers of *S. typhimurium* were reduced in the crop and more particularly the small intestine and caeca, where *Salmonella* invasion mainly occurs (Barrow, unpublished results). Although the phage might be expected to be less effective than would occur with the confluent layer of enterotoxigenic *E. coli* strains that colonize the small intestinal mucosa (Jones and Rutter, 1972; Smith and Huggins, 1983), the numbers of *Salmonella* were reduced sufficiently to limit invasion, with the result that smaller numbers of *S. typhimurium* were isolated from the liver. Although the reduction in the median count in this organ was less than 10-fold, the lethal dose 50% of F98 for such chicks by a parenteral route is approximately 10^3 organisms (Barrow *et al.*, 1987a), so that reductions in numbers from ($\log_{10}$) 2.9 to 2.0 might be significant. Although, in the short time of this experiment, the emergence of rough bacterial mutants was not observed, these should by their nature be of greatly reduced virulence.

A wider search might reveal more effective phages which might be used to reduce the numbers of *Salmonella* organisms present in the gut immediately prior to slaughter. Alternatively, phages might conceivably be adapted for greater efficiency in the gut. The phages isolated were not tested for activity on other *Salmonella* serotypes. It would be interesting to know their range of activity.

Acknowledgments

The authors would like to thank Mr. B. Wells and Miss V. Peters for assistance in different ways.

Activité de bactériophages lytiques pour *Salmonella typhimurium* dans le tube digestif du poulet

Des bactériophages lytiques pour *Salmonella typhimurium* ont été isolés en quantités considérables de poulets infectés par *S. typhimurium* et, en quantités moindres, de nourriture pour poulet. Des bactériophages lytiques ont été aussi isolés régulièrement d'eaux usées.

Nous avons inoculé un de ces isolats oralement ou *via* la nourriture à des poulets de 2 jours infectés par *S. typhimurium*. Le phage met plus de temps que *Salmonella* pour s'établir dans le cæcum, et il disparaît quand les numérations de *S. typhimurium* chutent à 10^6 UFC/ml (unité formatrice de colonie).

Aucun anticorps neutralisant antiphage n'a été détecté dans le sérum de ces poulets. Dans une seconde expérience, sur 30 poulets infectés par *S. typhimurium*, 5 ont reçu le phage; celui-ci a été isolé, dans les 3 jours suivants, de 72% des oiseaux «en contact».

Un second phage isolé d'eaux usées inoculé simultanément avec *S. typhimurium* à des poussins venant d'éclore, entraîne une réduction considérable de la mortalité chez les oiseaux. Cet effet se produit seulement si la concentration de phage inoculé est élevée ($> 10^{10}$ unités formatrices de plage/ml). Le phage réduit le nombre de *S. typhimurium* viables dans le jabot, l'intestin grêle et le cæcum de façon maximale jusqu'à la 12e heure après l'infection puis de façon moins importante, dans le foie, jusqu'à 24 h à 48 h après l'infection.

Mots-clés: Phagothérapie, *Salmonella typhimurium*, Bactériophage, Alimentation; Volaille, Intestin, Toxi-infection.

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