

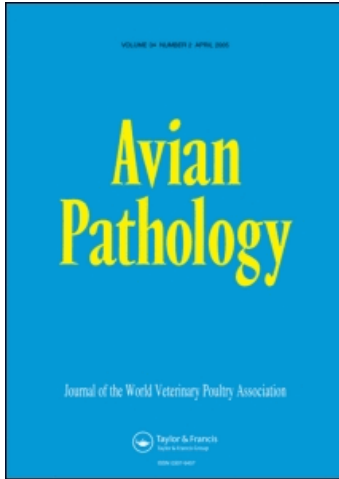
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Oral treatment with bacteriophages reduces the concentration of *Salmonella* Enteritidis PT4 in caecal contents of broilers

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Bacteriophages isolated from free-range chickens were tested as a therapeutic agent for reducing the concentration of *Salmonella enterica* serovar Enteritidis phage type 4 (*S. Enteritidis* PT4) in caeca of broilers. One-day-old broilers infected with *S. Enteritidis* PT4 by a seeder bird method were orally treated on the seventh day of age with a mixture of 10^{11} plaque-forming units of each of three bacteriophages. Five days after treatment the bacteriophage-treated group showed a reduction of 3.5 orders of magnitude on colony-forming units of *S. Enteritidis* PT4 per gram of caecal content. Samples collected at 10, 15, 20 and 25 days after treatment revealed that treated birds still had lower colony-forming units of *S. Enteritidis* PT4 per gram of caecal content. These data gave us compelling evidence that a mixture of bacteriophages may be efficacious in reducing *S. Enteritidis* PT4 concentration in broilers' caeca and therefore reducing contamination of poultry products by this food-borne pathogen.

Introduction

Concerns related to drug-resistant bacteria have stimulated interest in alternative treatments of bacterial infections (Cohen, 1994). Among these therapies, a special interest has been given to phage therapy, the use of bacteriophages to kill or otherwise control the bacterial population in infected hosts (Lorch, 1999; Sulakvelidze *et al.*, 2001; Joerger, 2003).

Early studies by Smith and Huggins with *Escherichia coli* (Smith & Huggins, 1982, 1983; Smith *et al.*, 1987) demonstrated that phage therapy can be as efficient as antibiotics. After these breakthrough studies had been presented, several other publications reported success of experimental phage therapy with different bacteria, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Soothill, 1992), *Vibrio vulnificus* (Cervený *et al.*, 2002), *Enterococcus faecium* (Biswas *et al.*, 2002), and *Pseudomonas aeruginosa* (Soothill, 1994); and even *Escherichia coli* in other animals such as calves (Barrow *et al.*, 1998) and chickens (Huff *et al.*, 2002a,b).

Major risks factors for food poisoning caused by salmonellae are related to poultry, such as eating raw or undercooked eggs (Molbak & Neiman, 2002). Food poisoning caused by salmonellae in man results in reduction of productivity, discomfort, expenditure on medication and sometimes death (Persson & Jendteg, 1992; Mead *et al.*, 1999). Contamination of frozen carcasses of broilers with salmonellae can reach rates as high as 53.5% (Santos *et al.*, 2000). Among the most important salmonellae associated with chicken meat and eggs is *Salmonella enterica* serovar Enteritidis

(*S. Enteritidis*) (Chung *et al.*, 2003). This is a murine serotype that has entered the intensive poultry industry and can cause infection and contamination of poultry products in the absence of a clinical disease (Gast & Beard, 1990). *S. Enteritidis* phage type 4 (*S. Enteritidis* PT4) is the salmonella of higher frequency in broilers in Brazil (dos Santos *et al.*, 2003; Fernandes *et al.*, 2003; Nunes *et al.*, 2003) and one of the most frequently isolated salmonella in other poultry-producing countries as well (Capita *et al.*, 2003; Cogan & Humphrey, 2003; Esaki *et al.*, 2004).

Control of salmonellae at the pre-harvest stage is of paramount importance. It can prevent the introduction of this bacterium into the food chain and consequently reduce food poisoning among consumers (Wegener *et al.*, 2003). Control of salmonellae at farm level is more likely to be effective through a multi-factorial approach. Good agricultural practices based on hazard analysis and critical control points (Rose *et al.*, 2002; Nayak *et al.*, 2003), vaccination (Zhang-Barber *et al.*, 1999; Yamame *et al.*, 2000; Cogan & Humphrey, 2003), probiotics, prebiotics and synbiotics (Van Immerseel *et al.*, 2002) have all been used as preventive measures when salmonella infection is likely to occur in broilers.

However, the use of antimicrobial drugs is often still required for reducing shedding and intestinal carriage of salmonellae, especially when preventive measures have not been sufficiently efficient (Davies *et al.*, 2003). In such cases, new concerns are born due to selection of resistant bacteria (Velonakis *et al.*, 2001; Molbak *et al.*, 2002; Chung *et al.*, 2003; Malorny *et al.*, 2003;

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Busani *et al.*, 2004), the possibility that residues of antimicrobial drugs may contaminate chicken meat and eggs consumed by man (Donoghue, 2003), and finally because antibiotic therapy alone will be unlikely to clear salmonellae from infected chickens (Fernandez *et al.*, 2001).

Alternatives to antimicrobial drugs are required for the control of salmonellae within poultry production. Besides reducing the use of antibiotics, these methods could also be used in conjunction with good agricultural practices in the multi-factorial approach for improvements on salmonella's control at a pre-harvest level. Bactericidal bacteriophages may provide a natural, non-toxic, feasible and non-expensive component for the pre-harvest control of salmonellae in poultry. Some preliminary work has indicated that salmonellae can be controlled by the use of bacteriophages (Barrow *et al.*, 1987; Berchieri *et al.*, 1991; Sklar & Joerger, 2001), although additional work has to be done in order to achieve more optimistic results.

In the study reported here we infected 1-day-old broilers with *S. Enteritidis* PT4 and orally treated them with a mixture of three bacteriophages isolated from faeces of free-range chickens.

Materials and Methods

Salmonella. *S. Enteritidis* PT4 isolate P125589, used throughout this study, was kindly provided by Dr Paul Barrow, from the Institute for Animal Health, England (Barrow & Lovell, 1991). This isolate was originally obtained from the Central Public Health Laboratory, London, UK following a case of human food poisoning. *S. Enteritidis* PT4 was grown on nutrient broth (NB) (Oxoid; 1 g/l beef extract, 2 g/l yeast extract, 5 g/l peptone, 5 g/l sodium chloride) or nutrient agar (NA) (NB was added at 15 g/l bacteriologic agar) at 37°C and kept frozen at -80°C unless stated. When used for bacteriophage isolation, *S. Enteritidis* PT4 was grown on NB or NA with added 5 mM MgSO₄ (NB-MgSO₄ or NA-MgSO₄). Inocula doses were measured by conventional plate counting on Brilliant Green Agar (BGA) (10 g/l proteose peptone, 3 g/l yeast extract, 10 g/l lactose, 10 g/l sucrose, 5 g/l sodium chloride, 0.08 g/l phenol red, 0.0125 g/l brilliant green, 12 g/l bacteriologic agar, pH 6.9). An antibiotic-resistant strain of salmonella was not necessary due to the high number of colony-forming units (CFU) present in the caecal contents, which allowed one to enumerate *S. Enteritidis* PT4 in dilutions rid of contaminants.

Bacteriophages. Bacteriophages lytic to *S. Enteritidis* PT4, denominated CNPSA1, CNPSA3 and CNPSA4, were isolated from free-range layers in Brazil and were morphologically characterized as described elsewhere (Fiorentin *et al.*, 2004). When stocks of equivalent titres were tested in overlay cultures to lyse *S. Enteritidis* PT4, these bacteriophages showed very similar performance and were chosen to be used as a combination to avoid selection of resistant *S. Enteritidis* PT4. Bacteriophage stocks were kept at -80°C in SM buffer with 7% dimethyl sulfoxide and 1% chloroform for long-term use. When used for treating birds, frozen stocks of bacteriophages were expanded by inoculation into 20 ml *S. Enteritidis* PT4 cultures in exponential growth in NB-MgSO₄, followed by incubation for approximately 18 h at 37°C with shaking (200 × g), treated with 5% chloroform to lyse all bacteria, centrifuged (12 000 × g for 5 min) and stored at 4°C as a supernatant with 1% chloroform. Inocula were titrated for plaque-forming units per millilitre (PFU/ml) in agar overlay cultures of *S. Enteritidis* PT4 as described by Kudva *et al.* (1999). Agar overlays were prepared by inoculating 10 µl of 10-fold dilutions of bacteriophage stocks in SM buffer (5.8 g/l NaCl, 2 g/l MgSO₄-7H₂O, 0.05 M Tris-HCl, pH 7.5, 0.1 g/l gelatin) into 250 µl *S. Enteritidis* PT4 culture in the exponential phase of growth, which was incubated for 20 min at 37°C and poured into 7 ml melted NB (45°C) containing 0.7% agarose. The melted agarose containing bacteria and diluted bacteriophage was then laid over a 10 cm diameter sterile NA-MgSO₄ Petri dish to solidify and

consequently be incubated for 24 h at 37°C. Plaques were identified as clear spots of lyse measuring approximately 1 mm in diameter.

Experimental infection with *S. Enteritidis* PT4. Three groups of 1-day-old chicks of the broiler-type Embrapa 021 breed were separately housed in air-filtered wire-mesh floor isolator cabinets, with freely available drug-free feed and water for 32 days (Table 1). Cloacal swabs collected from all birds as well as 10 g feed were negative for both salmonellae and bacteriophages lytic to *S. Enteritidis* PT4 on the first day of the experiment.

Infection with *S. Enteritidis* PT4 was performed under a seeder bird method as to simulate natural conditions. On the first day of life, five birds were marked with metal rings in their wings and orally inoculated with 100 µl fresh *S. Enteritidis* PT4 culture (10⁸ CFU per bird), while 30 birds were kept for infection by contact. On the third day post-infection (p.i.) all birds inoculated with the *S. Enteritidis* PT4 culture were eliminated and cloacal swabs were taken from the remaining birds to test for *S. Enteritidis* PT4 shedding.

Treatment with bacteriophages. On the seventh day of life (7 days p.i. of seeder birds), five birds of each group were necropsied to confirm the salmonella-free status of controls and to obtain frequencies of liver and spleen positive for *S. Enteritidis* PT4 as well as CFU *S. Enteritidis* PT4 per gram of caecal content (CFU/g) before treatment. All remaining birds from the treated group (Group 3) orally received 10¹¹ PFU of each of the three bacteriophages denominated CNPSA1, CNPSA3 and CNPSA4 diluted together in 300 µl SM buffer. Bacteriophages were administered as crude lysates of *S. Enteritidis* PT4 stored at 4°C and diluted 10 or 100 times in SM buffer to reach the necessary oral dose.

Addressing the effect of the treatment. Five birds of each group were necropsied at 5-day intervals, corresponding to days 0, 5, 10, 15, 20 and 25 post-treatment (p.t.) with bacteriophages or to 12, 15, 17, 22, 27 and 32 days of life, the equivalent to days p.i. of seeder birds. Fragments of about 5 mm from the liver, spleen and caeca (including the tonsils) were inoculated in 3 ml Rappaport-Vassiliadis Soya Peptone broth (Oxoid; 4.5 g/l soya peptone, 7.2 g sodium chloride, 1.26 g/l potassium dihydrogen phosphate, 0.18 g/l di-potassium hydrogen phosphate, 13.58 g/l magnesium chloride anhydrous, 0.036 g/l malachite green, pH 5.2) and incubated overnight at 42°C. Loopfuls were then transferred to BGA and incubated at 37°C for an additional 48 h to identify colonies by morphology and serum agglutination with polyvalent anti-somatic serum (Probac). Caecal contents were weight, diluted 10-fold in phosphate-buffered saline (pH 7.4) and aliquots of 100 µl inoculated onto BGA plates and incubated at 37°C for 24 h to obtain the CFU/g.

Fragments of the liver, spleen and caeca were also subjected to attempts of bacteriophage isolation by incubation in a culture of *S. Enteritidis* PT4. Fragments of tissues of approximately 5 mm were incubated overnight at 37°C with 2 ml *S. Enteritidis* PT4 at the exponential phase of growth on NB-MgSO₄. The culture was then made to 5% chloroform, vortexed, centrifuged at 12 000 × g for 5 min and 5 µl supernatant inoculated over a fresh lawn of *S. Enteritidis* PT4 grown on NB-MgSO₄ solidified with 0.7% agarose. The presence of bacteriophages was identified by transparent spots of the bacterial lawn on sample sites 24 h after incubation at 37°C. Quantitative analysis of bacteriophages on caeca was addressed by 10-fold dilutions of their

Table 1. Experimental design

Group	Number of birds	Treatment
1	30	Uninfected and non-treated control
2	30	<i>S. Enteritidis</i> PT4 infected ^a
3	30	<i>S. Enteritidis</i> PT4 infected ^a and treated with bacteriophages ^b

^aInfected by contact with five seeder birds from the first to the third day of age.

^b10¹¹ PFU of each of three bacteriophages orally administered at the seventh day of life.

contents in SM buffer followed by inoculation of 10 µl each dilution into 250 µl *S. Enteritidis* PT4 in the exponential phase of growth. This mixture was then incubated for 20 min at 37°C and poured into melted NB-MgSO₄ with added 0.7% agarose for preparing overlay cultures as already described. Cultures were incubated overnight at 37°C to obtain the PFU per gram (PFU/g).

Statistical analysis. Frequencies of *S. Enteritidis* PT4 isolation from the spleen, liver and caecal fragments of Group 2 and Group 3 were compared using Fisher's exact test. Means of CFU/g obtained from caecal contents of birds from Group 2 and Group 3 were subjected to variance analysis and compared with the Student *t* test weighted by the inverse of the mean standard errors. Analyses were performed using the SAS-GLM procedure (SAS Institute Inc., 2001).

Results

S. Enteritidis PT4 infection. Cloacal swabs collected from contact birds on the third day of life allowed us to isolate *S. Enteritidis* PT4 from 28/30 and 30/30 birds in Group 2 and Group 3, respectively. In the face of the apparently different levels of infection detected we treated Group 3 with bacteriophages, keeping the group with lower level of *S. Enteritidis* PT4 shedding as a control. Neither clinical sign nor mortality was observed in any group during the whole experiment. The control group remained both salmonella-free and bacteriophage-free all through the experiment.

Concentration of bacteriophages in caecal contents.

Counting of total bacteriophages was addressed by direct isolation from caecal contents after bacterial lysis using 5% chloroform. Samples were diluted in SM buffer and aliquots mixed with *S. Enteritidis* PT4, which was then poured into melted NB-MgSO₄ containing 0.7% agarose and plated over NA-MgSO₄ to form overlay cultures. Because all three bacteriophages showed morphologically similar plaques we could not obtain individual counts. No bacteriophage was isolated from any sample of birds from Group 1 (uninfected and non-treated control) or Group 2 (*S. Enteritidis* PT4-infected and non-treated control). Bacteriophages were present in caecal contents collected at necropsies performed on Group 3 from 5 to 20 days after treatment (Table 2 and

Table 2. *Log*₁₀ bacteriophage counting on caecal contents of treated birds^a

Days post-inoculation ^b	Days post-treatment	Bacteriophages × 10 ⁴ PFU/g ^c
7	0	Not done
12	5	5.9
17	10	5.17
22	15	6.51
27	20	Positive ^d
32	25	Negative ^e

^aBirds from Group 1 (uninfected and non-treated) and Group 2 (infected and non-treated) were negatives for bacteriophages in all tests.

^bInoculation of seeder birds. Equals to days of life on contact birds.

^cAverage of five birds.

^dLower than the sensitivity of the method used for counting (10³ PFU/g) but still positive in a qualitative test.

^eIn both counting and qualitative tests.

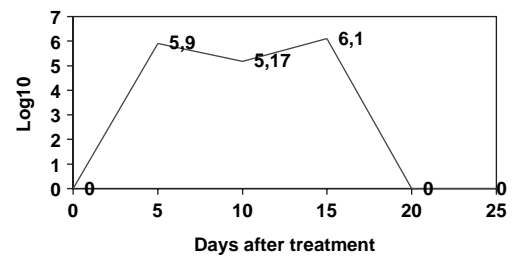


Figure 1. *Log*₁₀ PFU/g bacteriophages isolated from caecal contents of birds from Group 3. Data are the mean of five birds. On day 20 post-treatment all birds were positive in a qualitative assay but under the limit of detection for the technique used for enumeration. On day 25 all birds were negative in both qualitative and quantitative tests.

Figure 1). All samples of the liver and spleen from all three groups were negative for bacteriophages.

The effect of bacteriophages over *S. Enteritidis* PT4 concentration on caecal contents.

Group 3 had higher levels of infection by *S. Enteritidis* PT4 compared with Group 2, as addressed by the number of positive cloacal swabs after 3 days on contact with seeder birds as well as CFU/g caecal content at day 0 of bacteriophage treatment (Table 3). Five days after treatment, both groups had a similar mean of CFU/g, representing approximately a 3.5 times reduction (from 1682 × 10⁷ to 486 × 10⁷ CFU/g) of CFU evidently caused by the bacteriophages. Caecal contents collected at days 10, 15, 20 and 25 p.t. showed higher *P* values for differences between means between means in a comparison done with the Student *t* test (Table 3), thus not being statistically significant. However, samples collected 20 days p.t. showed a difference with the marginal *P* value of 0.06, thus indicating a tendency for the treated group to have lower levels of contamination on an extended period of time.

Invasion of *S. Enteritidis* PT4. We isolated *S. Enteritidis* PT4 from the liver (Table 4) and spleen (Table 5) in both treated and infected control groups from 5 days p.i. until the end of the experiment. Although *P* values were high there was a tendency for a lesser number of positive birds among the treated group in some instances, like the frequency of positive spleens observed on day 15 p.i. The high frequency of isolation of *S. Enteritidis* PT4 from caecal tonsils (Table 6) was in accordance with the presence of the bacterium in caecal contents as

Table 3. *Log*₁₀ means of *S. Enteritidis* PT4 on caecal contents of birds from Group 2 and Group 3^a

Days post-inoculation ^b	Days post-treatment	Group 2	Group 3	<i>P</i> value
7	0	9.48	10.23	0.0019
12	5	9.63	9.69	0.8900
17	10	8.52	8.82	0.8000
22	15	9.98	9.58	0.2200
27	20	9.81	7.97	0.0620
32	25	8.64	6.34	0.5700

^aBirds from Group 1 (uninfected and non-treated) were negatives for salmonellae in all tests. ^bInoculation of seeder birds. Equals to days of life on contact birds.

Table 4. Frequency of *S. Enteritidis* PT4-positive livers per group

Days post-inoculation	Days post-treatment	Group 2	Group 3	P value ^a
7	0	5/5	5/5	1.00
12	5	5/5	5/5	1.00
17	10	5/5	2/5	0.39
22	15	4/5	2/5	0.23
27	20	1/5	1/5	0.56
32	25	0/5	2/5	0.22

^aFisher's exact test.

verified by the quantitative test used for counting CFU/g (Table 3).

Discussion

The aim of this study was to see whether bacteriophages, originally isolated from free-range chickens, could be of any help in reducing the concentration of *S. Enteritidis* PT4 in caeca of experimentally infected broilers. The results obtained by us showed that bacteriophages acted as a factor inducing reduction in CFU/g caecal content. Results of CFU/g also showed that bacteriophages possibly reduced the cross-contamination that occurred within the isolator cabinets, indicating that benefits from the treatment may extend beyond the direct action of the oral dose administered.

Under the conditions in which this experiment was conducted, an apparent heavy infection occurred. Three days after inoculation of seeder birds almost every contact bird was shedding *S. Enteritidis* PT4, thus indicating that bacteriophages would act as therapeutic agents not prophylactic agents. We also detected a high frequency of liver and spleen positive for *S. Enteritidis* PT4 throughout the experiment, which indicates a high frequency of systemic infection. According to experiments conducted by others, *S. Enteritidis* PT4 is highly invasive for 1-day-old chicks (Barrow & Lovell, 1991; Berchieri *et al.*, 2001; Roy *et al.*, 2001) and oral infection with low doses of *S. Enteritidis* PT4 would not cause a systemic infection (Duchet-Suchaux *et al.*, 1997), thus indicating that the seeder birds method used by us resulted in high infection dose for contact birds. In this context, it is understandable that bacteriophages did not clear *S. Enteritidis* PT4 from infected chicks. However, the 3.5 orders of magnitude reduction caused on CFU/g treated birds clearly indicates that bacteriophages had an

Table 5. Frequency of *S. Enteritidis* PT4-positive spleens per group

Days post-inoculation	Days post-treatment	Group 2	Group 3	P value ^a
7	0	5/5	5/5	1.00
12	5	5/5	5/5	1.00
17	10	5/5	2/5	1.00
22	15	5/5	2/5	0.08
27	20	4/5	4/5	0.56
32	25	3/5	1/5	0.23

^aFisher's exact test.**Table 6.** Frequency of *S. Enteritidis* PT4-positive caecal tonsils per group

Days post-inoculation	Days post-treatment	Group 2	Group 3	P value ^a
7	0	5/5	5/5	1.00
12	5	5/5	5/5	1.00
17	10	5/5	5/5	1.00
22	15	5/5	5/5	1.00
27	20	4/5	4/5	1.00
32	25	5/5	4/5	0.50

^aFisher's exact test.

'*in vivo*' effect towards reduction of *S. Enteritidis* PT4 in caecal contents. In a similar work conducted by Sklar & Joerger (2001) the reduction obtained on CFU/g by the means of bacteriophage was 1.3 orders of magnitude. The use of a single dose with a high PFU of bacteriophages was probably the reason why we obtained a better result, in contrast to the continuous administration of bacteriophages used in the feed by Sklar & Joerger (2001). Continuous administration of bacteriophages may lead to resistant salmonellae. We used different bacteriophages, and that can also account for some of the difference. However, reduction with several logs of magnitude will be necessary to make this methodology viable as a practical standpoint. This will probably be achieved with additional research.

The almost complete elimination of bacteriophage from faeces 15 days p.t. could indicate a selection for *S. Enteritidis* PT4 strains resistant to the bacteriophages. We did not test for this purpose in this experiment because in a previous work all *S. Enteritidis* PT4 isolates obtained from faeces as long as 15 days after inoculation of bacteriophages remained sensitive (Fiorentin *et al.*, 2004). The use of three bacteriophages was also chosen to reduce the possibility of selection for resistance against one specific bacteriophage. Clearing of bacteriophages from faeces is more likely to be related to poor multiplication inside the chicken alimentary tract due to less contact with salmonella cells and the continuous expelling caused by intestinal motility. The scope of this experiment was to study a single flock because continuous use of bacteriophage in the same site could select resistant strains of salmonella (Sklar & Joerger, 2001).

Samples collected at 5 and 10 days after treatment showed a drop in total counts of bacteriophages per gram, while at day 15 after treatment the results showed an increase in total bacteriophage counting (Table 2). This observation coincides with the increase on CFU/g *S. Enteritidis* PT4 in both Group 2 and Group 3 (Figure 1), probably representing multiplication of bacteriophages inside the alimentary tract of the bird due to the increased salmonella population originated from cross-contamination within the isolators. The technique used for bacteriophage counting had a dilution factor of 1000, thus leading to the conclusion that after 20 days of treatment the bacteriophages were present in the caecal content at levels lower than 10³ PFU/g. As an attempt to increase the sensitivity of our method, qualitative tests were performed inoculating 1 ml *S. Enteritidis* PT4 culture with fragments of caeca, followed by incubation at 37°C for 24 h to allow bacteriophages to multiply, and identifying their

presence by plating over a fresh lawn of *S. Enteritidis* PT4 grown in NB with 7% agarose. This technique would theoretically detect a single particle of bacteriophages and allowed us to isolate bacteriophages from caecal contents of birds collected at day 20 after treatment while on the 25th day all samples were confirmed as negative (Table 2). In previous work (Fiorentin *et al.*, 2004) we noticed that these bacteriophages did not multiply in the alimentary tract of salmonella-free birds, thus not using any bacteria from the physiologic flora as a target. Taken together, the results obtained from counting bacteriophages and CFU/g *S. Enteritidis* PT4 suggest that bacteriophages will best be effective in a short period after administration and only in birds with high CFU/g. The fact that bacteriophages disappear will help to avoid resistant salmonellae arising.

The concentration of *S. Enteritidis* PT4 in the caecal content naturally decreases a few weeks after inoculation of 1-day-old chicks (Desmidt *et al.*, 1997; Berchieri *et al.*, 2001). A peak in CFU/g we observed 15 days p.i. probably represents cross-contamination within the isolator cabinets. In this view, bacteriophages not only speeded up the natural drop in CFU/g, but also opposed cross-contamination once treated birds had a lower peak (Figure 2).

The reduction in CFU/g was more evident 5 days after treatment, when both control and treated groups showed similar means even though treated birds had higher initial counts at day 0. We are tempted to conclude that the high multiplicity of infection, the number of particles of bacteriophage per cell of salmonella, is necessary for dropping caecal CFU. When PFU/g dropped over time the difference between means of CFU/g was no longer statistically significant. Further studies with higher PFU per dose in several treatments spaced 5 days apart may result in one even more evident drop in CFU/g.

We could not calculate the multiplicity of infection because it is impossible to estimate how many particles of bacteriophages from the oral dose actually reached the caeca. However, this number was probably high at day 1 p.t. because we administered an individual dose with about 18 times more bacteriophages than the CFU/g *S. Enteritidis* PT4 identified in the caeca of treated birds. Goode *et al.* (2003) observed that *S. Enteritidis*

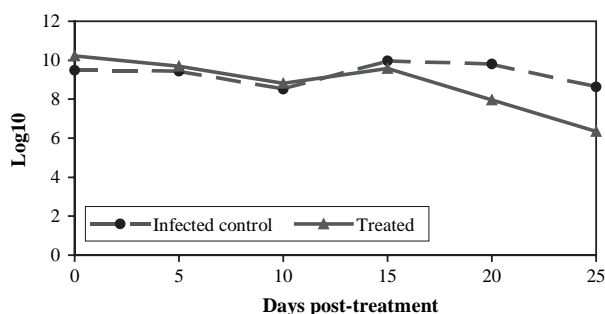


Figure 2. Log₁₀ CFU/g *S. Enteritidis* PT4 enumerated from caecal contents of birds from Group 2 and Group 3. Data are the mean of five birds as displayed in Table 3. On the x axis are days post-treatment, starting on day 7 post-infection by contact. Birds on Group 2 (dashed line) represent infected non-treated controls while birds of Group 3 were infected by contact and orally treated with bacteriophages. Uninfected and non-treated birds (Group 1) were negative for salmonellae in all tests. See Table 3 for P values for differences between means.

PT4 counting was reduced in experimentally contaminated chicken skin when the multiplicity of infection was as low as one, which has probably been achieved at the caecal level in our experiment.

This was our first attempt to control *S. Enteritidis* PT4 using these bacteriophages. New experiments with a higher number of birds, lower variability among birds and repeated treatments with the bacteriophages in a 5-day interval may show statistical differences beyond 5 days p.t. Overall, the results obtained with this experiment gave us an optimistic view over the possibilities of controlling *S. Enteritidis* PT4 by the use of bacteriophages once it confirms reduction of CFU/g at the caecal level.

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Translations of the abstract in French, German and Spanish are available on the *Avian Pathology* website.

Non-English Abstracts

Oral treatment with bacteriophages reduces the concentration of *Salmonella* Enteritidis PT4 in caecal contents of broilers

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Le traitement oral avec des bactériophages réduit la concentration de *Salmonella* Enteritidis PT4 dans les contenus cœcaux des poulets de chair

Les bactériophages isolés de poulets élevés en plein air ont été testés comme agent thérapeutique pour réduire la concentration de *Salmonella enterica* sérovar Enteritidis phage type 4 (*S. Enteritidis* PT4) dans les cæca de poulets de chair. Des poulets de chair, âgés d'un jour, infectés par l'intermédiaire d'animaux excréteurs de *S. Enteritidis* PT4, ont été traités oralement à l'âge de 7 jours avec un mélange de trois bactériophages tirant 10^{11} plages formant unité (PFU). Cinq jours après le traitement, le lot ayant reçu les bactériophages a présenté une réduction de l'ordre de 3,5 du nombre de colonies formant unité de *S. Enteritidis* PT4 par gramme de contenu cæcal (CFU/g). Les échantillons récoltés à 10, 15, 20 et 25 jours après le traitement ont révélé que les animaux traités avaient encore les plus faibles CFU/g. Ces données mettent en évidence que le mélange de bactériophages peut être efficace en réduisant la concentration de *S. Enteritidis* PT4 dans les cæca de poulets de chair et par conséquent réduire la contamination des produits avicoles par cet agent pathogène d'origine alimentaire.

Orale Behandlung mit Bakteriophagen reduziert die Konzentration von *Salmonella* Enteritidis PT4 im Zäkuminhalt von Broilern

Bakteriophagen aus im Auslauf gehaltenen Hühnern wurden als Therapeutikum zur Reduktion der Konzentration von *Salmonella enterica* serover Enteritidis Phagentyp 4 (*S. Enteritidis* PT4) im Zäkum von Broilern getestet. Boilerküken, die mittels eines Trägartieres mit *S. Enteritidis* PT4 am 1. Lebenstag infiziert worden waren, wurden am 7. Lebenstag mit einer Mischung von jeweils 10^{11} Plaque bildenden Einheiten (PFU) von drei Bakteriophagen oral behandelt. Fünf Tage nach der Behandlung zeigten die mit Bakteriophagen behandelten Gruppen eine Reduktion der Kolonie bildenden Einheiten von *S. Enteritidis* PT4 je Gramm Zäkalinhalt (CFU/g) in einer Größenordnung von 3,5. Proben, die 10, 15, 20 und 25 Tage nach der Behandlung gesammelt wurden, zeigten, dass die behandelten Tiere immer noch niedrigere CFU/g aufwiesen. Diese Daten gaben uns den zwingenden Beweis, dass eine Bakteriophagenmischung effektiv die *S. Enteritidis* PT4-Konzentration in Broilerzäka verringern und damit die Kontamination von Geflügelprodukten mit diesem durchs Futter übertragenen Erreger reduzieren kann.

El tratamiento oral con bacteriófagos reduce la concentración de *Salmonella* Enteritidis PT4 en contenido cecal de pollos de engorde

Se probaron bacteriófagos obtenidos de pollos en extensivo como agentes terapéuticos para reducir la concentración de *Salmonella enterica* serovar Enteritidis fagotipo 4 (*S. Enteritidis* PT4) en ciegos de pollos de engorde. Pollos de engorde infectados con *S. Enteritidis* PT4 mediante un método de ave semilla fueron tratados oralmente al 7º día de edad con una mezcla de 10^{11} unidades formadoras de placa (UFP) de cada uno de los tres bacteriófagos. Tras cinco días de tratamiento, el grupo tratado con los bacteriófagos mostró una reducción de 3.5 órdenes de magnitud de unidades formadoras de colonias de *S. Enteritidis* PT4 por gramo de contenido cecal (UFC/g). Las muestras recogidas a los 10, 15, 20 y 25 días tras el tratamiento revelaron que las aves tratadas aún tenían menores UFC/g. Estos datos evidencian que una mezcla de bacteriófagos pueden ser eficaces para reducir la concentración de *S. Enteritidis* en ciegos de pollos de engorde y, en consecuencia, para reducir la contaminación de productos derivados del pollo por parte de este patógeno alimentario.

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