

ISSN 1682-8356  
ansinet.org/ijps



INTERNATIONAL JOURNAL OF  
**POULTRY SCIENCE**

**ANSI***net*

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## Selection and Application of Bacteriophages for Treating *Salmonella enteritidis* Infections in Poultry

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**Abstract:** The objective of this study was to select appropriate bacteriophages that survive in the gastrointestinal tract of neonatal poultry and utilize those bacteriophages to reduce intestinal colonization of *Salmonella enteritidis* phage type 13A (SE) in challenged birds. Broiler chicks served as an *in vivo* biological filter to preferentially select bacteriophages from our bacteriophage library capable of surviving the gastrointestinal environment. A mixture of bacteriophage isolates designated PHL 1-71 was administered orally to three SE challenged chicks on three consecutive days. Each day, bacteriophages were recovered from the ileum, ileocecal junction and ceca for sequential administration the following day. The recovered bacteriophages were then administered to SE-infected turkey poults. In the first experiment, two-day old poults were challenged with  $10^4$  cfu SE and treated 48 h later with 5mM Mg (OH)<sub>2</sub> followed by  $2.5 \times 10^9$  plaque forming units (pfu) of bacteriophages in 1mM Mg (OH)<sub>2</sub> solution. This treatment numerically reduced SE recovered from cecal contents at 12 and 24 h after treatment as compared to untreated controls. In a second experiment, two-day old poults were challenged with  $1.6 \times 10^4$  cfu SE and treated with 5mM Mg (OH)<sub>2</sub> followed by  $7.5 \times 10^9$  pfu phage in 1mM Mg (OH)<sub>2</sub> solution 48 h post-challenge. We recovered 79,728 cfu of SE per g of cecal contents in the control group and 11,224 cfu/g in the phage treated group 24 h post treatment. These data were not significantly different, but they suggest that bacteriophages can be preferentially selected *in vivo* to increase survival in the avian gastrointestinal tract. However, improved efficacy is required prior to useful application of the approach for reducing *Salmonella* infection.

**Key words:** Bacteriophage, *Salmonella enteritidis*, selection, poultry

### Introduction

In the United States, it is estimated that 1.4 million humans contract salmonellosis and that the annual cost of this illness, including lost productivity, is \$3 billion annually (WHO, 2006). In the year 2004, surveillance data indicated that the greatest number of foodborne illnesses was caused by *Salmonella*, comprising 42% of all laboratory diagnoses (FoodNet, 2005). Because many of these illnesses are associated with poultry and poultry products (Bean and Griffin, 1990; Persson and Jendteg, 1992), the reduction of microbial contamination during the production of poultry is important. Currently we are exploring the use of bacteriophages as a method to control *Salmonella enteritidis* (SE) infections in poultry. We previously isolated bacteriophages that lyse SE (Higgins *et al.*, 2005) and here selected those that are most adapted to the gastrointestinal tract to potentially treat SE infected birds.

Poultry harboring *Salmonella* infections can be treated with antibiotics with some success (Goodnough and Johnson, 1991; Muirhead, 1994). However, Manning *et al.* (1992, 1994) reported increased *Salmonella* colonization when chickens are treated with selected antibiotics, possibly due to reduction of normal bacterial flora in the gastrointestinal tract that serve as a natural barrier to *Salmonella* infection. Additionally, Kobland

*et al.* (1987) and Gast *et al.* (1988) have recovered antibiotic resistant *Salmonella* from experimentally challenged birds treated with antibiotics. Recently, the United States Food and Drug Administration (FDA) has banned the use of enrofloxacin in poultry production because of the increase in resistant *Campylobacter* infections in humans (FDA, 2005). Thus, it is important that effective and inexpensive methods or products to treat bacterial infections in sick birds be developed.

One possible alternative to antibiotic treatment, is use of bacteriophages to eliminate or control bacterial infections. Bacteriophages are viruses that infect and replicate in prokaryotic cells rather than eukaryotic cells (Cann, 1993; Voyles, 1993). They are ubiquitous in the environment and readily isolated from water sources, sewage and soil. Bacteriophages are also host specific and usually can infect only one serotype within a bacterial species (Ackermann *et al.*, 1978).

Smith and Huggins (1983, 1987) have successfully used bacteriophages to eliminate *Escherichia coli* (*E. coli*) infections in calves, pigs and lambs. Bacteriophages have also been used with some success at eliminating poultry pathogens. Barrow *et al.* (1998) was able to prevent morbidity and mortality in chickens with bacteriophages lytic for *E. coli*. When chickens were challenged intramuscularly with *E. coli* and

simultaneously treated with  $10^6$ - $10^8$  pfu of bacteriophage the mortality was reduced by 100%. Berchieri *et al.* (1991) also demonstrated efficacy at reducing *Salmonella typhimurium* in chickens when the challenge and treatment were administered simultaneously. This experiment also clearly demonstrated that the bacteriophage increased in numbers as long as the host was present. Recently, Huff *et al.* (2002, 2003 and 2005) demonstrated efficacy in the use of bacteriophages to treat airsacculitis caused by *E. coli* in chickens. Marked efficacy was achieved when administering the phages with the bacterial challenge inoculum by injection in the thoracic air sac. Despite progress toward effective use of bacteriophages in poultry, there are no established methods for selecting specific bacteriophages that can readily amplify in the gastrointestinal tract of poultry. Additionally, there is a need to select efficacious bacteriophages that can lyse the pathogen even when administered after infection.

We hypothesized that effective bacteriophages against SE in turkey poult could be achieved by choosing bacteriophages that were better adapted to survive the gastrointestinal environment. As a selection tool, we used broiler chicks as a biological filter to select bacteriophages capable of surviving passage through the gastrointestinal tract. The selected bacteriophages were then evaluated *in vivo* in SE-challenged turkey poults to determine their effectiveness.

## Materials and Methods

**Bacteriophage isolation:** Wastewater samples were obtained from a local municipal wastewater treatment plant and filtered through a 0.2  $\mu$ m syringe filter<sup>2</sup>. A combination of 100  $\mu$ L of  $10^7$  cfu/mL SE and 1 mL of the wastewater sample filtrate was added to 1.5 mL of Tryptic Soy Agar<sup>3</sup> (TSA) and poured over a warm TSA petri plate. Plates were incubated overnight at 37 C and those with confluent lysis of SE were flooded with 15 mL of sterile 0.9% NaCl (saline). The fluid was then poured off the plate and filtered through a 0.2  $\mu$ m filter<sup>1</sup>. Serial ten-fold dilutions were made in saline. Thin agar overlay plates were poured as described above containing 1 mL of each bacteriophage dilution. Individual plaques resulting from this plating were differentiated on the basis of plaque morphology and selected by removing an agar plug. Each distinct plaque morphology was then sequentially passed on TSA plates three times to purify the isolate.

This technique yielded 71 bacteriophage isolates from four independent wastewater samples. Bacteriophage isolates were arbitrarily designated PHL 1-PHL 71 for identification purposes. While several different plaque morphologies were noted and isolates were obtained from four different wastewater samples, no attempts to

differentiate bacteriophages based on molecular or physiological characteristics have been made. Therefore, the 71 wild-type bacteriophage isolates utilized in these experiments may contain an unknown amount of redundancy.

**Bacteriophage preparation:** All bacteriophages were maintained on TSA soft agar plates as described above. Bacteriophages were resuspended in solution for use in experiments by obtaining a single agar plug of the desired isolate and mixing it in sterile saline. Briefly, a sterile Pasteur pipette was pushed into the center of a bacteriophage plaque resulting from lysis of SE. This bacteriophage plug was then resuspended in 1 mL of sterile 0.9% saline and filtered through a 0.2  $\mu$ m filter<sup>1</sup>.

**Amplification of bacteriophages:** All 71 bacteriophage isolates were obtained from each plate in an agar plug as above and amplified in Tryptic Soy Broth<sup>4</sup> (TSB) containing the mutagen ethidium bromide<sup>5</sup> (0.125  $\mu$ g/mL). Amplification of bacteriophages was accomplished by mixing the cell free bacteriophage suspension, a turbid SE culture (12h at 37°C) and fresh TSB combined at a ratio of 1:3:5 respectively and then incubated at 37°C for 1.5 h. This amplification procedure was repeated three times *in vitro*. The final titer of the bacteriophage mixture was approximately  $10^{10}$  pfu/mL as determined by soft overlay plating.

***In vivo* biological filter:** Ten, day-of-hatch Cobb 500 broiler chicks were orally challenged with  $10^4$  cfu of SE in a 0.25-mL volume of TSB. Chicks used in all experiments were cared for using procedures approved by the University of Arkansas Institutional Animal Care and Use Committee. The chicks were maintained on clean pine shavings in a floor pen at age-appropriate temperature with feed and water ad libitum. Immediately prior to bacteriophage administration (0.25 mL containing  $\sim 2.5 \times 10^9$  pfu), a feed passage marker, ferric oxide<sup>6</sup> (100 mg) was administered per os. When red feces were detected from an individual chick (1-3 h), that chick was humanely killed by CO<sub>2</sub> inhalation and samples from the gastrointestinal tract were obtained. The lower 4 cm of the ileum and ileocecal junction and half of both cecal pouches were removed aseptically from each chick. The combined tissues from each bird were minced with scissors and placed in a sterile tube containing 10 mL of TSB and incubated at 37°C for 1 h. Samples were stored on wet ice until samples were obtained from all three chicks and each sample had been incubated for 1 h.

Following recovery of surviving bacteriophages, these bacteriophages were re-amplified *in vitro* prior to re-passage through chicks. The samples were centrifuged for 10 min at 3000 rpm (1864 $\times$ g) and a total of 20 mL of

supernatant was pooled from all tubes and sterile filtered through a 0.2 µm syringe filter. Pooled filtrate was incubated with 10 mL of fresh TSB in the presence of  $3 \times 10^5$  cfu of SE at 37°C for 15 h. Following incubation, 20 mL was filtered and passed with 20 mL of fresh TSB and 0.4 mL of  $10^7$  cfu of SE and incubated for 5 h. The bacteriophages underwent two additional amplifications utilizing the 1:3:5 ratio for bacteriophage, SE and TSB respectively and incubation for 2 h at 37°C. The final bacteriophage titer after the first passage through chicks and subsequent amplification was  $10^{10}$  pfu/mL. This procedure for passaging bacteriophages through chicks was repeated two additional times.

Bacteriophage present in the tissue samples of the third *in vivo* passage were amplified by standard methods to generate a stock. These bacteriophages represent a heterogenous subpopulation from the original wild-type pool that are tolerant of low pH and other enteric conditions as evidenced by survival in three consecutive passages through the proventriculus of chicks. Ten soft agar plates with undiluted bacteriophage were created to retain samples of these bacteriophages.

***In vivo* evaluation:** In Experiment 1, One hundred twenty turkey poultts were obtained on day-of-hatch and randomly divided into three groups of 40. Poults were placed in brooder rings on clean pine shavings, provided feed and water ad libitum and maintained at an age-appropriate temperature for the duration of the experiment as above. The control group was maintained in a BSL-3 capable isolation room separate from the two groups of poults receiving bacteriophage treatment. All poults received a challenge of  $10^4$  cfu of SE by oral gavage (0.25 mL) 48 h after placement. Experimental treatment groups (groups 2 and 3) received bacteriophage and the control group (group 1) received sterile TSB 48 h following SE challenge. Group 2 was orally gavaged with  $2.5 \times 10^9$  pfu (0.25 mL) of the final bacteriophage mixture (passaged through chicks as above). Group 3 was first orally gavaged 30 min prior to bacteriophage treatment with 0.25 mL of 5mM Mg (OH)<sub>2</sub><sup>7</sup> in an attempt to buffer the acidity of the proventriculus. Then group 3 also received  $2.5 \times 10^9$  pfu bacteriophages in the presence of 1mM Mg (OH)<sub>2</sub> (0.25 mL).

Ten poults were randomly selected and humanely killed by CO<sub>2</sub> inhalation from each group 6 h, 12 h, 24 h, or 48 h post-bacteriophage treatment. At each time point, 0.2 g of cecal contents were aseptically collected and diluted in 1.8 mL of sterile saline in a sterile glass tube. Two serial ten-fold dilutions of each sample were made and 100µL of the three concentrations of cecal contents was spread plated on brilliant green agar<sup>8</sup> plates containing novobiocin<sup>9</sup> and nalidixic acid.<sup>10</sup>The plates were incubated for 18 h at 37°C and the number of colony

forming units of SE per gram of cecal contents was determined for each sample.

In Experiment 2, thirty day-of-hatch turkey poults were obtained and randomly divided into three groups of ten poults each. All poults were placed in brooder rings on litter, provided feed and water ad libitum and maintained at an age-appropriate temperature for the duration of the experiment. All poults received a challenge of  $1.6 \times 10^4$  cfu of SE by oral gavage 48 h after placement. The treatment groups received bacteriophage and the control group (group 1) received sterile TSB by oral gavage 48 h after SE challenge. Group 2 received 5mM Mg (OH)<sub>2</sub> by oral gavage 30 min prior to bacteriophage administration. Then,  $7.5 \times 10^9$  pfu of bacteriophages were administered by oral gavage with 1mM Mg (OH)<sub>2</sub> in a 0.25 mL volume. Group 3 received the same initial treatment as group 2, with subsequent oral administration of bacteriophages ( $7.5 \times 10^9$  pfu) every six h for 24 h. Additionally, groups 2 and 3 received 0.1% MgSO<sub>4</sub><sup>11</sup> in the drinking water until termination of the experiment. At 24 h post-treatment, all poults were killed and 0.2 g of cecal contents were aseptically collected and diluted in 1.8 mL of saline. Two serial ten-fold dilutions were made and 100 µL of all three concentrations were spread plated on brilliant green agar plates containing novobiocin and nalidixic acid. The plates were incubated for 24 h at 37°C, SE colonies were counted and the number of cfu of SE per gram of cecal contents were calculated.

**Statistical analysis:** All data were analyzed within time points using a one-way analysis of variance<sup>12</sup>. No means were found to be statistically significant.

## Results

In experiment 1, poults were challenged with SE and then treated with bacteriophages alone or in combination with Mg (OH)<sub>2</sub>. At 6, 12 or 24 h post-treatment, SE recovery was numerically less in bacteriophage-treated birds when compared to non-treated controls (Table 1). However, these differences were not statistically significant at any of the time points evaluated. Additionally, by 48 h post treatment the cfu's of SE in the control group had declined from 21,329 cfu of SE to 5,189 cfu of SE and the group that received Mg (OH)<sub>2</sub> in combination with bacteriophages had increased from 4,064 cfu to 227,527 cfu at 48 h.

In the group receiving bacteriophages in the absence of Mg (OH)<sub>2</sub>, no numerical difference in SE was demonstrated at any time point evaluated. From this group we also recovered SE in numbers exceeding the untreated control at 48 h post-treatment. Although the bacteriophages administered were selected for the ability to survive the gastrointestinal tract, there was no suggestion of efficacy noted in the absence of Mg (OH)<sub>2</sub> (Table 1).

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Table 1: Recovery of *Salmonella enteritidis* (SE) from cecal contents of turkey poult challenged with  $10^4$  cfu SE by oral gavage on day-of-hatch

Treatment <sup>2</sup>	Average cfu SE per g cecal contents <sup>1</sup>			
	6 h <sup>3</sup>	12 h	24 h	48 h
Control	21,329±12,694	11,294±5,699	29,812±21,506	5,189±2,739
Bacteriophage	20,840±6,758	44,162±27,811	30,437±18,118	55,288±13,557
Mg (OH) <sub>2</sub> and bacteriophage	4,402±3,616	1,437±765	4,064±2,299	227,527±131,210

<sup>1</sup>Poults were humanely killed by CO<sub>2</sub> inhalation for SE recovery at selected times post-treatment, <sup>2</sup>Poults were either untreated (control) or treated with  $2.5 \times 10^9$  pfu bacteriophages 48 h after SE challenge, <sup>3</sup>0.25 mL of 5mM Mg (OH)<sub>2</sub> solution was administered 30 min before bacteriophage suspension, which also contained 1mM Mg(OH)<sub>2</sub>

Table 2: Recovery of *Salmonella enteritidis* (SE) from the cecal contents of poults challenged with  $1.6 \times 10^4$  cfu of SE 48 h after placement and treated with  $7.5 \times 10^9$  pfu of selected bacteriophages

Treatment <sup>1</sup>	Average cfu SE per g cecal contents <sup>2</sup>
Control	79,728±25,893
Bacteriophage	11,224±9,110
Bacteriophage <sup>3</sup>	58,031±51,636

<sup>1</sup>Poults were either untreated (control) or treated with 0.25 mL of 5mM Mg(OH)<sub>2</sub> solution, then  $7.5 \times 10^9$  pfu 30 min later with 1mM Mg (OH)<sub>2</sub>, <sup>2</sup>Poults were humanely killed for SE recovery 24 h post-treatment, <sup>3</sup>Bacteriophage treatment was readministered every 6 h for 24 h (4x)

In experiment 2, we hypothesized that repeated administration of bacteriophages could reduce or possibly eliminate SE and potentially overcome the development of bacteriophage-resistant SE. In this experiment we compared a non-treated control group with poults treated once or every 6 h for 24 h with bacteriophages and Mg (OH)<sub>2</sub>. Additionally, we added 0.1% MgSO<sub>4</sub> to the water of poults in the treatment groups to provide a continuous source of Mg<sup>2+</sup>. Again, at 24 h post-treatment, we recovered numerically less SE from the group that received bacteriophage and Mg(OH)<sub>2</sub>, but the data was not significantly different than untreated controls (Table 2). Similarly, repeated administration of Mg (OH)<sub>2</sub> and bacteriophage treatment every 6 h for 24 h did not reduce the recoverable SE as compared to the control.

### Discussion

In these experiments we found that the selected bacteriophages were ineffective with co-administration of Mg (OH)<sub>2</sub> at reducing SE in poults. Recently Koo *et al.* (2001) described increased survival of bacteriophages against *Vibrio cholerae* in a simulated human gastrointestinal tract with antacid (aluminum hydroxide hydrate and magnesium hydroxide) present. Because of the buffering capabilities of the Mg (OH)<sub>2</sub>, perhaps more bacteriophages are intact after passing through the proventriculus than without the aid of an antacid. If so, lack of efficacy would then be a matter of too few bacteriophages to overcome the increasing SE

population. Additionally, the properties of the Mg<sup>2+</sup> ions may render the bacteriophages better able to infect the *Salmonella*. It is common practice to use Mg<sup>2+</sup> ions such as MgSO<sub>4</sub> in bacteriophage media to better enable adsorption of the bacteriophage to the host cell (Eisenstark, 1967). This interaction in the avian gastrointestinal tract could enhance the ability of the bacteriophages to adhere to host cells and thus proliferate.

We also observed in Experiment 1 increased SE recovery at 48 h in the treatment groups. This could be due to mutations in the SE that rendered it resistant to the bacteriophages administered. Although the numbers of SE in this treatment group dropped dramatically compared to controls, the bacteriophage treatment did not eliminate SE. Further, due to the populations of bacteriophage administered as treatments and their subsequent amplification in susceptible host SE, it is highly unlikely that the SE remaining in the ceca of treated poults did not encounter bacteriophages. Data from our laboratory indicates that resistant host bacteria occur in most *in vitro* cultures containing bacteriophages (unpublished data). This phenomena has also been reported by other investigators (Smith and Huggins, 1983). However, we did not evaluate the recovered SE to determine if it was still susceptible to the bacteriophages utilized as treatment in this study.

An alternative explanation for the observation of increased SE in the treated groups is lack of host specificity. Recently our laboratory has observed that our original wild-type bacteriophage library is not stringently host specific (unpublished data). Some of the bacteriophage isolates PHL 1-71 have demonstrated the ability to lyse non-pathogenic bacteria found as natural microflora of the cecae. This observation led us to consider the possibility that the pool of bacteriophages can potentially destroy normal microflora in addition to SE, giving bacteriophage-resistant SE the advantage of less competition. Although it is speculation, this could allow resistant SE to proliferate to high numbers as demonstrated at 48 h in the treatment groups.

In Experiment 2, re-administration of the bacteriophages

did not prove efficacious. Repeated handling of the poult every 6 h could have contributed to stress and possibly immunosuppression in that group. In fact, the group that received repeated administration of bacteriophages showed qualitative behavioral changes, including increased cannibalism which was not observed in the other two groups.

Little research on bacteriophage treatment of poultry diseases has focused on oral administration of bacteriophages. Bechieri *et al.* (1991) showed efficacy with oral administration of bacteriophages specific for *Salmonella typhimurium*, however the bacteriophages were only effective if administered simultaneously with the challenge. Recently Huff *et al.* (2002, 2003 and 2005) showed remarkable efficacy at treating airsacculitis caused by *E. coli* with bacteriophages administered intramuscularly. When the same bacteriophages were administered orally in the drinking water, they were not effective.

Possibly, oral administration of effective bacteriophages is difficult because of the harsh conditions encountered within the gastrointestinal tract. Enzymes and variable pH conditions are likely detrimental to the structure of the bacteriophages. Further research is needed to increase the efficacy of bacteriophage therapy for treating *Salmonella* infections.

## References

- Ackermann, H.W., A. Audurier, L. Berthiaume, L.A. Jones, J.A. Mayo and A.K. Vidaver, 1978. Guidelines for Bacteriophage Characterization. In: *Advances in Virus Res.*, pp: 1-24.
- Barrow, P., M. Lovell and A. Berchieri, Jr., 1998. Use of Lytic Bacteriophage for Control of Experimental *Escherichia coli* Septicemia and Meningitis in Chickens and Calves. *Clin. Diagn. Lab Immunol.*, 5: 294-298.
- Bean, N.H. and P.M. Griffin, 1990. Food-borne disease outbreaks in the United States, 1973-1987: Pathogens and trends. *J. Food Prot.*, 53: 804.
- Berchieri, Jr., A., M.A. Lovell and P.A. Barrow, 1991. The activity in the chicken alimentary tract of bacteriophages lytic for *Salmonella typhimurium*. *Res. Microbiol.*, 142: 541-549.
- Cann, A.J., 1993. *Principles of Molecular Virology*. Academic Press, Harcourt Brace and Company Publishers, NY.
- Eisenstark, A., 1967. Bacteriophage Techniques. In: *Methods in Virology*, pp: 449-524.
- Food and Drug Administration, U.S., 2005. FDA Announces Final Decision About Veterinary Medicine. P05-48, Docket No. 2000N-1571.
- FoodNet, 2005. Preliminary FoodNet Data on the Incidence of Infection with Pathogens. Transmitted Commonly Through Food---10 Sites, United States, 2004. *Morbidity and Mortality Weekly Report*, 54: 352-356.
- Gast, R.K., J.F. Stephens and D.F. Foster, 1988. Effect of kanamycin administration to poultry on the proliferation of drug-resistant *Salmonella*. *Poult. Sci.*, 67: 699.
- Goodnough, M.C. and E.A. Johnson, 1991. Control of *Salmonella enteritidis* infections in poultry by polymyxin B and trimethoprim. *Appl. Environ. Micro.*, 57: 785.
- Higgins, J.P., S.E. Higgins, K.L. Guenther, W. Huff, A.M. Donoghue, D.J. Donoghue and B.M. Hargis, 2005. Use of a specific bacteriophage treatment to reduce *Salmonella* in poultry products. *Poult. Sci.*, 84: 1141-1145.
- Huff, W.E., G.R. Huff, N.C. Rath, J.M. Balog, H. Xie, P.A. Moore Jr. and A.M. Donoghue, 2002. Prevention of *Escherichia coli* respiratory infection in broiler chickens with bacteriophage. *Poult. Sci.*, 81: 437-441.
- Huff, W.E., G.R. Huff, N.C. Rath and A.M. Donoghue, 2003. Bacteriophage treatment of severe *Escherichia coli* respiratory infection in broiler chickens. *Avian Dis.*, 47: 1399-1405.
- Huff, W.E., G.R. Huff, N.C. Rath, J.M. Balog and A.M. Donoghue, 2005. Alternatives to antibiotics: utilization of bacteriophage to treat colibacillosis and prevent foodborne pathogens. *Poult. Sci.*, 84: 655-659.
- Kobland, J.D., G.O. Gale, R.H. Gustafson and K.L. Simkins, 1987. Comparison of therapeutic versus subtherapeutic levels of chlortetracycline in the diet for selection of resistant *Salmonella* in experimentally challenged chickens. *Poult. Sci.*, 66: 1129.
- Koo, J., D.L. Marshall and A. DePaola, 2001. Antacid Increases Survival of *Vibrio vulnificus* and *Vibrio vulnificus* Phage in a Gastrointestinal Model. *Appl. Environ. Micro.*, 67: 2895-2902.
- Manning, J.G., B.M. Hargis, A. Hinton, D.E. Corrier, J.R. DeLoach and C.R. Creger, 1992. Effect of nitrofurazone or novobiocin on *Salmonella enteritidis* cecal colonization and organ invasion in leghorn hens. *Avian Dis.*, 36: 334.
- Manning, J.G., B.M. Hargis, A. Hinton, D.E. Corrier, J.R. DeLoach and C.R. Creger, 1994. Effect of selected antibiotics and anticoccidials on *Salmonella enteritidis* cecal colonization and organ invasion in Leghorn chicks. *Avian Dis.*, 38: 256.

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- Muirhead, S., 1994. Feed Additive Compendium. Miller Publishing, Minneapolis, MN.
- Persson, U. and S.I. Jendteg, 1992. The economic impact of poultry-borne salmonellosis: how much should be spent on prophylaxis? *Int. J. Food Micro.*, 15: 207.
- Smith, H.W. and M.B. Huggins, 1983. Effectiveness of Phages in Treating Experimental *Escherichia coli* Diarrhoea in Calves, Piglets and Lambs. *J. Gen. Micro.*, 129: 2659-2675.
- Smith, H.W., M.B. Huggins and K.M. Shaw, 1987. The Control of Experimental *Escherichia coli* Diarrhoea in Calves by Means of Bacteriophages. *J. Gen. Micro.*, 133: 1111-1126.
- Voyles, B.A., 1993. The Biology of Viruses. Mosby-Year Book, Inc., St. Louis, MO.
- WHO, 2006. Subject: Drug resistant Salmonella. <http://www.who.int/mediacentre/factsheets/fs139/en/> Accessed Nov.

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Abbreviation Key: SE = *Salmonella enteritidis* phage type 13A, TSA = tryptic soy agar, TSB = tryptic soy broth, BGA = brilliant green agar, NO = novobiocin, NA = naladixic acid

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