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Bacteriophage Treatment Reduces *Salmonella* Colonization of Infected Chickens

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SUMMARY. Three different lytic bacteriophages (BPs) were isolated from the sewage system of commercial chicken flocks and used to reduce *Salmonella* Enteritidis (SE) colonization from experimental chickens. Ten-day-old chickens were challenged with 9.6×10^5 colony-forming units (CFU)/ml of a SE strain and treated by coarse spray or drinking water with a cocktail of the three phages at a multiplicity of infection (MOI) of 10^3 plaque-forming units (PFU) 24 hr prior to SE challenge. Chickens were euthanatized at day 20 of age for individual SE detection, quantitative bacteriology, and phage isolation from the intestine and from a pool of organs. SE detection was performed by both bacteriologic culture and genome detection by polymerase chain reaction (PCR). Qualitative bacteriology showed that aerosol-spray delivery of BPs significantly reduced the incidence of SE infection in the chicken group ($P = 0.0084$) to 72.7% as compared with the control group (100%). In addition, SE counts showed that phage delivery both by coarse spray and drinking water reduced the intestinal SE colonization ($P < 0.01$; $P < 0.05$, respectively). BPs were isolated at 10 days postinfection from the intestine and from pools of organs from BP-treated chickens. We conclude that the phage treatment, either by aerosol spray or drinking water, may be a plausible alternative to antibiotics for the reduction of *Salmonella* infection in poultry.

RESUMEN. El tratamiento con bacteriófagos reduce la colonización de *Salmonella* en aves infectadas.

Tres tipos diferentes de bacteriófagos líticos fueron aislados del sistema de aguas residuales de granjas de aves comerciales y posteriormente se utilizaron para reducir la colonización de *Salmonella enteritidis* bajo condiciones experimentales. Aves de 10 días de edad fueron desafiadas con 9.6×10^5 unidades formadoras de colonia (UFC)/ml de una cepa de *Salmonella enteritidis* y tratadas vía aspersión con gota gruesa o agua de bebida con una mezcla de tres bacteriófagos a una multiplicidad de infección de 10^3 PFU, 24 horas antes del desafío con *Salmonella enteritidis*. Las aves fueron sometidas a eutanasia a los 20 días de edad con el fin de realizar detección individual de Salmonella, la bacteriología cuantitativa y el aislamiento de los fagos desde el intestino y de una mezcla de órganos. La detección de *Salmonella enteritidis* fue desarrollada mediante cultivo bacteriano e identificación del genoma usando la reacción en cadena por la polimerasa. La bacteriología cualitativa mostró que el suministro de bacteriófagos vía aspersión redujo significativamente la incidencia de infección con *Salmonella enteritidis* a 72.7% ($P = 0.0084$) comparada con el grupo control (100%). Adicionalmente, el recuento de *Salmonella enteritidis* mostró que el suministro del fago, tanto vía aspersión como por el agua de bebida, redujo la colonización por parte de la bacteria ($P < 0.01$ y $P < 0.05$, respectivamente). Los bacteriófagos fueron aislados 10 días postinfección a partir del intestino y de la mezcla de órganos de aves tratadas. Con este trabajo concluimos que el tratamiento con fagos, tanto por vía aspersión como por agua de bebida, puede ser una alternativa al uso de antibióticos para reducir la infección por Salmonella en aves comerciales.

Key words: *Salmonella*, poultry, bacteriophage

Abbreviations: BP = bacteriophage; CFU = colony-forming units; DW = drinking water; MOI = multiplicity of infection; *nal*^{r} = nalidixic acid resistant; PCR = polymerase chain reaction; PFU = plaque-forming units; *rif*^{r} = rifampicin resistant; SE = *Salmonella* Enteritidis

Food-borne diseases remain a major worldwide health problem. *Salmonella enterica* serovar Enteritidis (SE) is one of the most frequently identified bacteria in human outbreaks of disease and it has been epidemiologically associated with the consumption of contaminated avian meat or eggs. Preventive and control strategies in chicken commercial flocks include increased biosecurity, vaccination, competitive exclusion, and antimicrobials. However, no one approach has been shown to be 100% effective. The emergence of antibiotic-resistant strains has limited the use of therapeutic measures and stimulated the interest in alternative treatments, including the ancient concept of bacteriophage therapy.

Bacteriophages (BPs) infect and can kill bacteria (17). Lytic BPs target specific bacteria and often are limited to infection of serotypes within a single species, which is known to occur within *Salmonella enterica*. Because of host specificity, other microbial populations are not affected. Early experiences on antibacterial phage therapy showed reduced success and the rapid development of chemotherapy led to discontinuation of the research on phage therapy. Because the emergence of pathogenic bacteria resistant to most currently available antimicrobials has become a critical problem, phage therapy is newly considered as a clinical tool for therapy (18,20) and biocontrol of food-borne pathogens such as *Salmonella* and *Campylobacter* (5,6,11,13).

A few more recent reports indicate that oral phage therapy using a ''cocktail'' leads to significant reductions in *Salmonella* intestinal colonization and in the incidence of infection in live birds (4,16,19). In addition, phages have been successfully used to reduce the *Salmonella* contamination of poultry carcasses and chicken skin (5,6).

Phages can be delivered via the drinking water, coarse spray, or the intramuscular route (8,19), and may be an effective alternate to antibiotics when high titers of the bacteriophage cocktail are being administered to the critical site of the bacterial infection (10). Previously, we demonstrated that treatment with one phage applied via the oral route reduced the incidence of *Salmonella* Enteritidis infection in commercial broiler chickens (3). The aim of this study was to determine the efficacy of a treatment of a ''cocktail'' of three

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different bacteriophages delivered by coarse spray or via the drinking water to reduce *Salmonella* Enteritidis colonization in chickens.

MATERIALS AND METHODS

Chickens. One-day-old White Leghorn chicks free of *Salmonella* were obtained from a hatchery of *Salmonella*-free laying hens at the Agriculture and Livestock Service, Ministry of Agriculture (Chile) and housed in a controlled environment under strict biosecurity. The chickens were negative for antibodies against *Salmonella* by enzyme-linked-immunosorbent assay (ELISA) (IDEXX Laboratories). To ensure that the experimental birds remained free of natural infection, feces were obtained at 3 and 6 days of age and tested for *Salmonella* by polymerase chain reaction (PCR) as described by Malorny *et al*. (12). Feed and water were supplied *ad libitum*. Feed was negative for *Salmonella* by culture and PCR. All chicken trials were performed following international animal welfare regulations and approved by the institutional animal care and use committee.

Bacterial challenge strain. A *Salmonella* Enteritidis (SE) strain, originally obtained from a laying hen and kindly supplied by Dr. I. Acevedo (Laboratory of Bacteriology, Agriculture and Livestock Service, Ministry of Agriculture, Chile) was used. A spontaneous nalidixic acid and rifampicin resistant (*nal^r rif^r*) mutant of this strain was used for challenge purposes.

Isolation, propagation, and characterization of BP. Three lytic bacteriophages (BP) were isolated from 57 samples obtained from the sewage system of commercial chicken flocks. The BP isolation was performed as described by Santander and Robeson (14,15). Briefly, 1 ml of a diluted sample was mixed with 10 ml of Luria Bertoni (LB), rifampicin (100 μg/ml) and 0.5 ml of an exponential growth-phase culture of SE ATCC 13076 (rifampicin mutant) and incubated by 24 hr at 37 C. After overnight shaking, 1 ml was centrifuged for 5 min at 10,000 rpm and the supernatant treated with chloroform. The supernatant was plated on a SE ATCC 13076 culture with the use of the double agar layered method. Plaques that formed on the plates after incubation at 37 C were stabbed with a platinum loop and replicated on the same SE ATCC strain in LB agar. This procedure yielded phage stocks in concentrations higher than 10^{10} plaque-forming units (PFU). Phage stocks were screened for *Salmonella* specificity on bacterial lawns with the use of different serotypes of *Salmonella* (*Salmonella* Enteritidis, *Salmonella* Heidelberg, *Salmonella* Agona and *Salmonella* Senftenberg) and bacterial intestinal flora (200 positive and negative lactose strains) isolated from healthy chickens. Three BPs (BP1, BP2, and BP3) that showed clear lytic plaques on SE lawns but not on non-*Salmonella* spp. lawns were selected for further work.

Experimental infection. Groups of 22 10-day-old chicks were used to determine if treatment via coarse spray or drinking water BP reduces the incidence and *Salmonella* intestinal colonization (Table 1). Group 1 received SE challenge only and served as the positive control; group 2 did not receive treatment and served as the negative control; groups 3 and 4 received a cocktail of BP1, BP2, and BP3 (10^8 PFU/ml of each one of the three phages) by coarse spray (Spray) or drinking water (DW) 24 hr prior to challenge with SE *nal^r rif^r* (on day 9 of age) and group 5 received only BP. At 10 days of age, BP-treated and positive control chickens were challenged with a suspension of the SE *nal^r rif^r* strain containing 9.6×10^5 colony-forming units (CFU)/ml. On day 10 postchallenge (PI), chickens were euthanized by cervical dislocation (2) and necropsy was performed to obtain aseptic samples from intestine and heart, spleen and liver for bacterial detection (bacterial culture and genome detection by PCR) and BP isolation. Quantitative bacteriology (CFU/g) was performed in samples of intestine.

Bacteriology. Samples were weighed and transferred into a sterile plastic bag containing Rapapport-Vassiliadis broth (Difco, Franklin Lakes, NJ) (1:100), homogenized for 3 min, and incubated at 37 C for 24, 48, and 72 hr. After the initial incubation, samples were streaked onto XLD agar (Difco) supplemented with nalidixic acid and rifampicin (20 μg/ml) and incubated at 37 C for 48 hr. Black colonies were serologically confirmed with *Salmonella* O antiserum, poly A-I Vi (Difco). Rapapport-Vassiliadis broth negative samples were frozen and processed for *Salmonella* genome detection by PCR. Frequencies of SE isolation of groups 1, 3, and 4 were compared by chi-square testing.

Table 1. Experimental design.

Group	Number of birds	Treatment
1	22	SE infected[A] (positive control)
2	18	Uninfected and nontreated (negative control)
3	22	SE infected[A] and BP treated[B] by coarse spray
4	22	SE infected[A] and BP treated[B] by drinking water
5	22	Uninfected and oral BP treated[B]

[A]Infected by oral inoculation with 9.6×10^5 CFU/ml at 10 days of age.

[B]A dose of 10^8 PFU/ml of each of three phages (MOI 10^3) delivered at 9 days of age.

Quantitative bacteriology was performed from 1 ml of intestine samples that had been diluted in Rapapport-Vassiliadis broth. These samples were diluted 10-fold in sterile saline and 1 ml of each serial dilution was mixed onto XLD agar containing nalidixic acid and rifampicin (20 μg/ml). The XLD plates were incubated at 37 C for 24 hr before the typical *Salmonella* colonies were counted. When accurate colony counts were not possible, appropriate dilutions were prepared from the samples incubated for 24 hr at 37 C. Data were analyzed by analysis of variance and Tukey's multiple comparison post test.

PCR. The PCR was performed as described by Malorny *et al*. (12), targeting to the *invA* gen from *Salmonella* spp. with the use of the following primers*: InvA1*: 5′ GTG AAA TTA TCG CCA CGT TCG GGC AA 3′ (26 bp) and *InvA2*: 5′ TCA TCG CAC CGT CAA AGG AAC C 3′ (22 bp). The DNA extraction from frozen RV negative samples was performed with commercially available extraction kits (Fermentas, Vilnus, Lithuania). The reaction mixture contained 5 μl of sample DNA, 5 μl of each primers and 12.5 μl of Master Mix (Fermentas) which included dNTPs, *Taq* polymerase, buffer, and $MgCl_2$. The cycler was adjusted as follows: 95 C for 1 min for initial denaturation, 37 cycles at 95 C for 30 sec to denature, hybridization at 64 C for 30 sec and extension at 72 C for 30 sec. Cycles were continued for an additional extension at 72 C for 4 min. The PCR amplicon (284-bp fragment) was detected by electrophoresis (2% agarose in Tris buffer acetate EDTA, 90 V by 90 min) in the presence of ethidium bromide (0.5 μg/ml) and visualized in a UV transilluminator. The SE *nal^r rif^r* was used as a control strain and a 50-bp DNA ladder (Fermentas) was used as the molecular weight standard.

RESULTS

From 57 samples obtained from the sewage system of commercial chicken flocks we isolated eight lytic bacteriophages (14.03%). Three bacteriophages were selected (BP1, BP2, and BP3) according to size and clarity of lytic plaques on *Salmonella* lawns. None of these phages showed lytic activity against *Salmonella* Senftenberg (Table 2) nor against 200 lactose-negative and 200 lactose-positive strains isolated from healthy poultry feces. Qualitative bacteriology performed with intestine and a pool of heart, spleen, and liver showed a significant reduction ($P = 0.0084$) in the incidence of *Salmonella* Enteritidis in chickens (72.7%) (16/22) treated with BP by spray, as compared with positive control birds (100%) (22/22). The incidence of SE-positive birds in group 4 (18/22) treated with BP via the drinking water did not differ significantly as compared with the positive control group (Fig. 1). With the use of only the organ pool, excluding the intestines, SE was isolated in positive control chickens (17/22) and from the chicken groups treated with BP via coarse spray (13/22) and via the drinking water (9/22), data not shown. Only delivery via the drinking water determined

Table 2. Lytic activity of bacteriophages 1, 2, and 3 against different *Salmonella* serovars.

Salmonella serovar	Bacteriophage 1	Bacteriophage 2	Bacteriophage 3
Agona	+	+	+
Enteritidis	+	+	+
Heidelberg	+	+	+
Senftenberg	−	−	−

significant differences ($P = 0.0142$). PCR method detected only six positive samples from all samples that were negative by culture. Neither clinical signs nor mortality was observed in any group throughout the experiment. The negative control group (uninfected and nontreated) remained both *Salmonella* free and BP free during the experiment.

Chicken groups treated with BP by spray ($P < 0.001$) and drinking water ($P < 0.05$) showed significantly lower SE counts compared with the positive control (Fig. 2). Mean intestinal CFU/ml ($\log_{10}$) of SE were 4.04 for spray, 4.25 for drinking water, and 5.67 for positive controls.

As shown in Table 3, over 50% of chickens that received bacteriophages, i.e., group BP only, and groups 3 and 4 were positive for BP in intestinal samples. A lower percent of BP isolation was observed from the pools of heart, spleen, and liver samples. Percentages of BP isolation were as follows; group BP only: 76.7% of all samples of intestine, and 50% of all pools of heart, spleen, and liver; group 4: 90.9% and 36.4% respectively; and group 3: 59.1% and 45.5%, respectively. Because the plaques formed by the selected phages do not differ in morphology, it was not possible to identify the isolated BP. Further characterization of BP genomes is in progress.

DISCUSSION

In this study *Salmonella*-specific BP were isolated from 14% of 57 sewage samples, all of them associated with commercial chicken flocks. There are many reports available describing the isolation of phages from sewage systems of commercial chicken flocks or cecal content (1,4,15,19) and wastewater treatment plants (6,8) demonstrating their abundance in nature.

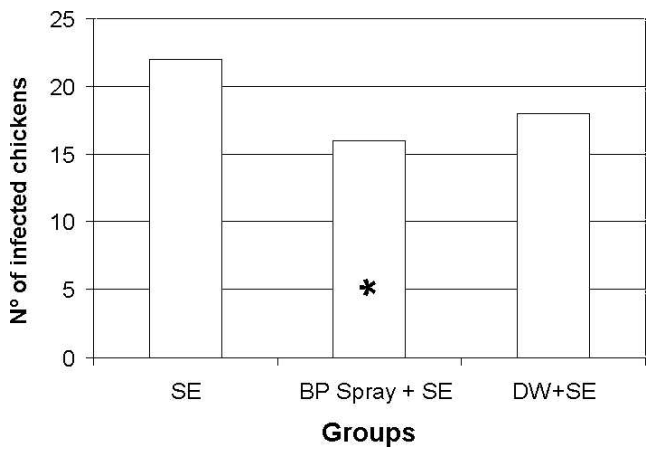

Fig. 1. Chickens ($n = 22$) positive for SE both by bacteriologic culture and/or PCR. Chickens were challenged with SE at 10 days of age and treated with phage (BP) via spray or drinking water (DW) 1 day prior to challenge (day 9 of age). Asterisk indicates significant difference ($P < 0.01$) between treated and positive control (SE alone).

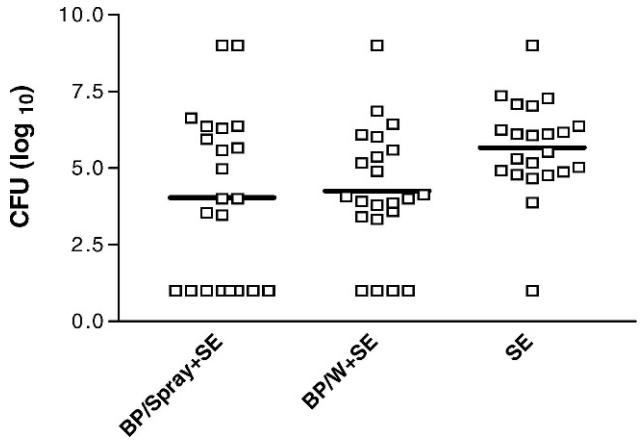

Fig. 2. *Salmonella* Enteritidis counts (SE) (CFU $\log_{10}$) detected in chickens challenged with SE at 10 days of age and treated with a bacteriophage combination (three different bacteriophages) on day 9 of age. Bacteriophage delivery was performed either via the drinking water or by coarse spray.

The host range of the three BP selected in the present study (Table 2) was reduced and highly specific against the more frequent *Salmonella* serovars isolated from animals in Chile (except of *Salmonella* Senftenberg).

The use of two or more bacteriophages reduces the possibility of selection for resistance against a specific bacteriophage. For this reason, phage cocktails have been chosen by different authors as a better tool to combat bacteria (4,5,8,19). In agreement with those authors, the present study used a phage cocktail of three phages and applied in a single-dose regime to reduce the risk of acquiring resistance as result of continuous administration (16) and to make this methodology more practical under commercial conditions.

The MOI (10^3 PFU/ml) selected for the present study was based on preliminary results (3) obtained with commercial chickens infected with SE and treated with 1 and 10^1 PFU/ml of the F3α SE phage. A 10^1 MOI reduced the *Salmonella* incidence from 86.6% to 46.6% and 1 MOI reduced it to 53.3%. Although phages replicate in the host cell, the more successful BP therapies in animal models have used high MOI. Huff *et al.* (9,10) demonstrated that a high titer of SPR02 phage (MOI 10^3 and 10^4) was the only consistently effective treatment that significantly reduced mortality in broiler chickens infected with *E. coli*.

The goal of the present study was bacteriophage treatment to prevent infection with *Salmonella* Enteritidis in chickens. Phages administered too early may result in clearing of the phage from the body before it reaches the replication threshold (17). Wagenaar *et al.* (20) showed that phage treatment 3 days prior to bacterial challenge could not prevent but did delay *Campylobacter* colonization in chickens and the numbers of *Campylobacter jejuni* detected in the ceca remained lower than in nontreated controls. Huff *et al.* (7) observed that phages delivered via spray on the same day of *E. coli*

Table 3. Frequency of bacteriophage isolation from chickens infected with SE and BP treated by spray or via the drinking water (DW) at 10 days postinfection.

Groups	% Intestine BP positive	% Organ BP positive
Uninfected and BP treated	76.7	50.0
SE Infected and BP treated by aerosol	59.1	45.5
SE Infected and BP treated by DW	90.9	36.4

challenge significantly reduced the mortality in chickens, but phages delivered 24 and 72 hr prior to *E. coli* challenge were not significantly different.

The results indicate that some bacteriophage treatment regimes can reduce the incidence of *Salmonella* infections and decrease intestinal *Salmonella* counts. The qualitative bacteriology performed with intestine and pool of heart, spleen, and liver, demonstrated that the BP delivered by aerosol spray significantly reduced ($P = 0.0084$) the incidence of *Salmonella* infections in chickens and reduced intestinal *Salmonella* counts. The incidence of SE in chickens treated with BP via the drinking water did not differ significantly from SE-only infected chickens but SE counts were reduced significantly. The route of BP administration has been shown to play a role on treatment success. Huff *et al*. suggest that the bacteriophage delivery via the drinking water offered no protection against *E. coli* respiratory infections in broiler chickens (9) but the phages' delivery early by aerosol spray and intramuscular injections reduced the mortality (7,8).

The SE positive control group showed mean SE counts of 5.67 $\log_{10}$ CFU. Chickens treated with BP by coarse spray showed values of 4.04 $\log_{10}$ CFU in the intestine and chickens treated via the drinking water showed mean SE counts of 4.25 $\log_{10}$ CFU. These results are similar to those reported by Sklar and Joerger (16), Fiorentin *et al*. (4), and Toro *et al.* (19).

In the present study BP isolation from intestine and pool of liver, spleen, and heart at 10 days post-SE infection in all groups that received phages indicate that they passed through the digestive tract, reached the infection site, replicated, and translocated to blood-streams. The frequency of BP isolation was higher in intestine samples than in pools of organs (Table 3). The persistence of BP in the samples from BP-only inoculated chickens through 10 days after inoculation (Table 3) was not expected because they should not have replicated without the host cell; all intestinal and internal organ pool samples from the control BP were negative for *Salmonella* by culture and PCR. Studies of bacteriophage dynamics demonstrated that they rapidly decrease the titer in absence of the cell target (8,20). Ongoing studies at this laboratory are aimed at further understanding the dynamics of bacteriophage populations in chickens infected with *Salmonella* Enteritidis and in uninfected birds.

In conclusion, the results described here show beneficial effect of bacteriophage treatment against *Salmonella* Enteritidis for reduction of bacterial loads. These results suggest that bacteriophage therapy may be a plausible alternative to antibiotics for the reduction of *Salmonella* infection in poultry.

REFERENCES

1. Atterbury, R. J., E. Dillon, C. Swift, P. L. Connerton, J. A. Frost, C. E. R. Dodd, D. Rees, and I. F. Connerton. Correlation of *Campylobacter* Bacteriophage with reduced presence of host in broiler chicken ceca. Appl. Environ. Microbiol. 71:4885–4887. 2005.

2. AVMA. American Veterinary Medical Association, Report of the AVMA Panel of Euthanasia. J. Am. Vet. Med. Assoc. 218:669–696. 2001.

3. Borie, C., P. Zurita, J. Santander, E. Krueger, M. L. Sanchez, S. Ramìrez, and J. Robeson. Efecto del bacteriófago F3α SE sobre la colonización de *Salmonella enteritidis* en un modelo aviar. XIX Panamerican Congress of Veterinary Science, October, Buenos Aires, Argentina. 2004.

4. Fiorentin, L., D. Nilson, and W. Barioni. Oral treatment with bacteriophages reduces the concentration of *Salmonella enteritidis* PT4 in caecal contents of broilers. Avian Pathol. 34:258–263. 2005.

5. Goode, D., V. M. Allen, and P. A. Barrow. Reduction of experimental *Salmonella* and *Campylobacter* contamination of chicken skin by application of lytic bacteriophage. Appl. Environ. Microbiol. 69:5032–5036. 2003.

6. Higgins, J. P., S. E. Higgins, K. L. Guebnther, W. Huff, A. M. Donoghue, D. J. Donoghue, and B. M. Hargis. Use of bacteriophage treatment to reduce *Salmonella* in poultry products. Poultry Sci. 84:1141–1145. 2005.

7. Huff, W. E., G. R. Huff, N. C. Rath, J. M. Balog, and A. M. Donoghue. Prevention of *Escherichia coli* infection in broiler chickens with a bacteriophage aerosol spray. Poult. Sci. 81:1486–1491. 2002.

8. Huff, W. E., G. R. Huff, N. C. Rath, J. M. Balog, and A. M. Donoghue. Evaluation of aerosol spray and intramuscular injection of bacteriophage to treat an *Escherichia coli* respiratory infection. Poult. Sci. 82:1108–1112. 2003.

9. Huff, W. E., G. R. Huff, N. C. Rath, J. M. Balog, H. Xie, P. A. Moore Jr., and A. M. Donoghue. Prevention of *Escherichia coli* respiratory infection in broiler chickens with bacteriophage (SPR02). Poult. Sci. 81:437–441. 2002.

10. Huff, W. E., G. R. Huff, N. C. Rath, and A. M. Donoghue. Evaluation of the influence of bacteriophage titer on the treatment of colibacillosis in broiler chicken. Poult. Sci. 85:1373–1377. 2006.

11. Leverentz, B., W. S. Conway, Z. Alavidze, W. J. Janisiewicvz, Y. Fuchs, M. J. Camp, E. Chigladze, and A. Sulakvelidze. Examination of bacteriophage as a biocontrol method for *Salmonella* on fresh-cut fruit: a model study. J. Food Prot. 64:1116–1121. 2001.

12. Malorny, B., J. Hoorfar, C. Bunge, and R. Helmuth. Multicenter validation of the analytical accuracy of *Salmonella* PCR: towards an International Standard. Appl. Environ. Microbiol. 69:290–296. 2003.

13. Modi, R., Y. Hirvi, A. Hill, and M. W. Griffiths. Effect of phage on survival of *Salmonella enteritidis* during manufacture and storage of cheddar cheese made from raw and pasteurized milk. J. Food Prot. 64:927–933. 2001.

14. Santander, J., and J. Robeson. Aislamiento y caracterización de bacteriófagos líticos contra *Salmonella* Enteritidis y su ensayo sobre *S.* Pullorum. Acta Microbiol. 8:17–22. 2002.

15. Santander, J., and J. Robeson. Bacteriophage prophylaxis against *Salmonella enteritidis* and *Salmonella pullorum* using *Caenorhabditis elegans* as an assay system. EJB Electron. J. Biotechnol. 7:206–209. 2004.

16. Sklar, I. B., and R. D. Joerger. Attempts to utilize bacteriophage to combat *Salmonella enterica* serovar *enteritidis* infection in chickens. J. Food Saf. 21:15–29. 2001.

17. Skurnik, M., and E. Strauch. Phage therapy: facts and fiction. Int. J. Med. Microbiol. 296:5–14. 2006.

18. Sulakvelidze, A., Z. Alavidze, and J. G. Morris. Bacteriophage therapy. Antimicrob. Agents Chemother. 45:649–659. 2001.

19. Toro, H., S. B. Price, S. McKee, F. J. Hoerr, J. Khreling, M. Perdue, and L. Bauersmeister. Use of bacteriophages in combination with competitive exclusion to reduce *Salmonella* from infected chickens. Avian Dis. 49:118–124. 2005.

20. Wagenaar, A., M. A. P. Van Bergen, M. A. Mueller, T. M. Wassenaar, and R. M. Carlton. Phage therapy reduces *Campylobacter jejuni* colonization in broilers. Vet. Microbiol. 109:275–283. 2005.

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