Efficacy of phage therapy in poultry: a systematic review and meta-analysis

S. Mosimann,[1] K. Desiree,[1] and P. Ebner[2]

Department of Animal Sciences, Purdue University, West Lafayette, IN 47907, USA

ABSTRACT The increasing prevalence of antimicrobial resistant bacteria has sparked a renewed interest in alternative bacterial control methods, including bacteriophage administration. In order to determine the overall efficacy of bacteriophage administration for the reduction of bacterial concentrations in poultry, a systematic literature review and a meta-analysis were conducted. The systematic review included studies in which 1) live chickens were challenged with a known quantity of bacteria; and 2) challenged chickens were administered a known quantity of bacteriophages; and 3) concentrations of the challenge bacteria were measured in tissue/fluid samples from both challenged and unchallenged chickens after phage administration; and 4) either standard deviation or standard error was reported. Results of a meta-analysis of the 12 studies included in this review (total inputs: n = 41; total observations: n = 711) indicated that concentrations of challenge bacteria were significantly lower ($P < 0.001$) in challenged, phage-treated chickens than in challenged, untreated chickens (effect size = -0.82 $\log_{10}$ cfu/g). Phage treatment effects were significantly greater ($P < 0.01$) in chickens administered phages via feed than in chickens administered phages via drinking water or aerosol spray. No significant differences were observed between subgroups when data were disaggregated by various other experimental characteristics, though some significant differences were observed across subgroups after further disaggregation by sampling time and animal age. As a whole, findings from the systematic review and meta-analysis indicate that phage administration can significantly lower concentrations of targeted bacteria in chickens and that, in some instances, the effect may be greater in the short-term vs. the long-term and in older vs. younger chickens.

Key words: phage, poultry, systematic review, meta-analysis

2021 Poultry Science 100:101472
https://doi.org/10.1016/j.psj.2021.101472

INTRODUCTION

Bacteriophages, or "phages", are viruses that infect specific host bacteria. Phages may be placed into two general categories: those that induce host cell lysis shortly after initial infection ("lytic" or "virulent" phages), and those that induce a lysogenic cycle and reside as prophages inside the host cell without immediately causing host lysis ("temperate" or "lysogenic" phages) (Sulakvelidze et al., 2001). Due to the direct antibacterial action of the lytic cycle, bacteriophage research aimed at the reduction of bacterial colonization has traditionally employed lytic phages. More recent studies have investigated the therapeutic potential of both temperate phages (Yosef et al., 2015; Park et al., 2017; Monteiro et al., 2019) and phage lysins (Fischetti, 2018; Vázquez et al., 2018), however. For pertinent, in-depth reviews of phage biology that discuss both lytic and temperate phages, see the works of Sulakvelidze et al. (2001), Guttman et al. (2005), and Ackermann and Węgrzyn (2014).

Phages have long been investigated for their potential as antimicrobials but have typically been passed over in favor of chemical antibiotics (Sulakvelidze et al., 2001). However, the increasing prevalence of antibiotic resistant bacteria has sparked a renewed interest in using bacteriophages as therapeutics or prophylaxes for both antibiotic resistant and antibiotic susceptible bacterial infections (CDC, 2019). There is a growing body of research on the use of bacteriophages as antibacterial alternatives in food animal production in particular. The systematic review and meta-analysis presented in this manuscript provide a summary and analysis of the aggregated results of 12 live animal research studies that focus on phage treatment in poultry production in order to determine if phage administration significantly reduces concentrations of specific challenge bacteria. Both a meta-analysis of the entire data set and analyses of data disaggregated by various experimental factors (e.g., sample collection time, phage protection strategy, and administration route) were performed in order to

Received April 1, 2021.
Accepted August 31, 2021.

[1]These authors contributed equally to this manuscript.

[2]Corresponding author: pebner@purdue.edu

identify if any particular factor significantly impacted phage treatment efficacy.

MATERIALS AND METHODS

A primary literature search for published research examining phage therapy in poultry between 1990 and present was conducted in the summer of 2020 using the PubMed and Google Scholar databases. Key search terms included "poultry + phage", "chicken + phage", "poultry + bacteriophage", and "chicken + bacteriophage". Articles were restricted to original research on live poultry; thus, review articles, non−peer-reviewed articles (e.g., theses/dissertations), "gray" literature, and articles utilizing models of the target species or nonpoultry animals were removed. This search resulted in a pool of 50 studies describing experiments measuring the antibacterial capacity of phages in live chickens. Works cited in these articles were screened to identify any additional, relevant articles not retrieved in the original search. The author(s), year published, a brief results summary, study location, challenge bacteria, a description of experimental animal characteristics, and whether or not an animal trial was performed was recorded for each article in the pool. Articles were then qualitatively assessed using a previously developed rubric (Supplementary Table 1). A review of the qualitative data indicated that further meta-analysis, with acceptable heterogeneity across studies, could be conducted using the concentration of challenge bacteria following phage treatment as the principle outcome variable by aggregating inputs from studies in which 1) live chickens were inoculated with a known quantity of a specific challenge bacteria; and 2) challenged chickens were treated with a known quantity of bacteriophage shown to be lytic against the challenge bacteria; and 3) concentrations of the challenge bacteria were quantified (e.g., CFU/g) in tissues or fluids of challenged chickens post-phage treatment; and 4) standard deviations (**SD**) or standard errors (**SE**) associated with post-phage treatment concentrations of challenge bacteria were reported. Studies employing natural challenges in which initial concentrations of the target bacteria were not known, studies reporting only qualitative or semi-quantitative results (e.g., frequency of shedding), and studies not reporting SD or SE were removed. To avoid over-representing any one study in the meta-analysis, the total number of inputs included per study was limited to one input per sampling period (0−7 d, 8−14 d, or >14 d post-treatment) per treatment. The resulting final data set included a total of 711 observations from 41 inputs (i.e., distinct phage treatments/experiments) across 12 studies.

Meta-analysis was performed following previously published guidelines (Harrer et al. 2019a,b) and processed using RStudio (RStudio Team, 2021). The final data set included 1) number of chickens in the phage treatment group; 2) number of chickens in the control group (i.e., chickens receiving challenge bacteria but not phage or any other antibacterial treatment); 3) mean concentration of the challenge bacteria in phage-treated chickens with SD; 4) and mean concentration of challenge bacteria in control chickens with SD. In cases where concentration means, SD, or SE values were included in graphs but not in the text, these values were estimated based on graphical data; estimations are noted in the systematic review when this occurred. When a single experiment contained one control group but multiple phage treatment groups or inputs, the number of animals in the control group was divided equally across the number of treatment groups to avoid over-representation of individual studies in the data set.

Data were analyzed using the dmetar package of R (Harrer et al., 2019a). The full data set was screened for "P-hacking" and "small-sample size bias". Subsequently, data were analyzed using a random effects model recognizing variation across studies (i.e., differences in chicken breed, chicken age, study methods, among others; Harrer et al., 2019b). Variance of distribution of effect size was estimated using Sidik-Jonkman tests and the model was adjusted using the Hartung-Knapp method (Harrer et al., 2019b). The experimental and data analysis methods of studies identified in R as outliers were re-examined to determine if removing them from the data set was justifiable. Data were disaggregated by various factors, for example, sampling time, sample type, phage delivery method (e.g., gavage, feed, and spray), challenge bacteria, among others, in attempt to identify if any of these factors significantly impacted phage treatment efficacy. Heterogeneity was assessed using the inconsistency index (I^2), or percentage of heterogeneity (Higgins and Thompson, 2002). Heterogeneity was considered low when I^2 values were between 0 and 25%, moderate between 26 and 50%, considerable between 51 and 75%, and significant between 76 and 100%. Identification of subgroups for data disaggregation was not random and therefore comparisons between subgroups were made using a mixed-effects model (i.e., a random-effects model within subgroups and a fixed-effects model between subgroups; Harrer et al., 2019a). Post-hoc power analyses (%) were conducted on the overall data set and on each disaggregated data set. Differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Systematic Review

Following the procedures described above, 12 individual studies were included in this systematic review. These studies all investigated the impact of bacteriophage administration on concentrations of challenge bacteria in live chickens and 1) challenged live chickens with a known quantity of bacteria; 2) administered a known quantity of phages to challenged chickens; 3) measured concentrations of challenge bacteria in tissue/fluid samples from both challenged and unchallenged chickens after phage administration; and 4) reported

either SD or SE. For the purposes of systematic review, these 12 studies were grouped for discussion based on similarities in experimental design. Discussion groupings were formed primarily on the basis of phage administration route (oral gavage, feed, drinking water, or aerosol spray) because meta-analytics suggested that administration route may significantly impact phage treatment efficacy. Due to the relatively large number of studies in which phages were administered via oral gavage, the discussion of these studies was further separated based on the age of chickens at the time of phage treatment (<14 days of age [**doa**] or >14 doa). Throughout the systematic review, experimental methods have been summarized and abbreviated for clarity and brevity. Further details regarding the studies' experimental methods, including phage preparation methods, treatment schedules, age of birds, and bacterial enumeration methods may be found in Table 1.

Oral Gavage Administration to Chickens >14 doa

Among the studies included in this review, oral gavage was a common administration route. Loc Carillo et al. (2005), Atterbury et al. (2007), and El-Shibiny et al. (2009) administered phages via oral gavage to chickens >14 doa challenged with various *Salmonella* spp.

In the study conducted by Atterbury et al. (2007), a single dose of phages was administered to chickens at a rate of 10^9 or 10^{11} pfu/bird 2 d following challenge with either *Salmonella* Enteritidis, *Salmonella* Typhimurium, or *Salmonella* Hadar. Administration of phages at 10^9 pfu/bird did not result in significant differences in concentrations of any of the challenge organisms between phage-treated and untreated birds at any sampling point. When phages were given at 10^{11} pfu/bird, phage-treated chickens challenged with *Salmonella* Enteritidis had significantly lower ($P < 0.05$) cecal concentrations of *Salmonella* Enteritidis (1.53 ± 2.38 $\log_{10}$ cfu/g) in comparison to untreated birds (5.77 ± 1.85 $\log_{10}$ cfu/g) at 2 d postchallenge. Similarly, birds challenged with *Salmonella* Typhimurium and treated with phages at 10^{11} pfu/bird had significantly lower ($P < 0.05$) cecal concentrations of *Salmonella* Typhimurium (3.48 ± 1.88 $\log_{10}$ cfu/g) than untreated birds (5.67 ± 0.41 $\log_{10}$ cfu/g) at 2 d postchallenge. However, there were no significant differences in *Salmonella* Hadar concentrations in phage-treated vs. untreated birds at any sampling point.

El-Shibiny et al. (2009) administered a single dose of bacteriophages (at 10^5, 10^7, or 10^9 pfu/bird) to chickens 5 d after challenging them with either *Campylobacter jejuni* or *Campylobacter coli*. Across all phage dosage rates and gastrointestinal sample sites, *Campylobacter jejuni* concentrations tended to be significantly lower ($P < 0.05$; 0.9−2.6 $\log_{10}$ cfu/g lower) in phage-treated vs. untreated birds (significant differences observed at 1, 2, and 5 d post-phage treatment). Phage-treated chickens challenged with *Campylobacter coli*, however, typically had significantly lower ($P < 0.05$; 0.9−1.9 $\log_{10}$ cfu/g lower) cecal concentrations of challenge bacteria compared to untreated chickens only when phages were administered at rates of 10^9 pfu/bird (significant differences observed at 2, 3, 4, and 5 d post-phage treatment). Significant differences in concentrations of *Campylobacter coli* were found only sporadically between phage-treated chickens administered 10^7 or 10^5 pfu/bird and untreated chickens.

Using comparable experimental methods, Loc Carillo et al. (2005) administered a single dose of bacteriophages (CP34 or CP8) to chickens previously challenged with either *Campylobacter jejuni* HPC5 or *Campylobacter jejuni* GIIC8. At 1 d post-treatment, cecal concentrations of *Campylobacter jejuni* HPC5 were significantly lower ($P < 0.05$) in chickens administered CP34 at 10^5 or 10^7 pfu/bird (10^7: 3.9 $\log_{10}$ cfu/g; 10^5: data not shown) compared to untreated chickens (~6.58 $\log_{10}$ cfu/g; estimation from graphical data). Birds receiving CP34 at 10^9 pfu/bird had significantly lower ($P < 0.001$) cecal concentrations of *Campylobacter jejuni* HPC5 in comparison to untreated birds at 4 d post-treatment. There were no significant differences in cecal concentrations of *Campylobacter jejuni* HPC5 between birds administered CP8 and untreated birds regardless of phage inoculum concentration. When *Campylobacter jejuni* GIIC8 was used as the challenge organism, however, chickens treated with CP8 at 10^7 pfu/bird had significantly lower ($P < 0.001$) cecal concentrations of *Campylobacter jejuni* GIIC8 in comparison to untreated chickens from 1 d (phage-treated: ~3.5 $\log_{10}$ cfu/g; untreated: ~7.8 $\log_{10}$ cfu/g) to 5 d (phage-treated: ~6.2 $\log_{10}$ cfu/g; untreated: ~8.2 $\log_{10}$ cfu/g) post-treatment (estimations from graphical data). To note, no other concentrations (e.g., 10^5 or 10^9 pfu/bird) of phage CP8 were administered to birds challenged with *Campylobacter jejuni* GIIC8 and no birds challenged with *Campylobacter jejuni* GIIC8 were treated with phage CP34.

Oral Gavage Administration to Chickens <14 doa

Oral gavage has also been used to administer bacteriophages to chickens <14 doa. In contrast to the studies in which phages were administered to adult birds, experiments in which phages were orally administered to chicks have often employed poly-phage treatments (i.e., phage cocktails) rather than single phage treatments. Additionally, many of these studies administered phages repeatedly rather than in a single dose.

In the first of 2 experiments, Fischer et al. (2013) administered a single dose of a bacteriophage cocktail to chicks 3 d after *Campylobacter jejuni* challenge. Significant differences ($P < 0.05$) in cecal concentrations of *Campylobacter jejuni* were observed between phage-treated and untreated birds at 1 and 3 d post-phage treatment, but not at 7 d post-treatment. At this sampling point phages were isolated from untreated birds, however, resulting in the exclusion of these birds from data analysis. In a second trial, in which chicks received one dose of either the phage cocktail or a single phage treatment, concentrations of *Campylobacter jejuni* were significantly lower ($P < 0.036$) in all phage-treated birds in comparison to untreated birds at 7, 14, 21, 28, and 35 d post-phage treatment. At these sampling times, average cecal concentrations of *Campylobacter jejuni* ranged from ~5.2 to ~6.6 $\log_{10}$ cfu/g in phage cocktail-treated birds, ~4.8 to ~7.7 $\log_{10}$ cfu/g in single phage-treated

Table 1. Description of studies included in systematic review and meta-analysis.

Reference	Challenge organism	Phage inoculum description	Chicken age	Phage delivery method	Phage delivery schedule	Sampling times included in meta-analysis	Significant ($P < 0.05$) effect observed	Bacterial enumeration method	Number of inputs; total observations
Adhikari et al. (2017)	*Salmonella enterica* Enteritidis	Two phage types, preparation methods not specified (**NS**)	> 14 doa	Feed	Phages delivered multiple times both prior to and after bacterial challenge	3, 7 d post-initial phage treatment	Yes	Viable cell count	2; 48
Atterbury et al. (2007)	*Salmonella enterica* Enteritidis	Single phage type suspended in PBS with 30% wt/vol $CaCO_3$	>14 doa	Oral gavage	Phages delivered once after bacterial challenge	3 d post-phage treatment	Yes, when phages given at 10^{11} pfu/bird for reduction of *Salmonella enterica* or *Salmonella typhimurium*	Viable cell count	3; 25
Bardina et al. (2012)	*Salmonella enterica* Typhimurium	Three phage types suspended in Luria Bertani medium	<14 doa	Oral[3]	Phages delivered multiple times both prior to and after bacterial challenge	6, 10, 17 d post-initial phage treatment	Yes, when phage treatment began less than 4 days after challenge	Viable cell count	3; 32
Borie et al. (2009)	*Salmonella enterica* Enteritidis	Three phage types, preparation method NS	<14 doa	Aerosol spray	Phages delivered twice prior to bacterial challenge	8 d post-initial phage treatment	No	Most probable number or similar	1; 30
Borie et al. (2008)	*Salmonella enterica* Enteritidis	Three phage types, preparation method NS	<14 doa	Aerosol spray or drinking water	Phages delivered once prior to bacterial challenge	11 d post-phage treatment	Yes	Most probable number or similar	3; 66
Colom et al. (2015)	*Salmonella enterica* Typhimurium	Three phage types suspended in $MgSO_4$ buffer or liposome-encapsulated[1] and suspended in $MgSO_4$ buffer	<14 doa	Oral[3]	Phages delivered multiple times both prior to and after bacterial challenge	4, 11, 16 d post-initial phage treatment	Yes	Most probable number or similar	6; 126
El-Shibiny et al. (2009)	*Campylo-bacter jejuni* and *Campylo-bacter coli*	Single phage type suspended in 30% wt/vol $CaCO_3$	>14 doa	Oral gavage	Phages delivered once after bacterial challenge	3 d post-phage treatment	Yes, treatment most effective at 10^9 pfu/bird; more effective when used to treat *C. jejuni* vs. *C. coli*	Viable cell count	2; 24
Fischer et al. (2013)	*Campylo-bacter jejuni*	Four phage types or single phage type suspended in SM buffer[2] with 33% wt/vol $CaCO_3$	<14 doa	Oral (into crop)	Phages delivered once after bacterial challenge	3, 14, 28 d post-phage treatment	Yes	Viable cell count	6; 99
Lim et al. (2012)	*Salmonella enterica* Enteritidis	Single phage type, preparation method NS	<14 doa	Feed	Phages delivered multiple times after bacterial challenge	7, 14, 21 d post-initial phage treatment	Yes	Viable cell count	3; 120
Loc Carillo et al. (2005)	*Campylo-bacter jejuni*	Single phage type suspended in 30% wt/vol $CaCO_3$	>14 doa	Oral gavage	Phages delivered once after bacterial challenge	3 d post-phage treatment	Yes	Viable cell count	3; 26

(*continued*)

Table 1 (*Continued*)

Reference	Challenge organism	Phage inoculum description	Chicken age	Phage delivery method	Phage delivery schedule	Sampling times included in meta-analysis	Significant ($P < 0.05$) effect observed	Bacterial enumeration method	Number of inputs; total observations
Luis Vaz et al. (2020)	*Salmonella enterica* Enteritidis	Three phage types, preparation methods NS	<14 doa or >14 doa	Drinking water	Phages delivered multiple times after bacterial challenge	4, 10 d post- initial phage treatment	Yes	Viable cell count	4; 67
Wagenaar et al. (2005)	*Campylo-bacter jejuni*	Two phage types or single phage type, preparation methods NS	<14 doa	Oral gavage	Phages delivered multiple times prior to challenge or multiple times after bacterial challenge	6, 13, 26 d or 5, 10, 17 d post- initial phage treatment	Significance not discussed	Viable cell count	6; 48

[1]Encapsulated via thin-film hydration method in a mixture of 1,2-dilauroyl-*rac*-glycero-3-phosphocoline, cholesteryl polyethylene glycol 600 sebacate, cholesterol, and cholesteryl 3*β*-*N*-(dimethylaminoethylcarbamate hydrochloride (cholesteryl at a 1:0.1:0.2:0.7 molar ratio);
[2]SM buffer = 5.8 g NaCl, 2.0 g $MgSO_4$ x 7 H2O, 50 ml 1M Tris (Sigma pH 7.5), 5 mL 2% gelatine, and distilled water.
[3]"gavage" not specified.

birds, and ~6.6 to ~7.6 $\log_{10}$ cfu/g in untreated birds (estimations based on graphical data). No differences in cecal *Campylobacter jejuni* concentrations were found across treatment groups on 1, 3, and 42 d post-phage treatment. Overall, the average cecal concentration of *Campylobacter jejuni* of phage-treated birds (5.9 $\log_{10}$ cfu/g, average of all phage-treated birds in both trials) was significantly lower ($P < 0.001$) than that of untreated birds (7.2 $\log_{10}$ cfu/g, average of untreated birds in the second trial).

Wagenaar et al. (2005) administered single phage treatments to chicks for 10 consecutive days beginning 3 d prior to *Campylobacter jejuni* challenge and found that concentrations of *Campylobacter jejuni* in the cecal contents of phage-treated birds (~6.7 to ~8.3 $\log_{10}$ cfu/g) were numerically lower than in untreated birds (~8.9 to ~9.5 $\log_{10}$ cfu/g; excluding day one postchallenge, when groups had comparable levels of *Campylobacter jejuni*). In a second trial, chicks were administered single phage treatments for 6 consecutive days beginning 5 d after bacterial challenge. Again, phage-treated birds typically had numerically lower cecal concentrations of *Campylobacter jejuni* than untreated birds. The authors point out a marked decrease in concentrations of *Campylobacter jejuni* in phage-treated birds at 7 d postchallenge (~5.4 $\log_{10}$ cfu/g); at this sampling time, concentrations of *Campylobacter jejuni* in untreated birds were ~8.8 $\log_{10}$ cfu/g. In a third trial, adult chickens (> 14 doa) were challenged with *Campylobacter jejuni* and administered a phage cocktail for 4 consecutive days beginning 7 d following the bacterial challenge. Phage-treated birds had numerically lower cecal concentrations of *Campylobacter jejuni* (~6.6 to ~7.6 $\log_{10}$ cfu/g) in comparison to untreated birds throughout this experiment (~8.3 to ~8.5 $\log_{10}$ cfu/g) (excluding day one post-phage treatment, when *Campylobacter jejuni* titers were similar across groups). To note, data presented here are approximations drawn from graphical data and no analyses to identify statistical differences were described in this study; as such, inferences drawn from this study regarding phage efficacy should be tempered.

Bardina et al. (2012) repeatedly treated chicks with a phage cocktail starting 1 d prior to challenge with *Salmonella* Typhimurium. Cecal concentrations of *Salmonella* Typhimurium were significantly lower ($P < 0.001$) in phage-treated vs. untreated birds from 2 d postchallenge until the conclusion of the experiment. Over this time period, average cecal concentrations of *Salmonella* Typhimurium ranged from ~3.92 to ~7.23 $\log_{10}$ cfu/g in phage-treated birds and from ~8.03 to ~9.03 $\log_{10}$ cfu/g in untreated birds. In a separate trial, chicks were challenged with *Salmonella* Typhimurium and intermittently treated with a phage cocktail beginning the day of bacterial challenge. *Salmonella* Typhimurium concentrations in phage-treated birds were lower than in untreated birds at 1 d (phage-treated: ~2.73 $\log_{10}$ cfu/g; untreated: ~6.75 $\log_{10}$ cfu/g) and 2 d (phage-treated: ~3.27 $\log_{10}$ cfu/g; untreated: ~8.32 $\log_{10}$ cfu/g) postchallenge (significance not discussed). Concentrations of *Salmonella* Typhimurium were significantly lower ($P <$ 0.001) in phage-treated vs. untreated birds from 6 d

postchallenge until the end of the experiment. To note, data presented here are estimations based on graphical data. An additional trial was performed in which naïve chickens were exposed to *Salmonella*-challenged chickens and subsequently treated with phages; data from this trial were not included in the meta-analysis as the chickens in this trial were not all administered a known quantity of the challenge organism.

Colom et al. (2015) orally administered phages to chicks for 8 d beginning 1 d prior to *Salmonella* Typhimurium challenge. Phages were given as either encapsulated or naked phages. Cecal concentrations of *Salmonella* Typhimurium were significantly different ($P < 0.05$) in all phage-treated chicks vs. untreated chicks at 1 d (naked phage: 2.9 ± 2.3 $\log_{10}$ cfu/g; encapsulated: 3.8 ± 1.2 $\log_{10}$ cfu/g; untreated: 5.8 ± 0.7 $\log_{10}$ cfu/g), 3 d (naked phage: 3.3 ± 2.7 $\log_{10}$ cfu/g; encapsulated: 3.3 ± 2.6 $\log_{10}$ cfu/g; untreated: 6.6 ± 0.5 $\log_{10}$ cfu/g), 6 d (naked phage: 4.1 ± 2.1 $\log_{10}$ cfu/g; encapsulated: 3.2 ± 2.6 $\log_{10}$ cfu/g; untreated: 6.9 ± 0.8 $\log_{10}$ cfu/g), and 8 d (naked phage: 5.2 ± 2.2 $\log_{10}$ cfu/g; encapsulated: 2.9 ± 2.8 $\log_{10}$ cfu/g; untreated: 6.7 ± 0.5 $\log_{10}$ cfu/g) postchallenge (to note, phage administration was ongoing through d 6). Chicks receiving encapsulated phages also had significantly lower ($P < 0.001$) cecal concentrations of *Salmonella* Typhimurium in comparison to untreated chicks at 10 d (encapsulated: 2.5 ± 2.8 $\log_{10}$ cfu/g; untreated: 6.4 ± 1.0 $\log_{10}$ cfu/g) and 15 d (encapsulated: 3.7 ± 1.4 $\log_{10}$ cfu/g; untreated: 5.2 ± 1.3 $\log_{10}$ cfu/g) postchallenge. In addition, concentrations of *Salmonella* Typhimurium were significantly lower ($P < 0.05$) in encapsulated phage-treated chicks vs. naked phage-treated chicks at 8, 10, and 15 d postchallenge.

Drinking Water, Aerosol Spray, and Feed Administration Routes Oral gavage is not the only method of bacteriophage administration to chickens that has been employed in research. Phages have also been administered via aerosol sprays, in feed, and in drinking water.

Luis Vaz et al. (2020) challenged chicks with *Salmonella* Enteritidis and administered a phage cocktail via drinking water. In an initial trial, the phage cocktail was administered for 5 d beginning 5 d postchallenge. Cecal concentrations of *Salmonella* Enteritidis were significantly lower ($P \leq 0.05$) in phage-treated (4.44 ± 0.16 $\log_{10}$ cfu/g) versus untreated (4.82 ± 0.13 $\log_{10}$ cfu/g) birds at all sampling times. In a second trial, the phage cocktail was administered for 5 d beginning 30 d post-challenge. At 1 d post-phage treatment, cecal *Salmonella* Enteritidis concentrations were higher in phage-treated birds ($\sim 3.5 \pm 0.9$ $\log_{10}$ cfu/g) than in untreated birds ($\sim 1.7 \pm 0.9$ $\log_{10}$ cfu/g; estimates based on graphical data, significance not discussed). However, the overall average concentration of *Salmonella* Enteritidis was significantly lower ($P \leq 0.05$) in phage-treated (0.80 ± 0.23 $\log_{10}$ cfu/g) vs. untreated birds (1.88 ± 0.37 $\log_{10}$ cfu/g) from d 4 to d 10 post-phage treatment. The incidence of *Salmonella* Enteritidis in liver, spleen, and cecal tonsil samples did not significantly differ ($P > 0.05$) between phage-treated and untreated birds in either trial.

Borie et al. (2008) administered a single dose of a phage cocktail to chicks via either aerosol spray or drinking water one day prior to challenge with *Salmonella* Enteritidis. At 10 d postchallenge, the overall incidence of *Salmonella* Enteritidis in organ samples (intestine, liver, spleen, and heart) was significantly lower ($P = 0.0084$) in chicks administered phages via aerosol spray (72.7%) than in untreated birds (100%). No significant difference ($P > 0.05$) was observed between the overall incidence of *Salmonella* Enteritidis in organ samples from birds receiving phages via drinking water versus untreated birds. When data from the pool of liver, spleen, and heart samples were analyzed without including intestinal sample data, however, the incidence of *Salmonella* Enteritidis was found to be significantly lower ($P < 0.05$) in chicks administered phages via drinking water (40.9%) than in untreated chicks (77.3%). Additionally, intestinal concentrations of *Salmonella* Enteritidis were significantly lower ($P < 0.001$) in all phage-treated chicks (aerosol spray: 4.04 $\log_{10}$ cfu/mL; drinking water: 4.25 $\log_{10}$ cfu/mL) in comparison to untreated chicks (5.67 $\log_{10}$ cfu/mL).

In a similar experiment, Borie et al. (2009) administered chicks 2 doses of a phage cocktail via aerosol spray 1 d prior to challenge with *Salmonella* Enteritidis. The authors observed significantly lower ($P < 0.05$) incidences of *Salmonella* Enteritidis in organ samples from phage-treated (80%) vs. untreated chicks (100%) at 7 d postchallenge (chicks were considered *Salmonella* Enteritidis-positive if the challenge organism was isolated in either a pool of the liver and spleen, in the cecum, or both). Cecal concentrations of *Salmonella* Enteritidis, however, were not found to be significantly different ($P > 0.05$) between phage-treated (~ 3.98 $\log_{10}$ cfu/g) and untreated chicks (~ 5.19 $\log_{10}$ cfu/g) at this sampling point.

Lim et al. (2012) challenged chicks with *Salmonella* Enteritidis and administered phage via feed (10^9, 10^7, or 10^5 pfu/g feed) for 21 d. Intestinal concentrations of *Salmonella* Enteritidis in challenged chicks receiving phages at 10^9 pfu/g feed were significantly lower ($P < 0.05$) than those in challenged, untreated chicks at 7 d (phage-treated: 5.53 $\log_{10}$ cfu/mL; untreated: 6.39 $\log_{10}$ cfu/mL), 14 d (phage-treated: 5.48 $\log_{10}$ cfu/mL; untreated: 6.55 $\log_{10}$ cfu/mL), and 21 d (~ 3.0 $\log_{10}$ cfu/mL; untreated: ~ 5.6 $\log_{10}$ cfu/mL) post-comingling. When phages were given at 10^7 pfu/g feed, intestinal concentrations of *Salmonella* Enteritidis were significantly lower ($P < 0.05$) in challenged, phage-treated chicks vs. challenged, untreated chicks at 7 d (phage-treated: 5.7 $\log_{10}$ cfu/mL; untreated: 6.39 $\log_{10}$ cfu/mL) and 21 d (phage-treated: ~ 3.3 $\log_{10}$ cfu/mL; untreated: ~ 5.6 $\log_{10}$ cfu/mL) post-comingling. Significant differences ($P < 0.05$) between intestinal concentrations of *Salmonella* Enteritidis in challenged chicks administered phages at 10^5 pfu/g feed (5.53 $\log_{10}$ cfu/mL) and in challenged, untreated chicks (6.39 $\log_{10}$ cfu/mL) were only observed at 7 d post-comingling. The concentrations of *Salmonella* Enteritidis at 21 d postchallenge presented here are estimations based on graphical data. To note,

the authors also measured *Salmonella* concentrations in naïve birds comingled with the *Salmonella*-challenged birds. As in the case with Bardina et al. (2012), concentrations of *Salmonella* in comingled birds were not included in our meta-analysis as such birds were not challenged with a known quantity of *Salmonella*.

Adhikari et al. (2017) also utilized a feed delivery route, administering a phage cocktail to adult chickens at a rate of either 0.1 or 0.2% of their diet. Chickens were given phages for 7 d and then challenged with *Salmonella* Enteritidis. Following bacterial challenge, chickens received phages for an additional 7 d. No significant differences ($P > 0.05$) were observed between the incidences or concentrations of *Salmonella* Enteritidis in fecal samples of phage-treated and untreated chickens at 3 d postchallenge. At 6 d postchallenge, however, fecal concentrations of *Salmonella* Enteritidis were significantly lower ($P < 0.05$) in 0.2% phage-treated birds (0.71 ± 0.34 $\log_{10}$ cfu/g) than in either 0.1% phage-treated (1.57 ± 0.37 $\log_{10}$ cfu/g) or untreated birds (1.57 ± 0.37 $\log_{10}$ cfu/g). The incidence of *Salmonella* Enteritidis in fecal samples from 0.2% phage-treated birds (37.5%) was also significantly lower ($P < 0.05$) than in 0.1% phage-treated birds (75%) and in untreated birds (75%) at this sampling point. At 7 d postchallenge, cecal concentrations of *Salmonella* Enteritidis were significantly lower ($P < 0.05$) in 0.2% phage-treated birds (2.0 ± 0.32 $\log_{10}$ cfu/g) than in either 0.1% phage-treated birds (2.9 ± 0.54 $\log_{10}$ cfu/g) or untreated birds (2.9 ± 0.40 $\log_{10}$ cfu/g). The incidence of *Salmonella* Enteritidis in cecal samples did not significantly differ ($P > 0.05$) across treatment groups, however. Neither incidence nor concentration of *Salmonella* Enteritidis significantly differed between the 0.1% phage-treated group and the untreated group at any sampling point.

Meta-Analysis

Following systematic review, data from the 12 included studies were aggregated for meta-analysis (total inputs: n = 41; total observations: phage-treated, n = 350, untreated, n = 261). Both a funnel plot and an Egger's test ($P = 0.008$) indicated that asymmetry was present in the data set. Asymmetry in these analyses is typically an indicator of small sample bias; however, because the biological properties of phages make it unlikely for phage treatment to cause increases in bacterial concentrations, asymmetry was expected and may not be a reliable indicator of bias. Results of a P-curve analysis to test for evidence of P-hacking indicated that evidential value was present and not absent or inadequate, that P values of the data set were right-skewed ($P < 0.05$), and that P values were not flat ($P > 0.05$), suggesting that P-hacking did not occur and that a true effect was present.

The meta-analysis performed using this data set indicated that, overall, phage treatment significantly reduced ($P < 0.0001$) concentrations of challenge bacteria in phage-treated vs. untreated chickens by an average of 0.82 $\log_{10}$ cfu/g. Data in the aggregate had only "moderate" heterogeneity ($I^2 = 34.5\%$) and had high statistical power (100%) in post-hoc power estimations. As the efficacy of phage treatment can be impacted by various environmental and biological factors, including pH, host range, heat, and the relative concentrations of host bacteria and phages (Iriarte et al., 2007; Huff et al., 2010; Knezevic et al., 2011; Hodyra-Stefaniak et al., 2015; Zhang et al., 2015; El-Dougdoug et al, 2019), after initial analyses data were disaggregated in an attempt to identify factors that significantly influenced phage treatment efficacy. When analyses of disaggregated data had low statistical power, this has been noted in the text. The complete results of the meta-analysis and of the analyses of difference in effect sizes between subgroups of disaggregated data are presented in Tables 2 and 3.

Effect of Sampling Time Phage-mediated lysis of bacterial cells and the subsequent release of progeny phages are time-dependent processes (Delbrück, 1940; Payne and Jansen, 2001, 2003; Kasman et al., 2002; Huff et al., 2006). For this reason, data were disaggregated by sampling time to investigate the impact of the length of time between phage treatment and the measurement of challenge bacteria concentrations on phage treatment efficacy. Phage treatment was found to significantly reduce concentrations of challenge bacteria in phage-treated vs. untreated chickens in samples collected 0 to 7 d ($P = 0.0001$; effect size = -0.99 $\log_{10}$ cfu/g) and 8 to 14 d post-treatment ($P = 0.002$; effect size = -0.74 $\log_{10}$ cfu/g), but not in samples collected >14 d post-treatment ($P = 0.174$; effect size = -0.59 $\log_{10}$ cfu/g). To note, heterogeneity was considerable ($I^2 = 63.6\%$) in the subgroup of samples collected >14 d post-treatment. Phage treatment effects did not significantly differ across sampling time subgroups ($P = 0.55$); however, this analysis of between-group differences had low statistical power (<15%).

Effect of Age All inputs in this meta-analysis utilized gastrointestinal bacteria to challenge chickens, but the gastrointestinal tracts of adult chickens and of chicks differ in their response to bacterial colonization (Sahin et al., 2003, 2015; Beal et al., 2004; Bar-Shira and Friedman, 2006; Han et al., 2016). As such, data were disaggregated by experimental bird age to determine the impact of age on phage treatment efficacy. All phage-treated chickens were observed to have significantly lower concentrations of challenge bacteria in comparison to untreated chickens (>14 doa adults: $P = 0.004$; effect size = -1.51 $\log_{10}$ cfu/g; <14 doa chicks: $P < 0.001$; effect size = -0.72 $\log_{10}$ cfu/g), and phage treatment effect sizes were found to differ only numerically between adult and chick subgroups ($P = 0.22$, statistical power = 23.5%). Both subgroups were then further disaggregated by sampling time. Among adult chickens, phage treatment significantly reduced concentrations of challenge bacteria in samples collected 0 to 7 d post-treatment ($P = 0.007$, effect size = -1.20 $\log_{10}$ cfu/g) but not in samples collected 8 to 14 d post-treatment ($P = 0.091$, effect size = -0.84 $\log_{10}$ cfu/g). To note, the latter subgroup contained only one input (total

Table 2. Results of a meta-analysis of studies measuring concentrations of challenge bacteria following phage administration to chickens.

Subgroup disaggregation factor	I^2 (%)	Effect size[1]	Effect significance (P)	Power[2] (%)	Number of observations, (Treatment; control)	Total observations
All observations together	34.5	−0.82 ± 0.14	< 0.0001	100	450; 261	711
Sample collection time						
Samples collected 0 to 7 d post phage treatment	21.6	-0.99 ± 0.20	0.0001	99.99	188; 112	300
Samples collected 8 to 14 d post phage treatment	22.0	−0.74 ± 0.20	0.002	99.98	170; 102	272
Samples collected >14 d post phage treatment	63.6	−0.59 ± 0.39	0.174	92.71	92; 47	139
Age of chickens						
Adults (> 14 days of age	1.2	−1.15 ± 0.32	0.004	98.84	95; 64	159
Adults, samples collected 0 to 7 d post phage treatment	10.0	−1.20 ± 0.36	0.007	97.75	86; 55	141
Adults, samples collected 8 to 14 d post phage treatment	–[3]	−0.84 ± 0.50	0.091	30.51	9; 9	18
Adults, samples collected >14 d post phage treatment	–[4]	–	–	–	–	–
Chicks (<14 days of age	42.6	−0.72 ± 0.15	< 0.001	100	355; 197	552
Chicks, samples collected 0 to 7 d post phage treatment	38.7	−0.83 ± 0.25	0.010	95.89	102; 57	159
Chicks, samples collected 8 to 14 d post phage treatment	28.0	−0.73 ± 0.22	0.007	99.95	161; 93	254
Chicks, samples collected >14 d post phage treatment	63.6	−0.59 ± 0.39	0.174	92.71	92; 47	139
Challenge bacteria						
Salmonella	44.3	−0.95 ± 0.17	< 0.001	100	331; 183	514
Salmonella, samples collected 0 to 7 d post phage treatment	0.0	−1.01 ± 0.16	< 0.0001	99.65	129; 72	201
Salmonella, samples collected 8 to 14 d post phage treatment	38.6	−0.93 ± 0.27	0.009	99.29	140; 83	223
Salmonella, samples collected >14 d post phage treatment	83.6	−1.01 ± 0.89	0.338	69.17	62; 28	90
Salmonella, adult chickens only	0.0	−0.77 ± 0.11	0.0004	93.73	66; 43	109
Salmonella, chicks only	59.5	−1.04 ± 0.24	0.0005	100	265; 140	405
Campylobacter	0.0	−0.60 ± 0.24	0.024	99.69	119; 78	197
Campylobacter, samples collected 0 to 7 d post phage treatment	43.3	−1.09 ± 0.53	0.076	84.64	59; 40	99
Campylobacter, samples collected 8 to 14 d post phage treatment	0.0	−0.29 ± 0.07	0.024	64.61	30; 19	49
Campylobacter, samples collected >14 d post phage treatment	0.0	−0.37 ± 0.10	0.035	64.61	30; 19	49
Campylobacter, adult chickens only	46.4	−2.13 ± 0.91	0.079	56.75	29; 21	50
Campylobacter, chicks only	0.0	−0.28 ± 0.05	0.0003	98.14	90; 57	147
Prophylactic vs. therapeutic						
Prophylactic[5]	54.1	−0.94 ± 0.21	0.0002	100	293; 153	446
Prophylactic, samples collected 0 to 7 d post phage treatment	0.0	−1.16 ± 0.20	0.0012	97.73	100; 50	150
Prophylactic, samples collected 8 to 14 d post phage treatment	48.3	−0.89 ± 0.34	0.034	81.16	66; 32	98
Prophylactic, samples collected >14 d post phage treatment	78.7	−0.80 ± 0.69	0.312	96.5	127; 71	198
Prophylactic, adult chickens only	0.0	−0.85 ± 0.09	0.065	61.07	32; 16	48
Prophylactic, chicks only	58.8	−0.96 ± 0.24	0.0008	99.99	261; 137	398
Therapeutic	0.0	−0.69 ± 0.18	0.001	99.98	157; 108	265
Therapeutic, samples collected 0 to 7 d post phage treatment	21.8	−0.92 ± 0.33	0.016	98.17	88; 62	150
Therapeutic, samples collected 8 to 14 d post phage treatment	0.0	−0.57 ± 0.16	0.021	82.71	43; 31	74
Therapeutic, samples collected >14 d post phage treatment	0.0	−0.42 ± 0.11	0.062	56.09	26; 15	41
Therapeutic, adult chickens only	18.2	−1.27 ± 0.41	0.012	94.62	65; 48	111
Therapeutic, chicks only	0.0	−0.37 ± 0.08	0.0012	98.55	94; 60	154
Frequency of phage administration						
Single dose	2.8	−0.82 ± 0.24	0.004	99.91	155; 85	240
Single dose, samples collected 0 to 7 d post phage treatment	37.2	−1.17 ± 0.45	0.028	86.91	67; 41	108
Single dose, samples collected 8 to 14 d post phage treatment	0.0	−0.58 ± 0.09	0.0085	89.59	66; 33	99
Single dose, samples collected >14 d post phage treatment	0.0	−0.50 ± 0.07	0.085	45.95	22; 11	33
Single dose, adult chickens only	17.6	−1.54 ± 0.52	0.021	82.72	45; 30	75
Single dose, chicks only	0.0	−0.46 ± 0.09	0.002	98.59	110; 55	165
Multiple doses	46.6	−0.83 ± 0.17	< 0.0001	100	295; 176	471
Multiple doses, samples collected 0 to 7 d post phage treatment	6.0	−0.93 ± 0.18	0.0006	99.56	121; 71	192
Multiple doses, samples collected 8 to 14 d post phage treatment	44.7	−0.87 ± 0.31	0.024	97.58	104; 69	173
Multiple doses, samples collected >14 d post phage treatment	73.9	−0.67 ± 0.56	0.289	77.86	70; 36	106
Multiple doses, adult chickens only	0.0	−0.72 ± 0.13	0.012	86.82	50; 34	84
Multiple doses, chicks only	54.5	−0.87 ± 0.21	0.0006	99.99	245; 142	387
Administration route						
Gavage	47.6	−0.88 ± 0.21	0.0002	99.99	235; 145	380

(*continued*)

Table 2 (*Continued*)

Subgroup disaggregation factor	I^2 (%)	Effect size[1]	Effect significance (P)	Power[2] (%)	Number of observations, (Treatment; control)	Total observations
Gavage, samples collected 0 to 7 d post phage treatment	33.0	−1.13 ± 0.29	0.002	99.36	109; 69	178
Gavage, samples collected 8 to 14 d post phage treatment	53.8	−0.82 ± 0.44	0.110	85.28	64; 39	103
Gavage, samples collected >14 d post phage treatment	58.9	−0.48 ± 0.44	0.323	83.7	62; 37	99
Gavage, adult chickens only	17.6	−1.54 ± 0.52	0.021	82.72	45; 30	75
Gavage, chicks only	51.6	−0.70 ± 0.22	0.005	99.93	190; 115	305
Feed	0.0	−1.05 ± 0.10	0.0004	97.83	122; 46	168
Feed, samples collected 0 to 7 d post phage treatment	0.0	−1.01 ± 0.14	0.020	83.73	62; 26	88
Feed, samples collected 8 to 14 d post phage treatment	–	−0.94 ± 0.38	0.014	47.2	30; 10	40
Feed, samples collected >14 d post phage treatment	–	−1.28 ± 0.40	0.0012	47.2	30; 10	40
Feed, adult chickens only	0.0	−0.85 ± 0.09	0.065	61.07	32; 16	48
Feed, chicks only	0.0	−1.15 ± 0.11	0.009	90.54	90; 30	120
Water	0.0	−0.65 ± 0.12	0.006	92.41	56; 44	100
Water, samples collected 0 to 7 d post phage treatment	0.0	−0.36 ± 0.03	0.050	51.28	17; 17	34
Water, samples collected 8 to 14 d post phage treatment	0.0	−0.82 ± 0.08	0.009	78.01	39; 27	66
Water, samples collected >14 d post phage treatment	–	–	–	–	–	–
Water, adult chickens only	0.0	−0.58 ± 0.25	0.263	53.58	18; 18	36
Water, chicks only	0.0	−0.69 ± 0.15	0.046	70.84	28; 26	54
Spray[6]	0.0	−0.44 ± 0.21	0.290	76.21	37; 26	63
Phage protection						
Protected	21.3	−0.95 ± 0.23	0.0009	99.9	153; 84	237
Protected, samples collected 0 to 7 d post phage treatment	36.6	−1.18 ± 0.39	0.013	91.86	81; 48	129
Protected, samples collected 8 to 14 d post phage treatment	42.2	−0.76 ± 0.40	0.200	56.41	36; 18	54
Protected, samples collected > 14 d post phage treatment	0.0	−0.70 ± 0.19	0.068	66.17	36; 18	54
Protected, adult chickens only	17.6	−1.54 ± 0.52	0.021	82.72	45; 30	75
Protected, chicks only	14.1	−0.66 ± 0.19	0.008	98.45	108; 54	162
Unprotected	43.1	−0.75 ± 0.17	0.0003	100	297; 177	474
Unprotected, samples collected 0 to 7 d post phage treatment	5.4	−0.87 ± 0.19	0.002	99.12	107; 64	171
Unprotected, samples collected 8 to 14 d post phage treatment	24.1	−0.74 ± 0.25	0.016	99.85	134; 84	218
Unprotected, samples collected >14 d post phage treatment	77.7	−0.61 ± 0.71	0.435	68.61	56; 29	85
Unprotected, adult chickens only	0.0	−0.72 ± 0.13	0.012	86.82	50; 34	84
Unprotected, chicks only	51.8	−0.76 ± 0.22	0.002	99.99	247; 143	390
Poly-phage vs. single phage						
Poly-phage	49.8	−0.84 ± 0.20	0.0004	100	258; 160.5	418.5
Poly-phage, samples collected 0 to 7 d post phage treatment	24.0	−0.90 ± 0.23	0.005	98.43	94; 58.5	152.5
Poly-phage, samples collected 8 to 14 d post phage treatment	40.8	−0.87 ± 0.29	0.016	98.79	121; 78.5	199.5
Poly-phage, samples collected >14 d post phage treatment	79.0	−0.77 ± 0.90	0.451	58.68	43; 23.5	66.5
Poly-phage, adult chickens only	0.0	−0.72 ± 0.13	0.012	86.82	50; 34	84
Poly-phage, chicks only	58.9	−0.89 ± 0.25	0.003	99.97	208; 126.5	334.5
Single phage	9.4	−0.80 ± 0.20	0.0008	99.98	192; 100.5	292.5
Single phage, samples collected 0 to 7 d post phage treatment	26.6	−1.09 ± 0.35	0.010	97.94	94; 53.5	147.5
Single phage, samples collected 8 to 14 d post phage treatment	0.0	−0.57 ± 0.20	0.066	78.11	49; 23.5	72.5
Single phage, samples collected >14 d post phage treatment	10.9	−0.70 ± 0.28	0.091	78.11	49; 23.5	72.5
Single phage, adult chickens only	17.6	−1.54 ± 0.52	0.021	82.72	45; 30	75
Single phage, chicks only	0.0	−0.62 ± 0.14	0.0013	99.73	147; 70.5	217.5

[1]All effect sizes are given as $\log_{10}$ cfu/g ± SE.
[2]For power analysis calculations heterogeneity was considered low between 0 and 33%, moderate between 34 and 66%, and high between 67 and 100%.
[3]Subgroups without an I^2 value contained only one input.
[4]Subgroups with no data contained no inputs.
[5]Bacteriophage administration on the same day as bacterial challenge was considered prophylactic treatment.
[6]Chicks were used and samples were collected 8 to 14 d post-treatment in all inputs involving the administration of phages via aerosol spray. Because of this, it was not necessary to disaggregate data by age or sample collection time for this subgroup.

observations: n = 18) and a post-hoc power analysis indicated that the statistical power of this subgroup was low (30.51%). Among chicks, phage treatment significantly reduced concentrations of challenge bacteria in samples collected 0 to 7 d ($P = 0.001$, effect size = −0.83 $\log_{10}$ cfu/g) and 8 to 14 d ($P = 0.007$, effect size = −0.73 $\log_{10}$ cfu/g) post-treatment, but not in samples collected >14 d post-treatment (P = 0.174, effect size = −0.59 $\log_{10}$ cfu/g). The >14 d post-treatment subgroup had adequate power (92.71%) but considerable heterogeneity ($I^2 = 63.6\%$). No significant differences in phage treatment effect sizes were observed across sampling time subgroups within either the adult ($P = 0.55$) or the chick subgroup ($P = 0.86$), though the statistical power of between-group difference analyses was low (<10%).

Table 3. Results of analyses of difference in effect size across subgroups of data from studies measuring concentrations of challenge bacteria following phage administration to chickens,

Subgroup 1 Subgroup 1A	Effect size[1]	Subgroup 1B	Effect size	Subgroup 1C	Effect size
All observations					
Samples collected 0 to 7 d post-treatment	-0.99 ± 0.20^{a}	Samples collected 8 to 14 d post-treatment	-0.74 ± 0.20^{a}	Samples collected >14 d post-treatment	-0.59 ± 0.39^{a}
Adults	-1.15 ± 0.32^{a}	Chicks	-0.72 ± 0.15^{a}		
Salmonella challenge	-0.95 ± 0.17^{a}	*Campylobacter* challenge	-0.60 ± 0.24^{a}		
Prophylactic	-0.94 ± 0.21^{a}	Therapeutic	-0.69 ± 0.18^{a}		
Single dose of phages	-0.82 ± 0.24^{a}	Multiple doses of phages	-0.83 ± 0.17^{a}		
Gavage	-0.88 ± 0.21^{ab}	Feed	-1.05 ± 0.10^{a}	Water -0.65 ± 0.12^{b}	Spray -0.44 ± 0.21^{b}
Protected	-0.95 ± 0.23^{a}	Unprotected	-0.75 ± 0.17^{a}		
Poly-phage	-0.84 ± 0.20^{a}	Single phage type	-0.80 ± 0.20^{a}		
Adults					
Samples collected 0 to 7 d post-treatment	-1.20 ± 0.36^{a}	Samples collected 8 to 14 d post-treatment	-0.84 ± 0.50^{a}	Samples collected >14 d post-treatment	–[2]
Chicks					
Samples collected 0 to 7 d post-treatment	-0.83 ± 0.25^{a}	Samples collected 8 to 14 d post-treatment	-0.73 ± 0.22^{a}	Samples collected >14 d post-treatment	-0.59 ± 0.39^{a}
Salmonella challenge					
Samples collected 0 to 7 d post-treatment	-1.01 ± 0.16^{a}	Samples collected 8 to 14 d post-treatment	-0.93 ± 0.27^{a}	Samples collected >14 d post-treatment	-1.01 ± 0.89^{a}
Adults	-0.77 ± 0.11^{a}	Chicks	-1.04 ± 0.24^{a}		
Campylobacter challenge					
Samples collected 0 to 7 d post-treatment	-1.09 ± 0.53^{a}	Samples collected 8 to 14 d post-treatment	-0.29 ± 0.07^{a}	Samples collected >14 d post-treatment	-0.37 ± 0.10^{a}
Adults	-2.13 ± 0.91^{a}	Chicks	-0.28 ± 0.05^{b}		
Prophylactic					
Samples collected 0 to 7 d post-treatment	-1.16 ± 0.20^{a}	Samples collected 8 to 14 d post-treatment	-0.89 ± 0.34^{a}	Samples collected >14 d post-treatment	-0.80 ± 0.69^{a}
Adults	-0.85 ± 0.09^{a}	Chicks	-0.96 ± 0.24^{a}		
Therapeutic					
Samples collected 0 to 7 d post-treatment	-0.92 ± 0.33^{a}	Samples collected 8 to 14 d post-treatment	-0.57 ± 0.16^{a}	Samples collected >14 d post-treatment	-0.42 ± 0.11^{a}
Adults	-1.27 ± 0.41^{a}	Chicks	-0.37 ± 0.08^{b}		
Single dose of phages					
Samples collected 0 to 7 d post-treatment	-1.17 ± 0.45^{a}	Samples collected 8 to 14 d post-treatment	-0.58 ± 0.09^{a}	Samples collected >14 d post-treatment	-0.50 ± 0.07^{a}
Adults	-1.54 ± 0.52^{a}	Chicks	-0.46 ± 0.09^{b}		
Multiple doses of phages					
Samples collected 0 to 7 d post-treatment	-0.93 ± 0.18^{a}	Samples collected 8 to 14 d post-treatment	-0.87 ± 0.31^{a}	Samples collected >14 d post-treatment	-0.67 ± 0.56^{a}
Adults	-0.72 ± 0.13^{a}	Chicks	-0.87 ± 0.21^{a}		
Gavage					
Samples collected 0 to 7 d post-treatment	-1.13 ± 0.29^{a}	Samples collected 8 to 14 d post-treatment	-0.82 ± 0.44^{a}	Samples collected >14 d post-treatment	-0.48 ± 0.44^{a}
Adults	-1.54 ± 0.52^{a}	Chicks	-0.70 ± 0.22^{a}		
Feed					
Samples collected 0 to 7 d post-treatment	-1.01 ± 0.14^{a}	Samples collected 8 to 14 d post-treatment	-0.94 ± 0.38^{a}	Samples collected >14 d post-treatment	-1.28 ± 0.40^{a}
Adults	-0.85 ± 0.09^{b}	Chicks	-1.15 ± 0.11^{a}		
Water					
Samples collected 0 to 7 d post-treatment	-0.36 ± 0.03^{B}	Samples collected 8 to 14 d post-treatment	-0.82 ± 0.08^{A}	Samples collected >14 d post-treatment	–[2]
Adults	-0.58 ± 0.25^{a}	Chicks	-0.69 ± 0.15^{a}		
Spray[3]					
Protected					
Samples collected 0 to 7 d post-treatment	-1.18 ± 0.39^{a}	Samples collected 8 to 14 d post-treatment	-0.76 ± 0.40^{a}	Samples collected >14 d post-treatment	-0.70 ± 0.19^{a}
Adults	-1.54 ± 0.52^{a}	Chicks	-0.66 ± 0.19^{a}		
Unprotected					
Samples collected 0 to 7 d post-treatment	-0.87 ± 0.19^{a}	Samples collected 8 to 14 d post-treatment	-0.74 ± 0.25^{a}	Samples collected >14 d post-treatment	-0.61 ± 0.71^{a}
Adults	-0.72 ± 0.13^{a}	Chicks	-0.76 ± 0.22^{a}		
Poly-phage					
Samples collected 0 to 7 d post-treatment	-0.90 ± 0.23^{a}	Samples collected 8 to 14 d post-treatment	-0.87 ± 0.29^{a}	Samples collected >14 d post-treatment	-0.77 ± 0.90^{a}
Adults	-0.72 ± 0.13^{a}	Chicks	-0.89 ± 0.25^{a}		
Single phage type					
Samples collected 0 to 7 d post-treatment	-1.09 ± 0.35^{a}	Samples collected 8 to 14 d post-treatment	-0.57 ± 0.20^{a}	Samples collected >14 d post-treatment	-0.70 ± 0.28^{a}
Adults	-1.54 ± 0.52^{a}	Chicks	-0.62 ± 0.14^{a}		

[1]All effect sizes are given as $\log_{10}$ cfu/g $\pm$ SE.
[2]No inputs available.
[3]All inputs in this subgroup involved the same sampling time and experimental bird age.
[a,b]Means within a row lacking a common superscript differ significantly ($P < 0.05$).
[A,B]Means within a row lacking a common superscript differ significantly ($P < 0.01$).

Effect of Challenge Bacteria Due to the inherent biological differences between *Salmonella* spp. and *Campylobacter* spp., the 2 genera of bacteria utilized as challenge organisms in the studies included in this meta-analysis, data disaggregation also occurred on the basis of challenge bacteria genus. Significantly lower concentrations of challenge bacteria were observed in phage-treated birds vs. untreated birds in both *Salmonella* spp. ($P < 0.001$; effect size = -0.95 $\log_{10}$ cfu/g) and *Campylobacter* spp. ($P = 0.024$; effect size = -0.60 $\log_{10}$ cfu/g) subgroups, and effect sizes did not significantly differ ($P = 0.24$, statistical power = 21.6%) between these subgroups. *Salmonella* and *Campylobacter* spp. subgroups were further disaggregated by both sampling time and age. Concentrations of challenge bacteria in phage-treated birds challenged with *Salmonella* spp. were significantly lower than in untreated birds in samples collected 0 to 7 d ($P < 0.0001$, effect size -1.01 $\log_{10}$ cfu/g) and 8 to 14 d post-treatment ($P = 0.009$, effect size = -0.93 $\log_{10}$ cfu/g), but not in samples collected >14 d post-treatment ($P = 0.338$, effect size = -1.0089 $\log_{10}$ cfu/g). To note, heterogeneity was high ($I^2 = 83.6\%$) and power was inadequate (69.17%) when samples were collected > 14 d post-treatment. Phage-treatment significantly reduced concentrations of *Salmonella* spp. in both the adult ($P = 0.0004$, effect size = -0.77 $\log_{10}$ cfu/g) and chick ($P = 0.0005$, effect size = -1.04 $\log_{10}$ cfu/g) subgroups, though heterogeneity was considerable ($I^2 = 59.5\%$) in the chick subgroup. No significant differences in effect size were observed across age ($P = 0.30$) or sample collection time subgroups ($P = 0.97$) within the *Salmonella* subgroup. However, the statistical power of these between-subgroup difference analyses was low (17.9%; <6%, respectively).

When *Campylobacter* spp. were utilized, phage treatment significantly reduced challenge bacteria concentrations in samples collected 8 to 14 d ($P = 0.024$, effect size = -0.29 $\log_{10}$ cfu/g) and >14 d ($P = 0.035$, effect size = -0.37 $\log_{10}$ cfu/g) post-treatment and in chicks ($P = 0.0003$, effect size = -0.28 $\log_{10}$ cfu/g). Phage treatment was not observed to have significant effects in samples collected 0 to 7 d post-treatment ($P = 0.076$, effect size = -1.09 $\log_{10}$ cfu/g) or in adult chickens ($P = 0.079$, effect size = -2.13 $\log_{10}$ cfu/g). Statistical power was low in the adult subgroup (56.75%), but adequate in the 0 to 7 d post-treatment sampling time subgroup (84.64%). Within the *Campylobacter* subgroup, phage treatment effects were found to be significantly greater ($P = 0.04$) in adult chickens (effect size = -2.13 $\log_{10}$ cfu/g) than in chicks (effect size = -0.28 $\log_{10}$ cfu/g) despite the low statistical power of this analysis (63.06%). No significant differences in effect size were found across sampling time subgroups ($P = 0.27$, statistical power <32%).

Effect of Prophylactic vs. Therapeutic Administration As bacteriophage replication requires the presence of sufficient concentrations of available host bacteria, the timing of phage treatment relative to bacterial challenge may impact phage replication. To examine the effect of phage administration timing on phage treatment efficacy, data were disaggregated into prophylactic and therapeutic administration subgroups. In this meta-analysis, phage administration beginning before or at the same time as bacterial challenge was considered prophylactic; phage administration beginning after bacterial challenge was considered therapeutic. Phage-treated chickens had significantly lower concentrations of challenge bacteria compared to untreated chickens in both prophylactic ($P = 0.0002$; effect size = -0.94 $\log_{10}$ cfu/g) and therapeutic ($P = 0.001$; effect size = -0.69 $\log_{10}$ cfu/g) subgroups, though heterogeneity was considerable ($I^2 = 54.1\%$) in the prophylactic group. No significant differences in effect size were found between these subgroups ($P = 0.37$, statistical power = 14.5%). As before, subgroups were further disaggregated by bird age and sampling time. When phages were prophylactically administered, phage treatment significantly reduced concentrations of challenge bacteria in samples collected 0 to 7 d ($P = 0.0012$, effect size = -1.16 $\log_{10}$ cfu/g) and 8 to 14 d post-treatment ($P = 0.034$, effect size = -0.89 $\log_{10}$ cfu/g), but not in samples collected >14 d post-treatment ($P = 0.312$, effect size = -0.80 $\log_{10}$ cfu/g). To note, there was high heterogeneity ($I^2 = 78.7\%$) across inputs with sampling times >14 d post-treatment. Prophylactic phage treatment also significantly reduced challenge bacteria concentrations in chicks ($P = 0.0008$, effect size = -0.96 $\log_{10}$ cfu/g), though this subgroup had considerable heterogeneity ($I^2 = 58.8\%$). No significant differences in challenge bacteria concentrations were found in prophylactically treated adult chickens ($P = 0.065$, effect size = -0.85 $\log_{10}$ cfu/g), though the statistical power of this analysis was low (61.07%). No significant differences in effect size were observed across prophylactic subgroups divided by sampling time ($P = 0.73$) or by age ($P = 0.67$), though the statistical power of between-group analyses was low in both cases (<11%; 7.1%, respectively).

When phages were administered therapeutically, phage treatment significantly reduced concentrations of challenge bacteria in phage-treated vs. untreated birds in samples collected 0 to 7 d ($P = 0.016$, effect size = -0.92 $\log_{10}$ cfu/g) and 8 to 14 d ($P = 0.021$, effect size = -0.58 $\log_{10}$ cfu/g) post-treatment and in both adults ($P = 0.012$, effect size = -1.27 $\log_{10}$ cfu/g) and chicks ($P = 0.0012$, effect size = -0.37 $\log_{10}$ cfu/g). In samples collected >14 d post-treatment ($P = 0.062$, effect size = -0.42 $\log_{10}$ cfu/g), challenge bacteria concentrations were only numerically lower in phage-treated vs. untreated birds; however, low statistical power (56.09%) may have contributed to the absence of significance in this group. The effect of therapeutic phage treatment was found to be significantly greater ($P = 0.03$) in adult chickens (effect size = -1.27 $\log_{10}$ cfu/g) than in chicks (effect size = -0.37 $\log_{10}$ cfu/g), though the statistical power of this analysis was low (58.0%). There were no significant differences in effect size across therapeutic subgroups divided by sampling time ($P = 0.30$, statistical power <31%).

Effect of Repeated Administration The number phage treatments administered could also impact overall treatment efficacy. For this reason, data were

disaggregated into a subgroup in which phages were given only once and a subgroup in which phages were administered repeatedly. In both subgroups, phage-treated chickens had significantly lower concentrations of challenge bacteria versus untreated chickens (single dose: $P = 0.004$, effect size = -0.82 $\log_{10}$ cfu/g; multiple doses: $P < 0.0001$, effect size = -0.83 $\log_{10}$ cfu/g). No significant difference in phage treatment effect size was found between these subgroups ($P = 0.98$), though the statistical power of this analysis was low (5.0%). These subgroups were further disaggregated by sampling time and by age. When phages were administered only once, phage treatment significantly reduced concentrations of challenge bacteria in phage-treated vs. untreated chickens in samples collected 0 to 7 d ($P = 0.028$, effect size = -1.17 $\log_{10}$ cfu/g) and 8 to 14 d ($P = 0.009$, effect size = -0.58 $\log_{10}$ cfu/g) post-treatment, but not in samples collected > 14 d post-treatment ($P = 0.085$, effect size = -0.50 $\log_{10}$ cfu/g; statistical power = 45.95%). Single dose phage administration also significantly reduced challenge bacteria concentrations in both adult ($P = 0.021$, effect size = -1.54 $\log_{10}$ cfu/g) and chick subgroups ($P = 0.002$, effect size = -0.46 $\log_{10}$ cfu/g). The effect of phage treatment was observed to be significantly greater ($P = 0.04$) in adults than in chicks, despite low statistical power in this analysis (53.9%). No significant differences in effect size were observed across single dose subgroups divided by sample collection time ($P = 0.28$, statistical power <32%).

When phages were administered repeatedly, concentrations of challenge bacteria were significantly lower in phage-treated vs. untreated birds in samples collected 0 to 7 d ($P = 0.0006$, effect size = -0.93 $\log_{10}$ cfu/g) and 8 to 14 d post-treatment ($P = 0.024$, effect size = -0.87 $\log_{10}$ cfu/g) and in both adults ($P = 0.012$, effect size = -0.72 $\log_{10}$ cfu/g) and chicks ($P = 0.0006$, effect size = -0.87 $\log_{10}$ cfu/g), though there was considerable heterogeneity ($I^2 = 54.5\%$) in the chick subgroup. Phage treatment effects were not significant in samples collected >14 d post-treatment ($P = 0.289$, effect size = -0.67 $\log_{10}$ cfu/g); however, heterogeneity in this subgroup was high ($I^2 = 73.9\%$) and statistical power was low (77.86%). Within the multiple dose subgroup, no significant differences in effect size were observed across sampling time ($P = 0.90$, statistical power <8%) or age subgroups ($P = 0.57$, statistical power = 8.8%).

Effect of Administration Route Inputs in this meta-analysis were drawn from studies that employed oral gavage, feed, drinking water, and aerosol spray administration routes. As different routes may be more or less conducive to enabling viable phages to reach the site of bacterial colonization (Carvalho et al., 2010; Lim et al., 2012), data were disaggregated by administration route in order to evaluate the impact of this factor on phage treatment efficacy. Significantly reduced concentrations of challenge bacteria were observed in phage-treated vs. untreated birds when phages were administered via oral gavage ($P = 0.0002$, effect size = -0.88 $\log_{10}$ cfu/g), feed ($P = 0.0004$, effect size = -1.05 $\log_{10}$ cfu/g), and drinking water ($P = 0.006$, effect size = -0.65 $\log_{10}$ cfu/g), but not when phages were given via aerosol spray ($P = 0.290$, effect size = -0.44 $\log_{10}$ cfu/g; statistical power = 76.21%). Phage treatment effects were significantly greater in the feed subgroup than in either the water ($P = 0.009$) or aerosol spray ($P = 0.009$) subgroups. No other significant differences between subgroup effect sizes were observed (feed vs. gavage: $P = 0.47$; gavage vs. water: $P = 0.33$; gavage vs. spray: $P = 0.14$; spray vs. water: $P = 0.39$). To note, all analyses of effect size differences between subgroups had low statistical power (feed vs. water: 74.7%; feed vs. spray: 74.4%; feed vs. gavage: 11.2%; gavage vs. water: 16.6%; gavage vs. spray: 32.1%; spray vs. water: 13.8%).

Following initial analyses, subgroups were further divided by sample collection time and experimental bird age. When phages were administered via oral gavage, phage-treated birds had significantly lower concentrations of challenge bacteria than untreated birds in samples collected 0 to 7 d post-treatment ($P = 0.002$, effect size = -1.13 $\log_{10}$ cfu/g) but not in samples collected 8 to 14 d ($P = 0.110$, effect size = -0.82 $\log_{10}$ cfu/g) or >14 d post-treatment ($P = 0.323$, effect size = -0.49 $\log_{10}$ cfu/g). Oral phage administration also resulted in significantly lower concentrations of challenge bacteria in both phage-treated adult chickens ($P = 0.021$, effect size = -1.54 $\log_{10}$ cfu/g) and chicks ($P = 0.005$, effect size = -0.70 $\log_{10}$ cfu/g) versus untreated birds. To note, heterogeneity was considerable in samples collected 8 to 14 d post-treatment ($I^2 = 53.8\%$), >14 d post-treatment ($I^2 = 58.9\%$), and among adult birds ($I^2 = 51.6\%$). Within the oral gavage subgroup, no significant differences in effect size were found across subgroups divided by sampling time ($P = 0.46$, statistical power <24%) or age ($P = 0.13$, statistical power = 32.2%).

When phages were given in feed, phage-treated birds had significantly lower concentrations of challenge bacteria versus untreated birds at all sampling times (0−7 d: $P = 0.02$, effect size = -1.01 $\log_{10}$ cfu/g; 8−14 d: $P = 0.014$, effect size = -0.94 $\log_{10}$ cfu/g; >14 d: $P = 0.0012$, effect size = -1.28 $\log_{10}$ cfu/g) and in the chick subgroup ($P = 0.009$, effect size = -1.15 $\log_{10}$ cfu/g). No significant differences in challenge bacteria concentrations were observed between phage-treated and untreated birds in the adult subgroup ($P = 0.065$, effect size = -0.85 $\log_{10}$ cfu/g; statistical power = 61.07%), however. Phage treatment was found to have a significantly larger ($P = 0.03$) effect in chicks (effect size = -1.15 $\log_{10}$ cfu/g) than in adult chickens (effect size = -0.85 $\log_{10}$ cfu/g), although the statistical power of this analysis was low (56.9%). No significant differences in phage treatment effect size were observed across sampling time subgroups ($P = 0.78$, statistical power <11%). To note, conclusions regarding phage administration via feed should be tempered as there were a limited number of inputs per subgroup when the feed subgroup was further disaggregated by sample collection time and age (total inputs/subgroup: 1−3; total observations/subgroup: 40−120).

When phages were administered via drinking water, significantly lower concentrations of challenge bacteria were observed in phage-treated vs. untreated chickens in

samples collected 0 to 7 d ($P = 0.050$, effect size = −0.36 $\log_{10}$ cfu/g) and 8 to 14 d ($P = 0.009$, effect size = −0.82 $\log_{10}$ cfu/g) post-treatment and in the chick subgroup ($P = 0.046$, effect size = −0.69 $\log_{10}$ cfu/g). Concentrations of challenge bacteria were only numerically different between phage-treated and untreated chickens in the adult subgroup ($P = 0.263$, effect size = −0.58 $\log_{10}$ cfu/g; statistical power = 53.58%). Within the drinking water subgroup, significantly greater ($P < 0.0001$) phage treatment effects were observed in samples collected 8 to 14 d post-treatment (effect size = −0.82 $\log_{10}$ cfu/g) than in samples collected 0 to 7 d post-treatment (effect size = −0.36 $\log_{10}$ cfu/g). No inputs in which samples were collected >14 d post-treatment were available. No significant differences in effect size were observed between the adult and chick subgroups ($P = 0.70$); however, statistical power of this analysis of between-group difference was low (6.7%). To note, further disaggregating inputs in the drinking water subgroup also resulted in a limited number of inputs per subgroup (total inputs/subgroup: 2−3; total observations/subgroup: 34−100). When phages were administered via aerosol spray, concentrations of challenge bacteria did not significantly differ between phage-treated and untreated birds ($P = 0.290$, effect size = −0.44 $\log_{10}$ cfu/g). Only 2 inputs (total observations: n = 63) were available in this subgroup; both involved samples collected 8 to 14 d post-treatment from chicks. Consequently, this subgroup was not further disaggregated by sampling time or animal age.

Effect of Phage Treatment Preparation Method Given the gastrointestinal colonization habit of the challenge bacteria employed in these studies as well as bacteriophages' sensitivity to low pH, which can lead to limited phage survival in the gastrointestinal tract (Ma et al., 2008; Knezevic et al., 2011), analyses of data disaggregated by phage preparation method were also performed. For the purposes of this meta-analysis, phages were considered "protected" if they were either microencapsulated or given in solution with calcium carbonate (~30% $CaCO_3$ wt/vol) and "unprotected" if they were administered without any protective preparation methods. All phage-treated birds were found to have significantly lower concentrations of challenge bacteria than untreated birds (protected: $P = 0.0009$; effect size = −0.95 $\log_{10}$ cfu/g; unprotected: $P = 0.0003$, effect size = −0.75 $\log_{10}$ cfu/g). No significant differences in phage treatment effect size were found between the 2 subgroups ($P = 0.49$), though statistical power for this between-group analysis of difference was low (10.8%). When data were further disaggregated by age and sample collection time, protected bacteriophage administration was found to significantly reduce concentrations of challenge bacteria in phage-treated vs. untreated birds in samples collected 0 to 7 d post-treatment ($P = 0.013$, effect size = −1.18 $\log_{10}$ cfu/g) and in both adult ($P = 0.021$, effect size = −1.54 $\log_{10}$ cfu/g) and chick ($P = 0.008$, effect size = −0.66 $\log_{10}$ cfu/g) subgroups. Concentrations of challenge bacteria did not significantly differ between protected phage-treated and untreated birds in samples collected 8 to 14 d ($P = 0.200$, effect size = −0.76 $\log_{10}$ cfu/g) or >14 d ($P = 0.068$, effect size = −0.70 $\log_{10}$ cfu/g) post-treatment, though low statistical power (56.41%, 66.17%, respectively) may have contributed to the lack of significant difference. Within the protected phage subgroup, no significant differences in effect size were observed across either age ($P = 0.11$) or sampling time subgroups ($P = 0.54$); the statistical power of these analyses of between-group difference was also low (35.4%; <20%, respectively).

Chickens receiving unprotected phages had concentrations of challenge bacteria that were significantly lower than those of untreated birds in samples collected 0 to 7 d ($P = 0.002$, effect size = −0.87 $\log_{10}$ cfu/g) and 8 to 14 d ($P = 0.016$, effect size = −0.74 $\log_{10}$ cfu/g) post-treatment, but not in samples collected >14 d post-treatment ($P = 0.435$, effect size = −0.61 $\log_{10}$ cfu/g). To note, the >14 d post-treatment subgroup had high heterogeneity ($I^2 = 77.7\%$) and low statistical power (68.61%). Unprotected phage treatment also resulted in significantly lower concentrations of challenge bacteria in adult ($P = 0.012$, effect size = −0.72 $\log_{10}$ cfu/g) and chick ($P = 0.002$, effect size = −0.76 $\log_{10}$ cfu/g) subgroups, though heterogeneity was considerable ($I^2 = 51.8\%$) in the chick subgroup. As before, no significant differences in phage treatment effect size were observed across subgroups divided by sampling time ($P = 0.88$) or by age ($P = 0.87$) and the statistical power of between-group analyses of difference was low (<8%; 5.3%, respectively).

Effect of Single vs. Poly-Phage Administration Poly-phage treatments (or phage "cocktails") were utilized in some of the studies included in this meta-analysis in an attempt to increase phage treatment efficacy, presumably by increasing host range and/or decreasing the potential impact of phage resistance in the challenge bacteria (Bardina et al., 2012; Fischer et al., 2013; Costa et al., 2019). Consequently, data were disaggregated into single and poly-phage treatment subgroups. Phage-treated chickens had significantly lower concentrations of challenge bacteria than untreated chickens in both poly-phage ($P = 0.0004$, effect size = −0.84 $\log_{10}$ cfu/g) and single phage ($P = 0.0008$, effect size = −0.80 $\log_{10}$ cfu/g) subgroups. No significant difference in effect size was observed between these subgroups ($P = 0.89$); however, the statistical power of this analysis was low (5.2%). These subgroups were then further disaggregated by sample collection time and experimental bird age. Poly-phage administration resulted in significantly lower concentrations of challenge bacteria in samples collected 0 to 7 d ($P = 0.005$, effect size = −0.90 $\log_{10}$ cfu/g) and 8 to 14 d ($P = 0.016$, effect size = −0.87 $\log_{10}$ cfu/g) post-treatment and in both adult chickens ($P = 0.012$, effect size = −0.72 $\log_{10}$ cfu/g) and chicks ($P = 0.003$, effect size = −0.89 $\log_{10}$ cfu/g), though heterogeneity was considerable in the chick subgroup ($I^2 = 58.9\%$). No significant differences in challenge bacteria concentrations were observed between phage-treated and untreated birds in samples collected >14 d

post-treatment ($P = 0.451$, effect size = -0.77 $\log_{10}$ cfu/g), though there was low statistical power (58.68%) and high heterogeneity ($I^2 = 79.0\%$) in this subgroup. Within the poly-phage subgroup, no significant differences in effect size were seen across age ($P = 0.55$) or sampling time subgroups ($P = 0.99$); to note, the statistical power of analyses of between-group difference was low in both cases (9.1%; <6%, respectively).

When a single phage type was administered, concentrations of challenge bacteria were significantly lower in phage-treated birds than in untreated birds in samples collected 0 to 7 d post-treatment ($P = 0.010$, effect size = -1.09 $\log_{10}$ cfu/g) but not in samples collected 8 to 14 d ($P = 0.066$, effect size = -0.57 $\log_{10}$ cfu/g) or >14 d ($P = 0.091$, effect size = -0.70 $\log_{10}$ cfu/g) post-treatment; post-hoc power analyses of the 8 to 14 d and >14 d subgroups indicated that statistical power in these groups was somewhat low (78.11%). Single phage administration also resulted in significantly lower concentrations of challenge bacteria in phage-treated vs. untreated birds among both adult chickens ($P = 0.021$, effect size = -1.54 $\log_{10}$ cfu/g) and chicks ($P = 0.0013$, effect size = -0.62 $\log_{10}$ cfu/g). Within the single phage type subgroup, no significant differences in phage treatment effect size were found across subgroups divided by sampling time ($P = 0.43$, statistical power <26%) or age ($P = 0.09$, statistical power = 40.1%).

CONCLUSIONS

As a whole, the results of this meta-analysis indicated that bacteriophage administration is able to significantly reduce concentrations of challenge bacteria in live chickens. The results further suggested that the effects of phage treatment may be greatest within 14 d of treatment. In addition, administering phages via feed appears to be as effective as administration via oral gavage and more effective than administration via either drinking water or aerosol spray. Across administration routes, significant reductions in challenge bacteria concentrations were observed when phages were administered via oral gavage, feed, or drinking water but not when phages were given via aerosol spray, though sample size was limited in the latter case (total inputs = 2; total observations = 63). Significant differences in phage treatment efficacy were not observed based on age alone. However, analyses of data after further disaggregation indicated that, when *Campylobacter* was the challenge organism, when phages were administered prophylactically, when a single dose of phages was given, or when a single phage type was used, bacteriophages reduced challenge bacteria concentrations more effectively in chickens >14 doa than in chicks <14 doa. Phage treatment was found to be more effective in chicks <14 doa than in chickens >14 doa only when phages were administered via feed. No significant differences in phage treatment efficacy were observed based on challenge bacteria genus (*Salmonella* spp. vs. *Campylobacter* spp.), phage administration timing (prophylactic vs. therapeutic), number of doses (single vs. repeated), phage protection method (protected vs. unprotected), or number of phage types (single- vs. poly-phage treatment).

When considering the results of the systematic review and meta-analysis, it is important to recognize the limitations of this study. In order to minimize heterogeneity and facilitate meaningful statistical analysis, data were drawn only from studies in which live chickens were challenged with a known quantity of bacteria and administered a known quantity of phages, challenge bacteria concentrations in tissues/fluids were measured following phage treatment, and either standard deviation or standard error were reported. Additionally, only one input per sampling period (0−7 d, 8−14 d, or >14 d post-treatment) per treatment method per study was included in the data set for meta-analysis in order to avoid over-representing any particular study. Though these delimitations effectively minimized overall heterogeneity, they also led to low statistical power in some cases; this may have contributed to the absence of significance in many between-group comparisons after data disaggregation. Study inclusion requirements also hindered some analyses due to resultant data scarcity; for instance, analyses are not reported for data disaggregated by sample type because of the paucity of inputs involving non-cecal samples. To this point, additional research concerning understudied aspects of phage administration aimed at reducing bacterial loads in poultry would be a valuable addition to existing knowledge regarding phage treatment efficacy. It is also relevant to note that, though several inputs were identified as outliers in preliminary analyses, no inputs were removed from the data set as additional justification (e.g., flaws in experimental methods in the studies from which the inputs were drawn) for their removal was not found.

ACKNOWLEDGMENTS

This research is possible through the financial support from UK Government − Department of Health and Social Care (DHSC), the Global AMR Innovation Fund (GAMRIF) and the International Development Research Centre, Ottawa, Canada.

DISCLOSURES

The authors do not have any conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2021.101472.

REFERENCES

Ackermann, H. W., and G. Węgrzyn. 2014. General characteristics of bacteriophages. Pages 3-22 in Phage Therapy: Current Research and Applications. J. Borysowski, R. Międzybrodzki and A. Górski, eds. Caister Academic Press; Northfolk, UK.

Adhikari, P. A., D. E. Cosby, N. A. Cox, J. H. Lee, and W. K. Kim. 2017. Effect of dietary bacteriophage supplementation on internal organs, fecal excretion, and ileal immune response in laying hens challenged by *Salmonella* Enteritidis. Poult. Sci 96:3264–3271.

Atterbury, R. J., M. A. P. Van Bergen, F. Ortiz, M. A. Lovell, J. A. Harris, A. De Boer, J. A. Wagenaar, V. M. Allen, and P. A. Barrow. 2007. Bacteriophage therapy to reduce *Salmonella* colonization of broiler chickens. Appl. Environ. Microbiol. 73:4543–4549.

Bardina, C., D. A. Spricigo, P. Cortés, and M. Llagostera. 2012. Significance of the bacteriophage treatment schedule in reducing *Salmonella* colonization of poultry. Appl. Environ. Microbiol. 78:6600–6607.

Bar-Shira, E., and A. Friedman. 2006. Development and adaptations of innate immunity in the gastrointestinal tract of the newly hatched chick. Dev. Comp. Immunol. 30:930–941.

Beal, R. K., P. Wigley, C. Powers, S. D. Hulme, P. A. Barrow, and A. L. Smith. 2004. Age at primary infection with *Salmonella enterica* serovar Typhimurium in the chicken influences persistence of infection and subsequent immunity to re-challenge. Vet. Immunol. Immunopathol. 100:151–164.

Borie, C., I. Albala, P. Sánchez, M. L. Sánchez, S. Ramírez, C. Navarro, M. A. Morales, A. J. Retamales, and J. Robeson. 2008. Bacteriophage treatment reduces *Salmonella* colonization of infected chickens. Avian Dis. 52:64–67.

Borie, C., M. L. Sánchez, C. Navarro, S. Ramírez, M. A. Morales, J. Retamales, and J. Robeson. 2009. Aerosol spray treatment with bacteriophages and competitive exclusion reduces *Salmonella enteritidis* infection in chickens. Avian Dis. 53:250–254.

Carvalho, C. M., B. W. Gannon, D. E. Halfhide, S. B. Santos, C. M. Hayes, J. M. Roe, and J. Azeredo. 2010. The in vivo efficacy of two administration routes of a phage cocktail to reduce numbers of *Campylobacter coli* and *Campylobacter jejuni* in chickens. BMC Microbiol. 10:232.

CDC. 2019. Antibiotic resistance threats in the United States. Accessed Mar. 2021. https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf

Colom, J., M. Cano-Sarabia, J. Otero, P. Cortés, D. Maspoch, and M. Llagostera. 2015. Liposome-encapsulated bacteriophages for enhanced oral phage therapy against *Salmonella* spp. Appl. Environ. Microbiol. 81:4841–4849.

Costa, P., C. Pereira, A. T. P. C. Gomes, and A. Almeida. 2019. Efficiency of single phage suspensions and phage cocktail in the inactivation of *Escherichia coli* and *Salmonella* Typhimurium: an in vitro preliminary study. Microorganisms. 7:94.

Delbrück, M. 1940. The growth of bacteriophage and lysis of the host. J. Gen. Physiol. 23:643–660.

El-Dougdoug, N. K., S. Cucic, A. G. Abdelhamid, L. Brovko, A. M. Kropinski, M. W. Griffiths, and H. Anany. 2019. Control of *Salmonella* Newport on cherry tomato using a cocktail of lytic bacteriophages. Int. J. Food Microbiol. 293:60–71.

El-Shibiny, A., A. Scott, A. Timms, Y. Metawea, P. Connerton, and I. Connerton. 2009. Application of a group II *Campylobacter* bacteriophage to reduce strains of *Campylobacter jejuni* and *Campylobacter coli* colonizing broiler chickens. J. Food Prot. 72:733–740.

Fischer, S., S. Kittler, G. Klein, and G. Glünder. 2013. Impact of a single phage and a phage cocktail application in broilers on reduction of *Campylobacter jejuni* and development of resistance. PLoS One. 8:e78543.

Fischetti, V. A. 2018. Development of phage lysins as novel therapeutics: a historical perspective. Viruses. 10:310.

Guttman, B., R. Raya, and E. Kutter. 2005. Basic phage biology. Pages 29-66 in Bacteriophages: Biology and Applications. E. Kutter and A. Sulakvelidze, eds. CRC Press; Boca Raton, FL.

Han, Z., C. Pielsticker, L. Gerzova, I. Rychlik, and S. Rautenschlein. 2016. The influence of age on C*ampylobacter jejuni* infection in chicken. Dev. Comp. Immunol. 62:58–71.

Harrer, M., P. Cuijpers, T. A. Furukawa, and D. D. Ebert. 2019a. Doing meta-analysis in R: a hands-on guide. Accessed Mar. 2021. https://bookdown.org/MathiasHarrer/Doing_Meta_Analysis_in_R/

Harrer, M., P. Cuijpers, T. Furukawa, and D. D. Ebert. 2019b. dmetar: Companion R package for the guide 'Doing Meta-Analysis in R'. R package version 0.0.9000. Accessed Mar. 2021. http://dmetar.protectlab.org/

Higgins, J. P., and S. G Thompson. 2002. Quantifying heterogeneity in a meta-analysis. Stat. Med. 21:1539–1558.

Hodyra-Stefaniak, K., P. Miernikiewicz, J. Drapała, M. Drab, E. Jończyk-Matysiak, D. Lecion, Z. Kaźmierczak, W. Beta, J. Majewska, M. Harhala, B. Bubak, A. Kłopot, A. Górski, and K. Dąbrowska. 2015. Mammalian host-versus-phage immune response determines phage fate in vivo. Sci. Rep. 5:14802.

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

Huff, W. E., G. R. Huff, N. C. Rath, and A. M. Donoghue. 2010. Immune interference of bacteriophage efficacy when treating colibacillosis in poultry. Poult. Sci. 89:895–900.

Iriarte, F. B., B. Balogh, M. T. Momol, L. M. Smith, M. Wilson, and J. B. Jones. 2007. Factors affecting survival of bacteriophage on tomato leaf surfaces. Appl. Environ. Microbiol. 73:1704–1711.

Kasman, L. M., A. Kasman, C. Westwater, J. Dolan, M. G. Schmidt, and J. S. Norris. 2002. Overcoming the phage replication threshold: a mathematical model with implications for phage therapy. J. Virol. 76:5557–5564.

Knezevic, P., D. Obreht, S. Curcin, M. Petrusic, V. Aleksic, R. Kostanjsek, and O. Petrovic. 2011. Phages of *Pseudomonas aeruginosa:* response to environmental factors and in vitro ability to inhibit bacterial growth and biofilm formation. J. Appl. Microbiol. 111:245–254.

Lim, T. H., M. S. Kim, D. H. Lee, Y. N. Lee, J. K. Park, H. N. Youn, H. J. Lee, S. Y. Yang, Y. W. Cho, J. B. Lee, S. Y. Park, I. S. Choi, and C. S. Song. 2012. Use of bacteriophage for biological control of *Salmonella* Enteritidis infection in chicken. Res. Vet. Sci. 93:1173–1178.

Loc Carillo, C., R. J. Atterbury, A. El-Shibiny, P. L. Connerton, E. Dillon, A. Scott, and I. F. Connerton. 2005. Bacteriophage therapy to reduce *Campylobacter jejuni* colonization of broiler chickens. Appl. Environ. Microbiol. 71:6554–6563.

Luis Vaz, C. S., D. Voss-Rech, L. Alves, A. Coldebella, L. Brentano, and I. M. Trevisol. 2020. Effect of time of therapy with wild-type lytic bacteriophages on the reduction of *Salmonella* Enteritidis in broiler chickens. Vet. Microbiol. 240:108527.

Ma, Y., J. C. Pacan, Q. Wang, Y. Xu, X. Huang, A. Korenevsky, and P. M. Sabour. 2008. Microencapsulation of bacteriophage Felix O1 into chitosan-alginate microspheres for oral delivery. Appl. Environ. Microbiol. 74:4799–4805.

Monteiro, R., D. P. Pires, A. R. Costa, and J. Azeredo. 2019. Phage therapy: going temperate? Trends Microbiol. 27:368–378.

Park, J. Y., B. Y. Moon, J. W. Park, J. A. Thornton, Y. H. Park, and K. S. Seo. 2017. Genetic engineering of a temperate phage-based delivery system for CRISPR/Cas9 antimicrobials against *Staphylococcus aureus*. Sci. Rep. 7:44929.

Payne, R. J. H., and V. A. A. Jansen. 2001. Understanding bacteriophage therapy as a density-dependent kinetic process. J Theor Biol. 208:37–48.

Payne, R. J. H., and V. A. A. Jansen. 2003. Pharmacokinetic principles of bacteriophage therapy. Clin. Pharmacokinet. 42:315–325.

RStudio Team. 2021. RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA. http://www.rstudio.com/.

Sahin, O., I. I. Kassem, Z. Shen, J. Lin, G. Rajashekara, and Q. Zhang. 2015. Campylobacter in poultry: ecology and potential interventions. Avian Dis. 59:185–200.

Sahin, O., N. Luo, S. Huang, and Q. Zhang. 2003. Effect of *Campylobacter*-specific maternal antibodies on *Campylobacter jejuni* colonization in young chickens. Appl. Environ. Microbiol. 69:5372–5379.

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

Vázquez, R., E. García, and P. García. 2018. Phage lysins for fighting bacterial respiratory infections: a new generation of antimicrobials. Front. Immunol. 9:2252.

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

Yosef, I., M. Manor, R. Kiro, and U. Qimron. 2015. Temperate and lytic bacteriophages programmed to sensitize and kill antibiotic-resistant bacteria. Proc. Natl. Acad. Sci. U. S. A. 112:7267–7272.

Zhang, J., Y. Hong, M. Fealy, A. Singh, K. Walton, C. Martin, N. J. Harman, J. Mahlie, and P. D. Ebner. 2015. Physiological and molecular characterization of *Salmonella* bacteriophages previously used in phage therapy. J. Food Prot. 78:2143–2149.