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ORIGINAL ARTICLE

Study of a lytic bacteriophage as a tool for the control of *Salmonella* Gallinarum in layer poultry

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Abstract

One of the leading diseases affecting laying poultry in Argentina is fowl typhoid, caused by the pathogen *Salmonella enterica* serovar Gallinarum (*S.* Gallinarum). Although this disease was eradicated from industrial hatcheries in several developed countries, it is still present in commercial farms in Latin America, causing substantial economic losses due to high mortality rates, limited production of quality eggs, high veterinary costs, and the need to renovate infected facilities. To develop a new *S.* Gallinarum biocontrol method, in the present study, we characterized a bacteriophage in terms of host specificity, growth kinetics, and stability under different environmental conditions. The phage could infect an ample range of bacterial hosts and had no lytic activity against the vaccine strain 9R. Moreover, the phage was stable at pH 3, chloroform presence did not significantly reduce its titer, and its activity was not affected by antibiotics or various chemical agents often used in aviculture. Application of a phage suspension on *S.* Gallinarum-contaminated surfaces resulted in a significant reduction of bacterial load ($p < 0.05$). These results show that lytic bacteriophages such as the one studied here are innovative alternatives with potential applications to control *S.* Gallinarum in laying poultry farms.

Keywords: Bacteriophage; *Salmonella* Gallinarum; fowl typhoid; laying poultry; biocontrol.

Abbreviations ATB= antibiotic; CFU= Colony forming units; OD= Optical density; PBS = phosphate buffered saline; PFU= Plaque forming units

1. Introduction

The Argentine poultry industry has grown steadily since 2003, and investments have become progressively higher. Thus, the outlook of poultry and egg production is favorable, and the strategy outlined for the following years will lead to increased productivity, which will satisfy both local and international demands [1]. Such a production plan will lead to greater competitiveness with inflexible sanitary controls. One of the challenges of poultry and egg production is the development of improved methods to prevent and control bacterial infections, including fowl typhoid, a disease of economic importance caused by *Salmonella* Gallinarum.

In the poultry sector, the prevention and treatment of bacterial diseases frequently rely on antibiotic administration. Furthermore, chickens may receive antibiotics as food additives and growth promoters. However, antibiotic use is currently restricted because of its link with emerging antibiotic-resistant strains in humans, as antibiotics accumulate in poultry and eggs [2]. Thus, researchers turn to natural bactericidal agents that are economically viable and less risky for human health to be used both as growth promoters and as control agents of some microorganisms such as *Salmonella* [3].

Bacteriophages, also known as phages, are viruses that specifically infect bacteria. They were initially described by Frederick Twort in 1915 and later by Félix d'Hérelle, who contributed substantially to these organisms' study [4, 5]. Since 2021, the taxonomy of bacterial viruses changed, establishing one class, seven orders, 31 families, 214 genera, and 858 species. The new class *Caudoviricetes* groups all tailed bacterial and archaeal viruses with icosahedral capsids and a double-stranded DNA genome [6, 7]. Lytic bacteriophages are those that induce bacterial lysis during their multiplication cycle. Due to their strict host specificity and infection kinetics (exponential unit increase within infected cells), lytic bacteriophages appear as novel bacterial infection biocontrol tools. Indeed, lytic phages have proven to be effective against *Enterococcus* and *Staphylococcus* biofilms [8, 9], and phage therapy has been developed to control fish [10], livestock [10, 11, 12, 13], and poultry [14, 15, 16, 17, 18, 19] pathogens. Recently, several bacteriophage-based products have been approved for their use in food and food animals [20, 21].

To develop a tool for the biocontrol of *S*. Gallinarum, in the present study, we characterized a bacteriophage isolated from the feces of a hen infected with fowl typhoid. The phage was phenotypically analyzed to determine its host specificity and lytic cycle parameters. We also evaluated the phage's lytic activity on surfaces experimentally contaminated with *S*. Gallinarum and studied its stability in the presence of disinfectants commonly used in poultry farms and solutions with varying degrees of acidity and veterinary antibiotics.

2. Materials and methods

2.1. Bacteriophage

The bacteriophage used in this study was previously isolated from the feces of a laying hen infected with fowl typhoid [22]. Briefly, the fecal sample was placed in nutrient broth (Laboratorios Britania, Buenos Aires, Argentina) inoculated with an *S*. Gallinarum culture and incubated overnight at 35 °C. An aliquot was then decontaminated with chloroform, and the presence of the phage was checked by the double agar layer method [23]. Subsequently, several filtration and centrifugation steps were performed to purify the phage and prepare stocks, which were preserved in nutrient broth supplemented with chloroform. The phage is not inactivated by chloroform under these conditions (see Section 3.4). Five repetitions of the double agar layer method [23] were performed by lifting a well-isolated lysis plaque with a loop handle each time to obtain a phage clone. To preserve a stock, its Routine Test Dilution (RTD) was calculated to achieve confluent lysis on its propagating strain. Once the bacteriophage was propagated, the plaque was flooded with 15 mL of nutrient broth and collected by dragging it with a Pasteur pipette. After centrifugation for one hour at 8000 x g, the supernatant was collected and filtered through a 0.22 µm Millipore membrane. The already propagated phage in the filtrate was titrated again in its propagating strain and stored in SM buffer (50 mM Tris-HCl; 0.1 M NaCl; 8 mM $MgSO_4 \cdot 7H_2O$; 0.01 % gelatin) at 4 ºC.

2.2. Host range

Several *S*. Gallinarum (n=4) and *S*. Enteritidis (n=17) strains were used to determine host specificity along with other *Salmonella* isolates (*S*. Westhampton [n=5]; *S*. Orion [n=1]), vaccine strain 9R (n=2), *Pseudomonas* spp. (n=3), and *E. coli* (n=1). All bacterial strains were cultured in nutrient broth and incubated at 37 °C to obtain fresh overnight cultures, and the *Salmonella* strain from which the bacteriophage was isolated served as positive control. Strains were streaked on nutrient agar (Laboratorios Britania, Buenos Aires, Argentina) plates at 10^8 CFU/mL in a

delimited area. Next, 10 µl of a phage suspension at a concentration of 10^9 PFU/mL was added to determine if the strain was susceptible to the phage after incubation at 37 °C for 48 h. Lysis efficiency in susceptible bacterial strains was assessed with the method by Mahmoud *et al* [24] with minor modifications. Briefly, serial dilutions of the phage were dropped on previously seeded susceptible host bacteria (spot test [25]), recording the highest phage dilution leading to individual lysis plaques. Lysis plaque appearance (clear or turbid – CP and TP, respectively) was registered.

2.3. Phage growth parameters

Bacteriophage adsorption constant and lytic cycle parameters (eclipse period and burst size) were determined via adhesion and single-cycle curves, respectively [26]. Briefly, 0.1 mL of a 20 h culture of *S*. Gallinarum was inoculated in 20 mL nutrient broth (Laboratorios Britania, Buenos Aires, Argentina), and the suspension was incubated at 37 °C with shaking (180 rpm) until reaching an optical density (OD) at 650 nm of 0.1. Then, 9 mL of the nutrient broth was placed in a 100-mL Erlenmeyer flask (designated C), whereas another 9 mL of the *S*. Gallinarum culture was placed in another Erlenmeyer flask (designated A). The remaining culture was kept in an ice bath to count the CFU numbers. Both Erlenmeyer flasks were incubated at 37 °C for 5 min with shaking (60 rpm), and then 1 mL of phage suspension, at a concentration approximately equivalent to a multiplicity of infection (MOI) of 0.001, was added to each flask. Duplicate samples of 100 µL were taken from Erlenmeyer A at one-minute intervals for 15 minutes, and at minute 15, two samples of equal volume were taken from Erlenmeyer C. Each sample was transferred to a microtube containing 900 µL of nutrient broth and 50 µL of $CHCl_3$, previously kept on ice. The tubes were mixed by shaking and were left on ice to determine the phage titer on nutrient agar (Laboratorios Britania, Buenos Aires, Argentina) by the double layer method [23]. Bacteria were separated from free phage by low-speed centrifugation. Likewise, the titer of the *S*. Gallinarum culture was determined in duplicate utilizing the colony counting method in nutrient agar plates. The phage adsorption constant was determined with the formula:

$$K = \frac{2.3}{Bt} \log \frac{P_0}{P} \tag{1}$$

Where K is the adsorption constant (mL/min), B is the concentration of bacteria, P_0 and P are the initial and final titers of non-adsorbed bacteriophages, respectively, and t is the time interval between P_0 and P.

To construct the one-cycle lysis curve and to determine phage lytic cycle parameters, a 0.1 mL phage suspension (10^3 PFU/mL) was incubated at 37 °C in a water bath and added with 0.1 mL of the *S*. Gallinarum culture in exponential phase grown in nutritive broth with stirring ($OD_{650nm} = 0.1$). The mixture was homogenized by shaking and incubated for 10 min to infect the bacterial culture. Then, bacteria were separated from free phages by low-speed centrifugation, and 10 mL of a nutritive broth at 37 °C was added to the precipitate. The suspension was mixed, and 0.4 mL was separated. Immediately, two aliquots of 0.1 mL were seeded in nutrient agar plates to determine the number of infected bacteria by plate count.

The remaining suspension was separated into two other aliquots of 0.1 mL, which were added to a tube containing 0.9 mL of nutrient broth and 50 µL of $CHCl_3$, mixed by shaking, and kept on ice until the corresponding counts were made (these aliquots correspond to time zero of the lysis curve in one cycle). Aliquots of 0.1 mL of the infected bacterial suspension were taken every 5 min for 80 minutes and then transferred to tubes containing nutrient broth/$CHCl_3$. Finally, phage titers were determined in all samples by the double-layer method on nutrient agar [23].

2.4. Phage stability in acid medium

Phage suspensions (0.5 ml; 10^9 PFU/mL) were incubated in test tubes containing 4.5 mL of phosphate-buffered saline (PBS) 1x adjusted to pH 2, pH 3, and pH 7 for one hour at 37 °C [27]. Then, a 0.1-mL aliquot of each pH-adjusted suspension was taken to quantify plaque numbers in triplicate by the double layer method [23] after 24 h of incubation at 37 °C. One-way ANOVA and Tukey's multiple comparison tests assessed the differences between mean PFU/mL values (SPSS software version 21.0). Statistical significance was considered at $p \leq 0.05$.

2.5. Phage stability in chloroform

A 0.5-mL phage suspension (10^9 PFU/mL) was dispensed in test tubes containing 0.1 mL of $CHCl_3$ and immediately mixed for 1 hour at room temperature [28]. Then, test tubes were centrifuged at 3.300 x g at 4 °C for 10 minutes, and their supernatant was employed to determine the final phage titer by the double layer method [23]. The same procedure was conducted with control test tubes in which the chloroform volume was replaced with a sterile 0.85 % saline solution. Treatments were performed in triplicate. Mean PFU values for phages in $CHCl_3$ and saline solution were compared using the Student's t-test (SPSS software version 21.0). Statistical significance was considered at $p \leq 0.05$.

2.6. Effect of temperature on phage

One milliliter of phage suspension (10^9 PFU/mL) was dispensed in test tubes, which were placed at temperatures of 20, 30, 40, 50, and 60 ± 2 °C in a water bath for 1 hour [27]. After incubation, the final titer of the bacteriophages was determined by the double-layer method [23]. Treatments were performed in triplicate. One-way ANOVA and Tukey's multiple comparison tests were used to examine differences between the mean PFU/mL values (SPSS software version 21.0). Statistical significance was considered at $p \leq 0.05$.

2.7. Phage activity in the presence of antibiotics

To determine the lytic activity of phages in the presence of aminoglycoside antibiotics (ATB) commonly used in veterinary medicine, 4.5 mL ATBs (Kanamycin 10 % (w/v) and Gentamicin 5 % (w/v) solutions in a 1:1 mixture) was mixed with 0.5 mL phage (10^9 PFU/mL) and incubated for 1 h at 37 ºC. Next, 0.1 mL of 10^{-6}, 10^{-7}, and 10^{-8} dilutions of the ATBs: phage mixture were added to 0.5 mL of *S*. Gallinarum (10^9 CFU/mL) and mixed, and the double layer method [23] was performed to count the number of plaques obtained after 4 h incubation at 37 °C. The mean PFU values for antibiotics/phage and the control phage were compared using Student's t-test (SPSS software version 21.0). Statistical significance was considered at $p \leq 0.05$.

To evaluate whether phage presence affects ATB efficacy, 0.5 mL of *S*. Gallinarum (10^8 CFU/mL) was incubated in 4 mL of nutrient broth with 0.5 mL of the kanamycin/phage mixture and, as a control, 0.5 mL of *S*. Gallinarum (10^8 CFU/mL) was inoculated in 4 mL of nutrient broth with 0.5 mL of kanamycin (without phage). After 2 hours at room temperature, 0.1 mL of 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} dilutions were plated on Petri dishes with nutrient agar. The plates were incubated at 37 °C for 24 h and the CFU/mL were counted.

2.8. Phage activity in the presence of disinfectants

A volume of 0.5 mL of phage suspension (10^9 PFU/mL) was incubated with 4.5 mL disinfectants commonly used in poultry production (iodine, creolin, CID 20®, and quaternary ammonium) for 2 h at 37 °C. Disinfectants were diluted according to the manufacturer's indications. The phage/disinfectant mixtures were conserved at room temperature for one week. Subsequently, 100 µL of each disinfectant/phage suspension was mixed with 500 µL of 10^8 CFU/mL of *S*. Gallinarum and the double layer method was used to determine the number of lysis plaques after 5 h incubation at 37 °C [23]. Experiments were performed in triplicate and mean values were compared using One-way ANOVA and Tukey's multiple comparison test (SPSS software version 21.0). Statistical significance was considered when $p \leq 0.05$.

2.9. Phage lytic activity on different surfaces

The lytic activity of the phage was evaluated on six different surfaces usually in contact with the birds and made of the following materials: polypropylene, PVC, zinc, low-density polyethylene, and PVC-coated fabrics. The test was divided into trials, each corresponding to different surfaces (**Table 1**)

Table 1. Different materials used for the test

Trial	Surface	Area	Number of pieces
1	zinc rectangles taken from feeders	66 cm^2	11
2	PVC containers taken from drinking fountains	50 cm^2	11
3	low-density polypropylene bases taken from cages	330 cm^2	11
4	pieces of polypropylene egg conveyor belt	150 cm^2	11
5	pieces of metal grids taken from cages	50 cm^2	11
6	polypropylene tubes for water circulation	30 cm^2	11

Before trials began, the absence of *S*. Gallinarum in each of the surfaces was verified by bacterial culture of samples taken with a sterile swab according to standard microbiological sampling procedures [29]. Subsequently, the different materials were immersed for 1 min in a container with an *S*. Gallinarum culture (10^8 CFU/mL) with manual agitation, dried in a laminar flow, and incubated for 60 min at 37 °C to allow bacterial growth. Next, the microorganisms present in one piece of each contaminated material were sampled with a sterile swab and their number (CFU/mL) was determined.

Each trial was divided into two treatments (**Table 2**).

Table 2. Treatments applied to the materials

Treatment	Method	Number of pieces
1	material sprayed with 1×10^9 PFU/mL phage	5
2	material sprayed with saline solution eliminate1×10^9 PFU/mL phage	5

Each piece of material was sprayed three times on each side. One spray-on is equivalent to a volume of 1 ml. After the respective treatment, the surfaces were incubated at 37 °C for 3 h. Then, with a sterile swab, samples were taken from the surfaces of each of the trials with their different treatments, and incubated in 150 mL of peptone water at 37 °C for 24 h. For the isolation

of S. Gallinarum, 0.1 mL aliquots were seeded in 10 mL of Rapapport-Vassiliadis broth (Difco) and incubated for 24 ± 1 h at 42 °C. Counting was performed in the selective and differential medium XLD agar (Difco). Likewise, 0.1 mL of each of the containers containing the swabs was taken from the surfaces that received Treatment 1 and the presence of phages was determined by the double-layer method [23]. The mean CFU values for each treatment and the control were compared using Student's t-test or Welch test (SPSS software version 21.0). Statistical significance was considered when $p \leq 0.05$.

3. Results

3.1. Host range

The *Salmonella* strains used to evaluate the phage's host range were isolated from surfaces in contact with the birds or on the birds themselves in poultry farms located in the Argentine Luján and Mercedes areas, within the Province of Buenos Aires. As expected, the phage caused total lysis of the *Salmonella* strain from which it was originally isolated (strain 88). Furthermore, total lysis was evidenced in *S*. Gallinarum strains INTA and 527 and in five additional *S.* Enteritidis strains, whereas a partial lytic effect was observed after 48 h incubation in one *S*. Gallinarum strain and in eight strains of *S*. Enteritidis. Four *S*. Enteritidis strains were not susceptible to the phage (**Table 3**). In turn, four *S*. Westhampton strains (ID 2 si, 2 sw, 2 fec, 1 sw, 8 fee), one *S*. Orion strain as well as two vaccine strains of the rugose type *S*. Gallinarum 9R were not susceptible to the phage. Of note, there was a partial lytic effect in the *S*. Westhampton strain 6 fee at a high phage MOI, but no individual lysis plaques were observed when the phage was diluted. Finally, no lytic activity was observed against *E. coli* or *Pseudomonas*.

Table 3. (+) indicates that the strain is susceptible to the phage and produces lysis plaques, (-) indicates that no plaques were observed, PL indicates partial lysis after 48 h incubation, LW indicates lysis from without observed plaques, TP indicates turbid plaque, CP indicates clear plaque. The average dilution at which the formation of individual lysis plaques was observed in the spot test is indicated in parentheses. * *S*. Gallinarum (88) is the host cell from which the phage was isolated.

Bacterial Strain	sample ID	Source of isolate	Lysis	Lysis efficiency by Spot test
S. Gallinarum	88*	Feces, Luján, Arg.	+	CP (10^{-8})
S. Gallinarum	INTA	Balcarce, Arg.	+	CP (10^{-8})
S. Gallinarum	9R-A	Vaccine manufacturer, La Plata, Arg.	-	
S. Gallinarum	9R-B	Vaccine manufacturer, Del Viso, Arg	-	
S. Gallinarum	53	Farm, Luján, Arg.	PL	
S. Gallinarum	527	Feeder, Mercedes, Arg	+	CP (10^{-6})
S. Enteritidis	68	Farm, Luján, Arg.	PL	
S. Enteritidis	102	Farm, Luján, Arg.	-	
S. Enteritidis	132	Farm, Mercedes, Arg.	PL	
S. Enteritidis	134	Farm, Luján, Arg.	+	
S. Enteritidis	141	Farm, Luján, Arg.	PL	
S. Enteritidis	147	Farm, Luján, Arg.	+	TP (10^{-5})
S. Enteritidis	160	Farm, Luján, Arg.	+	CP (10^{-5})
S. Enteritidis	169	Farm, Mercedes, Arg.	PL	
S. Enteritidis	175	Farm, Luján, Arg.	PL	

S. Enteritidis	187	Farm, Luján, Arg.	-	
S. Enteritidis	198	Farm, Luján, Arg.	PL	
S. Enteritidis	199	Farm, Luján, Arg.	PL	
S. Enteritidis	269	Farm, Mercedes, Arg.	-	
S. Enteritidis	276	Farm, Luján, Arg.	PL	
S. Enteritidis	278	Farm, Luján, Arg.	+	CP (10^{-6})
S. Enteritidis	354	Farm, Mercedes, Arg.	-	
S. Enteritidis	823	Farm, Luján, Arg.	+	TP (10^{-4})
S. Westhampton	2 si	Farm 2 silo, Luján, Arg.	-	
S. Enteritidis	2 sw	Farm 2 swab, Luján, Arg.	-	
S. Enteritidis	2 fec	Farm 2 feces, Luján, Arg.	-	
S. Orion	1 sw	Farm 1 swab, Luján, Arg.	-	
S. Enteritidis	6 fee	Farm 6 feeder; Luján, Arg.	PL	LW
S. Enteritidis	8 fee	Farm 8 feeder, Luján, Arg.	-	
E. coli	INTA	Balcarce, Arg.	-	
Pseudomonas spp.	P1	Farm land, Luján, Arg.	-	
Pseudomonas spp.	P3	Farm land, Luján, Arg.	-	
Pseudomonas spp.	P7	Farm land, Luján, Arg.	-	

3.2. Lytic cycle parameters

Phage adhesion to *S*. Gallinarum cells was assessed. The phage's adsorption constant (K) was 5.96 x 10^{-10} mL/min, and its growth parameters were determined via one-step curves. The burst size (b), representing the number of viral particles released by each infected bacterium, was 40 PFU/CFU, as calculated with the equation described by Wang [30]. The eclipse period, *i.e*., the time from infection until the formation of the complete (infective) phage particles, was 25 minutes (**Fig. 1**)

3.3. Phage tolerance to acidity

PBS solutions adjusted to pH 2, 3, and 7 helped to evaluate the phage's tolerance to acidity. Acid media (pH 2 and 3) recreated the chicken's upper digestive tract conditions. Phage suspension incubation at pH 2 resulted in significantly fewer lysis plaques, with a low phage titer of 5.99 $\log_{10}$ (PFU/mL). Besides, we observed that some bacterial colonies in the center of the plaques were not lysed, suggesting that medium acidity affected the lytic activity of the phage. In contrast, in phage suspension incubated at pH 3 or 7, the lytic activity was unaffected, with plaque counts of 8.35 and 9.29 $\log_{10}$ (PFU/mL), respectively (**Fig. 2**).

3.4. Phage stability in chloroform

Chloroform completes bacteriophage purification by lysing the bacterial remnant. To evaluate phage stability in the presence of chloroform, lysis plaque numbers were counted and compared between suspensions with and without chloroform. **Fig. 3** reveals no statistically significant reduction in viral titer with chloroform, confirming phage stability in this solvent.

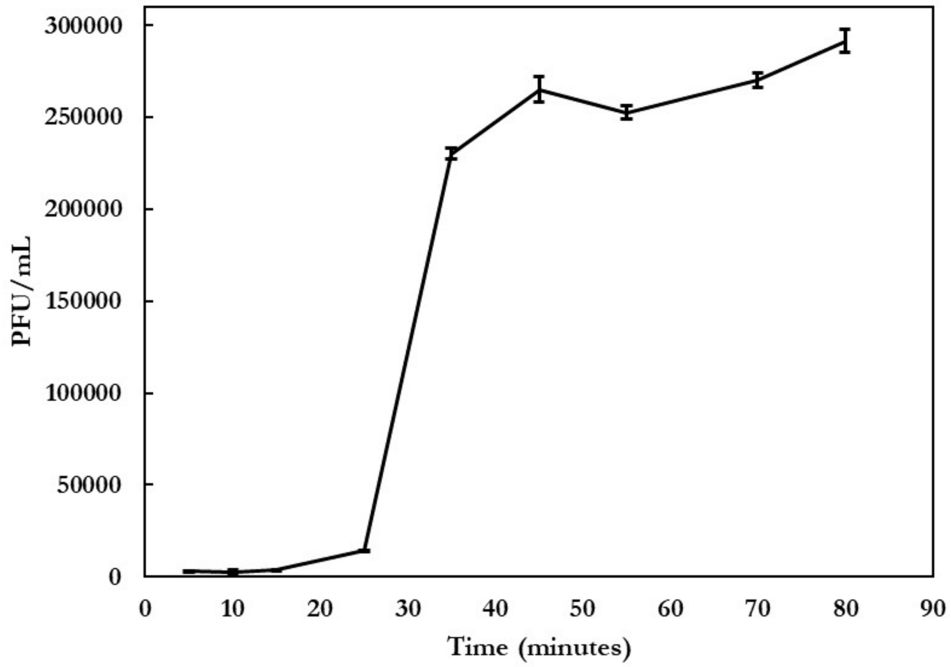

Figure 1. Phage one-step growth curve in the presence of the host *S*. Gallinarum (strain 88). Values represent the mean of three experiments; error bars represent the standard deviation. The eclipse period was 25 minutes.

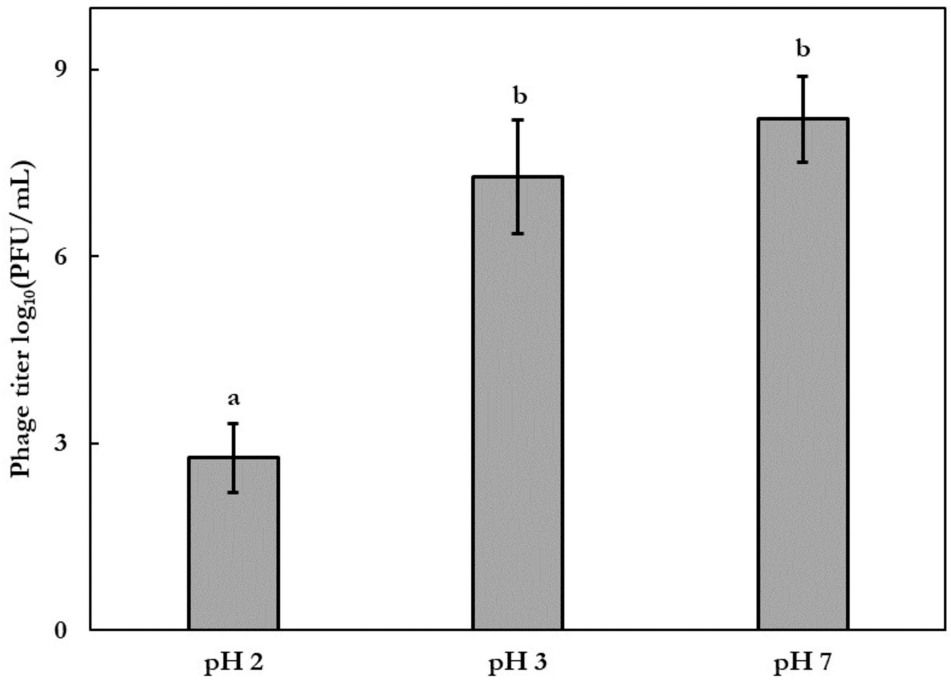

Figure 2. Phage Tolerance to acidity. Phage suspensions ($5x10^8$ UFP) were treated at different pH levels (2, 3, 7) for one hour, followed by the assessment of the phage titer. The values represent the mean of three experiments; error bars represent the standard deviation. The different letters represent significantly different means ($p < 0.05$).

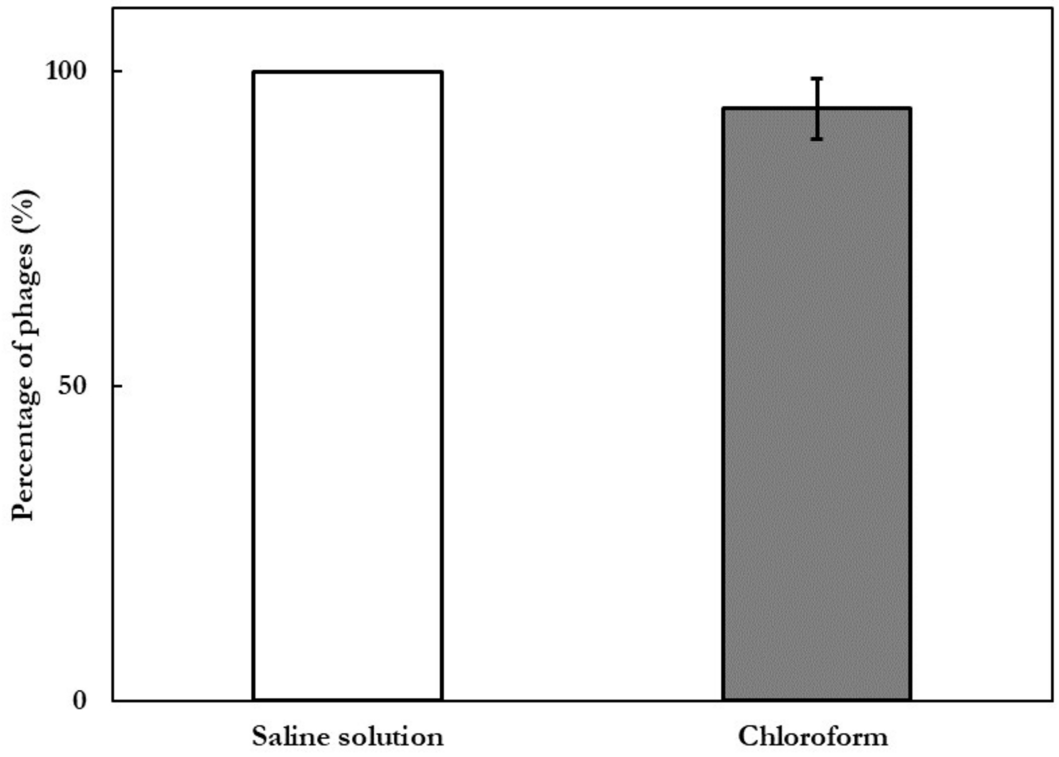

Figure 3. Phage stability in the presence of chloroform. Bar heights represent the average phage counts (as percentages) across three experiments.

3.5. Temperature-dependent phage stability

A thermal stability test was conducted to determine phage resistance to heat. As shown in **Fig. 4**, phage titers did not differ significantly after 1 h incubation at 20 °C to 60 °C.

3.6. Phage lytic activity in the presence of aminoglycoside antibiotics used in veterinary medicine

To evaluate a potential interaction of the phage with aminoglycoside antibiotics (ATB), which are widely used in poultry production and might interfere with the lytic activity of the phage, *in vitro* tests were performed. Phage suspensions were incubated with and without antibiotic addition, and PFUs were counted at 4 h after *S*. Gallinarum infection. Phage effectiveness was significantly reduced in the presence of antibiotics compared to controls, thus indicating that phage activity was affected in the presence of aminoglycoside antibiotics (**Fig. 5**).

To test whether phage presence could affect antibiotic efficacy, *S*. Gallinarum was incubated in nutrient broth with kanamycin with and without the phage, and CFUs were counted after 24 h. Bacterial colonies were absent in all Petri dishes. Therefore, the phage did not affect antibiotic (kanamycin) activity.

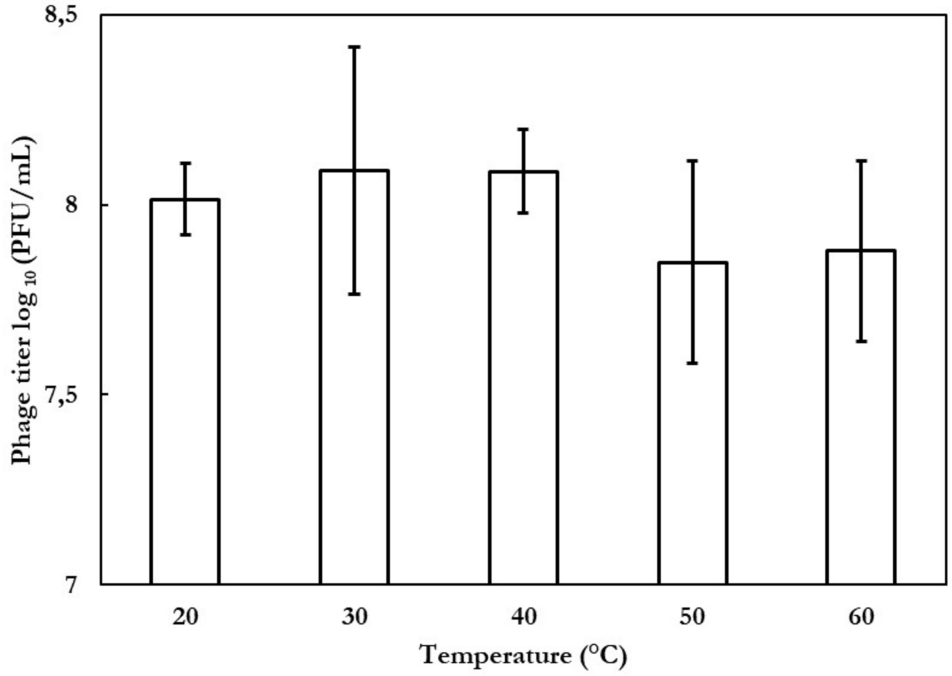

Figure 4. Phage thermal stability test. Bar heights represent average page titers (expressed as $\log_{10}$ PFU per milliliter) across three repetitions of tests at five different temperatures from 20 °C to 60 °C; error bars represent the standard deviation

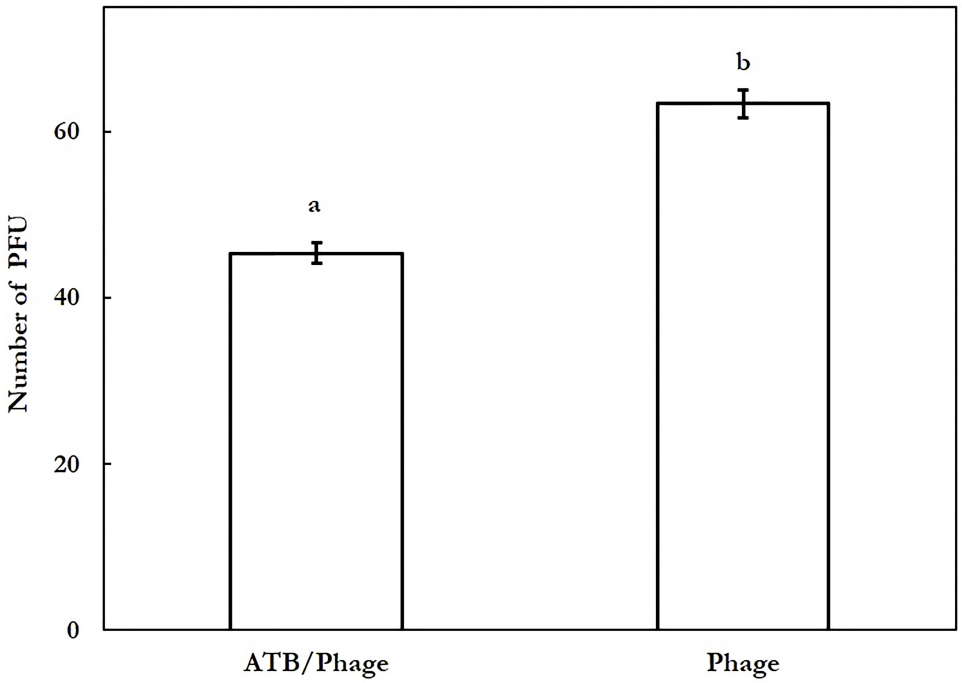

Figure 5. Phage lytic activity in the presence of aminoglycoside antibiotics (ATB) Bar heights represent the average PFU value of three experiments; error bars represent the standard deviation. The different letters represent that the means are significantly different ($p < 0.05$).

3.7. Phage lytic activity in the presence of disinfectants

To determine the lytic activity of the phage in the presence of disinfectants used in poultry, we prepared and tested an equal share of commercially available disinfectants and phage suspension mixes. Each includes one of four different commercially available disinfectants. The double-layer method revealed that the disinfectant-phage mix inhibited bacterial growth due to the antimicrobial action of the disinfectant and prevented the phage activity from being noticed. However, when the disinfectant-phage suspension was prepared seven days before use, the disinfectants decreased their antimicrobial power, allowing bacterial growth and lysis plaque formation. Phage lytic activity was evaluated in 7-day-old disinfectant-phage suspensions, and lysis plaques were counted after 5 hours of incubation. As shown in **Fig. 6**, the lytic activity of the phage was not affected by the presence of disinfectants used in poultry production. However, we observed a statistically significant decrease in lysis plaque numbers counted in the iodine/phage combination.

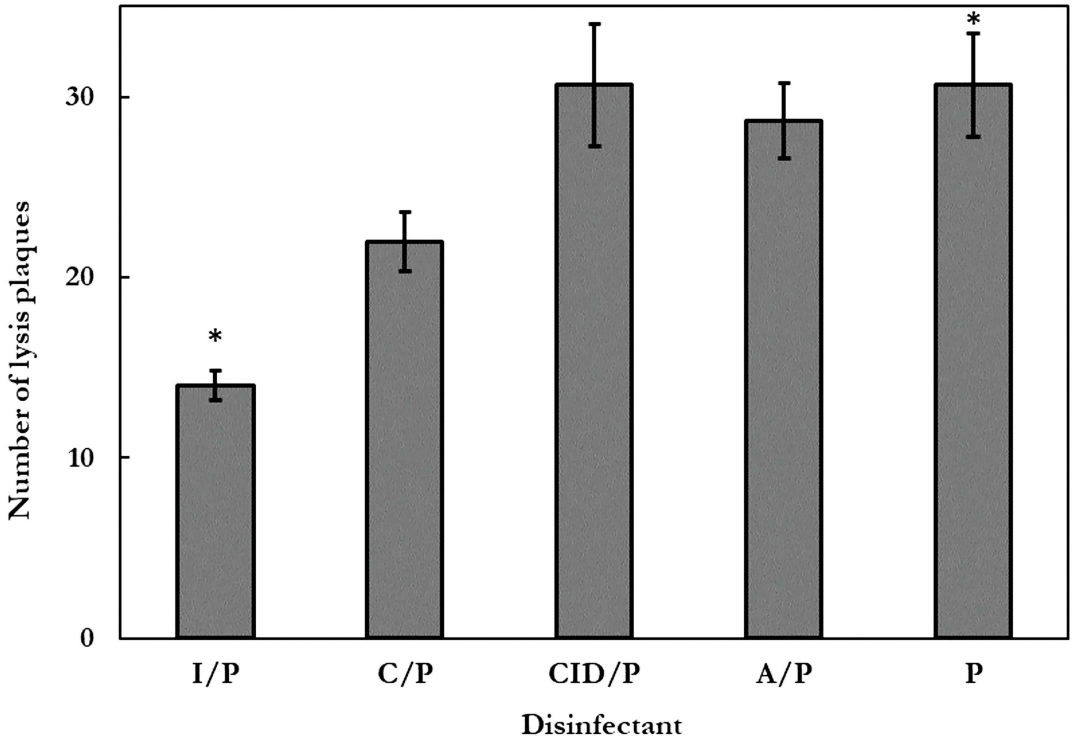

Figure 6. Phage lytic activity in the presence of disinfectants Values represent the mean of three experiments; error bars represent the standard deviation. Disinfectants: I (iodine), P (phage), C (Creolin), CID (CID 20 ®), A (quaternary ammonium). The asterisk represents means that are significantly different ($p < 0.05$).

3.8. Phage lytic activity on different surfaces

To determine the potential use of phage suspensions as biological tools for the control of *S.* Gallinarum, the lytic activity of the bacteriophage on contaminated surfaces was determined. After experimental contamination with *S.* Gallinarum for 60 min, the tested surfaces revealed high bacterial counts (between $4x10^5$ and $1.28x10^8$ CFU/cm^2). As shown in **Fig. 7**, the bacterial load was significantly lower ($p < 0.05$) on the surfaces that received Treatment 1 (phage suspension)

than on those that received Treatment 2 (saline solution). Moreover, of all phage-treated surfaces, bacteriophages were still present after three hours. Of note, bacteriophages could be recovered from the treated surfaces after three-hour exposure (data not shown).

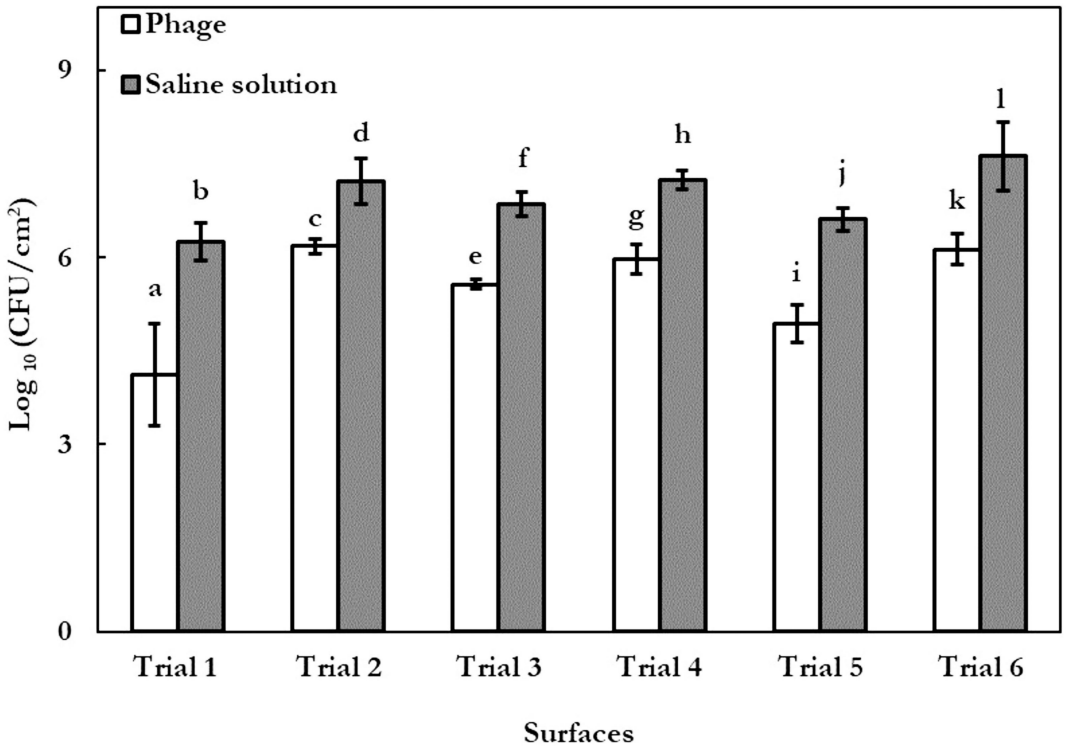

Figure 7. Lytic activity of the phage on different surfaces
Average counts of *Salmonella* Gallinarum were assessed on six different surfaces. Bar heights are the average of five repetitions on each tested surface, after three hours of incubation before treatment. The treated surfaces were: Trial 1 (zinc rectangle: 66 cm^2), Trial 2 (plastic container: 50 cm^2), Trial 3 (plastic bases: 330 cm^2), Trial 4 (collection tape: 150 cm^2), Trial 5 (metal grid: 50 cm^2), and Trial 6 (plastic tube: 30 cm^2). Different letters indicate significant differences ($p < 0.05$) between treatments on the same surface.

4. Discussion

In veterinary medicine, the indiscriminate use of antimicrobials is associated with the emergence of new varieties of resistant microorganisms, which arise as a result of genetic changes that allow them to tolerate high concentrations of the substances that would normally inhibit their growth.

At present, under the influence of the decisions of the European Commission and the FDA [2], the global aim is to reduce and, in some cases, eliminate the use of antimicrobials in production activities [31]. Thus, researchers are looking for natural bactericidal agents, featuring economical viability and minimal risk to human health, to promote animal growth and to control some microorganisms such as *Salmonella* [32, 33].

Lytic bacteriophages constitute an alternative tool to control *Salmonella*. These phages are remarkable for their infection specificity, their kinetics in reaching target cells with a mechanism of action that is different from that of antibiotics, and their low production cost [34, 35, 36, 37].

Due to their minimal environmental impact, bacteriophages are recommended as an alternative for the control of *S.* Gallinarum. However, to know if a phage is useful for application in poultry environments it must be tested under conditions that replicate commercial practices.

In this work, one of the first phage features we addressed was its infection capacity across different bacterial genera and among different *Salmonella* serotypes. Our results indicate that the phage tested is specific for the host serovar, somewhat specific to strains, and able to cross-infect other serovars. The latter probably occurred due to a phenomenon known as lysis from without. This phenomenon was exemplified by *S.* Westhampton sample 6 fee featuring sensitivity to phage at a high MOI while revealing no individual lysis plaques when dilutions of phage were seeded. The distinct profiles of *Salmonella* susceptibility may be explained by the non-specific binding receptors on the bacterial host or different resistance mechanisms during phage infection [37, 38, 39]. Most dsDNA phage genomes are cased into an icosahedral protein shell attached to a tail [40]. The tail-end contains fiber proteins which help them to recognize the receptor on the bacterial cell wall and also restrain them to bind onto non-specific bacterial cells [41, 42].

Also, bacteria could resist phage infections through superinfection exclusion (SIE) systems. SIE systems are likely membrane-associated proteins that prevent the phage DNA from entering bacterial hosts. Although the molecular mechanisms are not yet fully understood, the prophages of some Enterobacteriaceae species have genes encoding the Sim and SieA proteins. The virulent coliphage T4 possesses two different SIE systems, which offer resistance against other T-even-like phages by blocking the phage DNA injection in Gram-negative bacteria. Interestingly, *S. enterica* subsp. *Enterica* serovar Typhimurium carrying lysogenic phage P22 was also reported to have SieA systems and to be insensitive to other phage (*e.g.*, L, mG178, and mG40) infections [43, 44].

Regarding the phage's lytic cycle parameters, the adsorption test revealed a K value of 5.96 x 10^{-10} mL/min. This rate can be considered low compared to the rate of adsorption found by other authors with other *Salmonella* phages [24, 45, 46, 47]. K values are usually in the order of 10^{-9} mL/min for phages such as coliphage T4 [48] that recognizes several hundred cellular receptors, up to values of the order of 10^{-11} mL/min for phages like M13 that recognize 2 or 3 receptors per cell. The leading factors influencing K values among different phages are the presence of salts, organic compounds, receptor counts on the cell surface, agitation, and temperature, among others [48]. As for the one-step curve, we found eclipse period and explosion size values similar to those reported by other authors who worked with bacteriophages infecting the genus *Salmonella* [24, 45, 46, 47]. However, these parameters are characteristic of each virus, and the conditions under which the test is conducted (e.g., host used, culture medium, temperature [41]) will influence the results obtained [49].

Phage stability at different pH values is another important aspect, since acidity, among other physicochemical environmental conditions affecting the virus, could modify a phage's lytic activity [50]. Several authors reported that a low pH affects phages [18, 51, 52, 53], and in our case, we also observed that PFU numbers decreased significantly at pH 2. With these data, the perspective of administering phages orally, as additives in bird food or in drinking water, should be initially ruled out given the stomach's acidity, the presence of digestive enzymes, bile, and the pH of 2.5 that is recorded in the proventriculus and gizzard in commercial chickens [54]. According to our results, at pH 2, we also observed the presence of small bacterial colonies in the center of the lysis plaques, which some authors explain as the appearance of phage-resistant bacterial mutants. Therefore, when applying a phage treatment, a bacteriophage cocktail should be administered so that bacterial mutants resistant to one phage can remain sensitive to the other cocktail components [55]. Another interesting result is that chloroform and high temperature did not significantly

change lysis plaque numbers, which is a fact to consider when storing, purifying, or manipulating phages. Chloroform plays a central role during bacteriophage purification by lysing bacterial remnants, and its use does not significantly decrease phage activity. On the other hand, the phage showed a range of stability at temperatures of 20 °C to 60 °C for one hour. Some authors propose that this behavior could have some connection with the natural habitat from which the phage was isolated [56].

The studied phage did not interfere with the 9R vaccine strains, and its lytic activity was not affected by antibiotics, which shows that the phage can be combined with other antimicrobial agents. Moreover, phages did not affect antibiotic activity either. Studies revealed that phage use with antibiotics can reduce the number of bacteria and regulate the emergence of those resistant to ATB [57, 58, 59]. While bacteria may show resistance to phages, such as developing resistance to ATB, a cocktail of phages that recognize different receptors on their target cell surface could overcome this challenge. However, the rate at which bacteria develop resistance to phages is about ten times lower than that of antibiotics, and the selection and testing of a new phage is faster than the development of new antibiotics, which can take up to several years [60, 61, 62]. Bacteriophages have been used in phage therapies in humans and animals since the beginning of the 20th century, demonstrating their harmlessness [63, 64]. In addition, there is a variety of bibliographic documentation of toxicity tests in laying birds that indicates that animals treated with phages do not show signs of disease [65, 66].

Concerning the effects of disinfectants for veterinary use on the lytic activity of the phage, none was observed, thus favoring its use as a bactericidal agent on different surfaces. Besides, since the presence of organic matter, sunlight, or elevated temperatures inactivate most disinfectants, or these have a limited shelf life after initial dilution [67], phage-based treatments on contaminated surfaces would help disinfectants improve their effectivity. With our assay, we verified that after incubating the phage for a week in a dilution of different disinfectants, the number of lysis plaques was not significantly different from that of phage alone, except for the case of the mixture of iodine/phage, which led to an increase in the size of the lysis halo, likely explaining the lower amount of lyses plaques counted.

Phage-driven reduction of *Salmonella* concentrations on surfaces is another alternative when employing bacteriophages. In our work, the measured lytic activity of phages sprayed on surfaces of different materials significantly reduced *S.* Gallinarum counts compared to surfaces sprayed with a saline solution. Currently, trials applying bacteriophages onto food surfaces to reduce their microbial load have yielded successful results, for instance, against *Salmonella* [68, 69, 70]. Phage applications in the form of spray would help to control the *Salmonella* typical of the environment as a complement in disinfection and hygiene programs.

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6. Conflict of Interests

The authors declare having no conflicts of interest.

References

[1] Lamelas K, Beczcowski G, Mair G, Heredia A. Anuario 2010. Ganados y carnes. Ministerio de Agricultura, Ganadería y Pesca de la Nación. Subsecretaría de Ganadería, Buenos Aires, Argentina 2011.

[2] Regulation (EC) N° 1831/2003 of the European parliament and of the council of 22 September 2003 on additives for use in animal nutrition. *Official Journal of the European Communities*, 268: 29-43, 2003.

[3] Steiner, T. Managing gut health: natural growth promoters as a key to animal performance, Nottingham University Press, UK 2006.

[4] d'Hérelle F. Sur un microbe invisible antagoniste des bacilles dysentériques. *Acta Kravsi*, 2: 40-42, 1961.

[5] Twort FW. An investigation on the nature of ultramicroscopic viruses. *Lancet*, 2: 1241–1243, 1915.

doi:10.1016/S0140-6736(01)20383-3.

[6] Walker PJ, Siddell SG, Lefkowitz EJ, *et al*. Recent changes to virus taxonomy ratified by the International Committee on Taxonomy of Viruses (2022). *Archives of Virology*,167(11):2429-2440, 2022.

doi:10.1007/s00705-022-05516-5

[7] Turner D, Shkoporov AN, Lood C, *et al*. Abolishment of morphology-based taxa and change to binomial species names: 2022 taxonomy update of the ICTV bacterial viruses subcommittee. *Archives of Virology*. 168(2):74, 2023

doi: 10.1007/s00705-022-05694-2

[8] Gutiérrez D, Vandenheuvel D, Martínez B, Rodríguez A, *et al*. Two phages, phiIPLA-RODI and phBiIPLA-C1C, lyse mono- and dual-species staphylococcal biofilms, *Appllied and Environmental Microbiology*, 81: 3336-48, 2015.

doi: 10.1128/AEM.03560-14

[9] Khalifa L, Brosh Y, Gelman D, Coppenhagen-Glazer S, *et al*. Targeting *Enterococcus faecalis* biofilms with phage therapy, *Appllied and Environmental Microbiology*, 81: 2696-2705, 2015.

doi: 10.1128/AEM.00096-15

[10] Nakai T, Sugimoto R, Park K-H, Matsuoka S, *et al*. Protective effects of bacteriophage on experimental *Lactococcus garvieae* infection in yellowtail, *Diseases of Aquatic Organism*, 37(1): 33-41, 1999.

doi: 10.3354/dao037033

[11] Smith HW, Huggins MB, Shaw KM. The control of experimental *Escherichia coli* diarrhea in calves by means of bacteriophages, *Journal of General Microbiology* ,133:1111-1126, 1987.

doi:10.1099/00221287-133-5-1111

[12] Smith H. W., Huggins M. B. Effectiveness of phages in treating experimental *Escherichia coli* diarrhea in calves, piglets and lambs, *Journal of General Microbiology*,129:2659-2675, 1983.

doi:10.1099/00221287-129-8-2659

[13] Stanford K, Niu YD, Johnson R. Oral delivery systems for encapsulated bacteriophages targeted at *Escherichia coli* O157:H7 in feedlot cattle, *Journal of Food Protection*, 73: 1304-1312, 2010

doi:10.4315/0362-028x-73.7.1304

[14] Atterbury RJ, Van Bergen MAP, Ortiz F, Lovell MA, *et al*. Bacteriophage therapy to reduce *Salmonella* colonization of broiler chickens, *Appllied and Environmental Microbiology*, 73(14):4543-4549, 2007.

doi:10.1128/AEM.00049-07

[15] Wagenaar JA, Bergen MAPV, Mueller MA, Wassenaar TM, Carlton RM. Phage therapy reduces *Campylobacter* jejuni colonization in broilers, *Veterinary Microbiology*, 109(3-4):275-283, 2005.

doi:10.1016/j.vetmic.2005.06.002

[16] Andreatti Filho RL, Higgins JP, Higgins SE, Gaona G, *et al*. Ability of bacteriophages isolated from different sources to reduce *Salmonella* enterica serovar enteritidis *in vitro* and *in vivo*, *Poultry Science*, 86(9):1904-1909, 2007.

doi:10.1093/ps/86.9.1904

[17] Huff WE, Huff GR, Rath NC, Balog JM, Donoghue AM. Bacteriophage treatment of a severe *Escherichia coli* respiratory infection in broiler chickens, *Avian Disease*, 47(4):1399-1405, 2003.

doi:10.1637/7041

[18] Huff WE, Huff GR, Rath NC, Balog JM, Donoghue AM. Evaluation of aerosol spray and intramuscular injection of bacteriophage to treat an *Escherichia coli* respiratory infection, *Poultry Science*, 82(7):1108-1112, 2003.

doi:10.1093/ps/82.7.1108

[19] Huff WE, Huff GR, Rath NC, Balog JM, Donoghue AM. Prevention of *Escherichia coli* infection in broiler chickens with a bacteriophage aerosol spray, *Poultry Science*, 81(10):1486-1491, 2002.

doi:10.1093/ps/81.10.1486

[20] Colavecchio A, Goodridge LD. Phage Therapy Approaches to Reducing Pathogen Persistence and Transmission in Animal Production Environments: Opportunities and Challenges, *Microbiology Spectrum Journal* 5(3):5.3.25, 2017.

doi:10.1128/microbiolspec.PFS-0017-2017

[21] Cooper IR. A review of current methods using bacteriophages in live animals, food and animal products intended for human consumption, *Journal of Microbiological* Methods, 130: 38-47, 2016.

doi:10.1016/j.mimet.2016.07.027

[22] Prosdócimo F, Anselmo R, Olivetto N, Gomez I, *et al*., Evaluación de un bacteriófago para la prevención de *Salmonella Gallinarum* en aves de postura, *Revista Argentina de Microbiología*, 42 (1): 114, 2010.

[23] Kropinski AM, Mazzocco A, Waddell TE, Lingohr E, Johnson RP. Enumeration of bacteriophages by double agar overlay plaque assay, *Methods in Molecular Biology*, 501:69-76, 2009.

doi:10.1007/978-1-60327-164-6_7

[24] Mahmoud M, Askora A, Barakat AB, *et al*. Isolation and characterization of polyvalent bacteriophages infecting multi drug resistant *Salmonella* serovars isolated from broilers in Egypt. *International Journal of Food Microbiology*, 266:8-13, 2018.

doi:10.1016/j.ijfoodmicro.2017.11.009

[25] Mazzocco A, Waddell TE, Lingohr E, Johnson RP. Enumeration of bacteriophages using the small drop plaque assay system, *Methods in Molecular Biology*, 2009, 501:81-85.

doi:10.1007/978-1-60327-164-6_9

[26] Hyman P, Abedon ST. Practical methods for determining phage growth parameters, *Methods in Molecular Biology*, 501:175-202, 2009.

doi:10.1007/978-1-60327-164-6_18.

[27] Haq U, Chaudhry W, Andleeb S, *et al*. Isolation and Partial Characterization of a Virulent Bacteriophage IHQ1 Specific for *Aeromonas punctata* from Stream Water, *Microbial Ecology*, 63 (4):954–963, 2012.

doi:10.1007/s00248-011-9944-2

[28] Fernández Espinel C., V. Flores Dominick, M. Medina Morillo. Aislamiento y caracterización del bacteriófago Va1 específico a *Vibrio Alginolyticus*, *Revista Peruana de Biología*, 24(1):93-100, 2017.

doi:10.15381/rpb.v24i1.13103

[29] Manual of food quality control. 12. Quality assurance in the food control microbiological laboratory, *FAO food and nutrition paper*,14(12):1-154, 1991.

http://www.fao.or

[30] Wang IN. Lysis timing and bacteriophage fitness, *Genetics*, 172 (1):17-26, 2006

[31] OIE. Organización Mundial de Sanidad Animal-Una sola salud

http://www.oie.int/es/para-los-periodistas/onehealth-es/

[32] Summers WC. Bacteriophage therapy, *Annual Review of Microbiology*, 55:437-451, 2001.

doi:10.1146/annurev.micro.55.1.437

[33] Wernicki A, Nowaczek A, Urban-Chmiel R. Bacteriophage therapy to combat bacterial infections in poultry, *Virolgy Journal*, 14(1):179, 2017.

doi:10.1186/s12985-017-0849-7

[34] [34] Joerger RD. Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages, *Poultry Science*, 82(4):640-647, 2003.

doi:10.1093/ps/82.4.640

[35] Lim TH, Lee DH, Lee YN, *et al*. Efficacy of bacteriophage therapy on horizontal transmission of *Salmonella Gallinarum* on commercial layer chickens, *Avian Disease*, 55(3):435-438, 2011.

doi:10.1637/9599-111210-Reg.1

[36] Matsuzaki S, Rashel M, Uchiyama J, *et al*. Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases, *Journal of Infection Chemotherapy*, 11(5):211-219, 2005.

doi:10.1007/s10156-005-0408-9

[37] Bielke L, Higgins S, Donoghue A, Donoghue D, Hargis BM. *Salmonella* host range of bacteriophages that infect multiple genera, *Poultry Science*, 86(12):2536-2540, 2007.

doi:10.3382/ps.2007-00250

[38] Shin H, Lee JH, Kim H, Choi Y, Heu S, Ryu S. Receptor diversity and host interaction of bacteriophages infecting *Salmonella* enterica serovar Typhimurium, *Public Library of Science* (PLoS One), 7(8): e43392, 2012.

doi:10.1371/journal.pone.0043392

[39] Duckworth DH, Glenn J, McCorquodale DJ. Inhibition of bacteriophage replication by extrachromosomal genetic elements, *Microbiological Reviews*, 45(1):52-71, 1981.

doi:10.1128/mr.45.1.52-71.1981

[40] Calendar R, Inman R. Phage biology. Phages: Their Role in Bacterial Pathogenesis and Biotechnology, American Society for Microbiology Press, Washington DC, USA, 2005

[41] Kutter E, R. Raya R, Carlson K. Bacteriophages: Biology and Applications, CRC Press, Boca Raton, Florida, USA, 2005

[42] Singh A, Arya SK, Glass N, Hanifi-Moghaddam P, *et al*. Bacteriophage tailspike proteins as molecular probes for sensitive and selective bacterial detection, *Biosensors and Bioelectronics*, 26(1):131-8, 2010.

doi:10.1016/j.bios.2010.05.024.

[43] Hofer B, Ruge, M, Dreiseikelmann B. The Superinfection Exclusion Gene (sieA) of Bacteriophage P22: Identification and Overexpression of the Gene and Localization of the Gene Product, *Journal of Bacteriology*, 177(11):3080-3086, 1995.

doi:10.1128/jb.177.11.3080-3086.1995.

[44] Labrie SJ, Samson JE, Moineau S. Bacteriophage resistance mechanisms, *Nature Reviews Microbiology*, 8(5):317-327, 2010.

doi: 10.1038/nrmicro2315 PMID: 20348932.

[45] Hong SS, Jeong J, Lee J, Kim S, *et al*. Therapeutic effects of bacteriophages against *Salmonella* Gallinarum infection in chickens, *Journal of Microbiology Biotechnology*, 23(10): 1478-1483, 2013.

doi:10.4014/jmb.1304.04067

[46] Carey-Smith GV, Billington C, Cornelius AJ, Hudson JA, Heinemann JA. Isolation and characterization of bacteriophages infecting *Salmonella* spp., *FEMS Microbiology Letters*, 258(2):182-186, 2006.

doi:10.1111/j.1574-6968.2006.00217.x

[47] Pereira C, Moreirinha C, Lewicka M, *et al*. Bacteriophages with potential to inactivate *Salmonella* Typhimurium: Use of single phage suspensions and phage cocktails, *Virus Research*, 220:179-192, 2016.

doi:10.1016/j.virusres.2016.04.020

[48] Kasman LM, Kasman A, Westwater C, Dolan J, *et al*. Overcoming the phage replication threshold: a mathematical model with implications for phage therapy, *Journal of Virology*, 76(11):5557-5564, 2002.

doi:10.1128/jvi.76.11.5557-5564.2002

[49] Hyman P, Abedon ST. Practical methods for determining phage growth parameters, *Methods in Molecular Biology*, 501:175-202, 2009.

doi:10.1007/978-1-60327-164-6_18

[50] Jończyk E, Kłak M, Międzybrodzki R, Górski A. The influence of external factors on bacteriophages–review. *Folia Microbiologica*, 56(3):191-200, 2011.

doi:10.1007/s12223-011-0039-8

[51] Huang C, Virk SM, Shi J, *et al*. Isolation, Characterization, and Application of Bacteriophage LPSE1 Against *Salmonella* enterica in Ready to Eat (RTE) Foods, *Frontiers in Microbiology*, 9:1046, 2018.

doi:10.3389/fmicb.2018.01046

[52] Tang F, Zhang P, Zhang Q, *et al*. Isolation and characterization of a broad-spectrum phage of multiple drug resistant *Salmonella* and its therapeutic utility in mice, *Microbial Pathogenesis*, 126:193-198, 2019.

doi:10.1016/j.micpath.2018.10.042

[53] Soto MJ, Retamales J, Palza H, Bastías R. Encapsulation of specific *Salmonella* Enteritidis phage f3αSE on alginate-spheres as a method for protection and dosification, *Electronic Journal of Biotechnology*, 31:57-60, 2018.

doi:10.1016/j.ejbt.2017.11.006

[54] Ghise A. The pH of the Digesta in the Gastrointestinal Tract in Laying Hens. Bulletin UASVM, *Veterinary Medicine*, 66: 491-492, 2009.

doi:10.15835/buasvmcn-vm:66:1:4061

[55] Sillankorva S, Pleteneva E, Shaburova O, *et al*. *Salmonella* Enteritidis bacteriophage candidates for phage therapy of poultry, *Journal of Applied Microbiology*, 108(4):1175-1186, 2010.

doi:10.1111/j.1365-2672.2009.04549.x

[56] Ul Haq I, Chaudhry WN, Andleeb S, Qadri I. Isolation and partial characterization of a virulent bacteriophage IHQ1 specific for *Aeromonas punctata* from stream water, *Microbial Ecology*, 63(4):954-63, 2012.

doi: 10.1007/s00248-011-9944-2

[57] Tagliaferri TL, Jansen M, Horz HP. Fighting Pathogenic Bacteria on Two Fronts: Phages and Antibiotics as Combined Strategy, *Frontier in Cellular and Infection Microbiology*, 9: 22, 2019.

doi:10.3389/fcimb.2019.00022

[58] Valério N, Oliveira C, Jesus V, *et al*. Effects of single and combined use of bacteriophages and antibiotics to inactivate *Escherichia coli*, *Virus Research*, 240:8-17., 2017.

doi:10.1016/j.virusres.2017.07.015

[59] Torres-Barceló C. The disparate effects of bacteriophages on antibiotic-resistant bacteria, *Emerging Microbes and Infections*, 7(1):168, 2018.

doi:10.1038/s41426-018-0169-z

[60] Ly-Chatain MH. The factors affecting effectiveness of treatment in phages therapy, *Frontiers in Microbiology*, 5:51, 2014.

doi:10.3389/fmicb.2014.00051

[61] Moelling K, Broecker F, Willy C. A Wake-Up Call: We Need Phage Therapy Now, *Viruses*, 10(12):688, 2018.

doi:10.3390/v10120688

[62] Saha D, Mukherjee R. Ameliorating the antimicrobial resistance crisis: phage therapy, *IUBMB Life*, 71(7):781-790. 2019.

doi:10.1002/iub.2010

[63] Skurnik M, Strauch E. Phage therapy: facts and fiction, *International Journal of Medical Microbiology*, 296(1):5-14, 2006.

doi:10.1016/j.ijmm.2005.09.002

[64] Wernicki A, Nowaczek A, Urban-Chmiel R. Bacteriophage therapy to combat bacterial infections in poultry, *Virology Journal*, 14(1):179-192, 2017.

doi:10.1186/s12985-017-0849-7

[65] Sulakvelidze A, Alavidze Z, Morris JG Jr. Bacteriophage therapy, *Antimicrobial Agents and Chemotherapy*, 45(3):649-659, 2001.

doi:10.1128/AAC.45.3.649-659.2001

[66] Xie H, Zhuang X, Kong J, Ma G, Zhang H. Bacteriophage Esc-A is an efficient therapy for *Escherichia coli* 3-1 caused diarrhea in chickens, *Journal of General and Applied Microbiology*, 51(3):159-163, 2005.

doi:10.2323/jgam.51.159

[67] Stringfellow K, Anderson P, Caldwell D, *et al*. Evaluation of disinfectants commonly used by the commercial poultry industry under simulated field conditions, *Poultry Science*, 88(6):1151-1155, 2009.

doi:10.3382/ps.2008-00455

[68] Duc H M, Son, H M, Honjoh K I, Miyamoto T. Isolation and application of bacteriophages to reduce *Salmonella* contamination in raw chicken meat, *LWT – Food Science and Technology*, 91:353-360, 2018.

doi:10.1016/j.lwt.2018.01.072

[69] Hooton SP, Atterbury RJ, Connerton IF. Application of a bacteriophage cocktail to reduce *Salmonella Typhimurium* U288 contamination on pig skin, *International Journal of Food Microbiology*, 151(2):157-163, 2011.

doi:10.1016/j.ijfoodmicro.2011.08.015

[70] Bai J, Jeon B, Ryu S. Effective inhibition of *Salmonella Typhimurium* in fresh produce by a phage cocktail targeting multiple host receptors, *Food Microbiology*, 77:52-60, 2019.

doi:10.1016/j.fm.2018.08.011

Estudio de un bacteriófago lítico como herramienta para el control de *Salmonella* Gallinarum en aves ponedoras

Resumen: La fiebre tifoidea aviar, causada por el patógeno *Salmonella enterica* serovar Gallinarum (*S*. Gallinarum) es una de las principales enfermedades que afectan a las aves ponedoras en Argentina. Si bien esta enfermedad fue erradicada de los criaderos industriales de varios países desarrollados, aún afecta a granjas comerciales de América Latina, causando altas tasas de mortalidad, producción limitada de huevos de calidad, altos costos veterinarios y la necesidad de renovar las instalaciones contaminadas con la bacteria, lo cual lleva a pérdidas económicas sustanciales. En el presente estudio, caracterizamos un bacteriófago en términos de especificidad del hospedador, cinética de crecimiento y estabilidad bajo diferentes condiciones ambientales con el fin de desarrollar un nuevo método de control biológico de *S.* Gallinarum. El fago logró infectar una amplia gama de huéspedes bacterianos y no mostró actividad lítica contra la cepa de vacuna 9R. Además, el fago se mantuvo estable a un pH de 3. La presencia de cloroformo no disminuyó de manera significativa su título, y su actividad no se vio afectada por antibióticos o compuestos químicos comúnmente empleados en la avicultura. Aplicar una suspensión de fagos en superficies contaminadas con *S.* Gallinarum resultó en una reducción significativa de la carga bacteriana ($p < 0.05$). Estos resultados muestran que los bacteriófagos líticos como el aquí estudiado son alternativas innovadoras con aplicaciones potenciales para el control de *S.* Gallinarum en granjas de aves ponedoras.

Palabras Clave: Aves ponedoras; Bacteriófago; Biocontrol; Fiebre tifoidea aviar; *Salmonella* Gallinarum.

Estudo de um bacteriófago lítico como ferramenta para o controle de *Salmonella* Gallinarum em aves poedeiras

Resumo: A febre tifoide aviária, causada pelo patógeno *Salmonella enterica* sorovar Gallinarum (*S.* Gallinarum) é uma das principais doenças que afetam as aves poedeiras na Argentina. Embora essa doença tenha sido erradicada de granjas industriais em vários países desenvolvidos, ela ainda afeta granjas comerciais na América Latina, causando altas taxas de mortalidade, produção limitada de ovos de qualidade, altos custos veterinários e a necessidade de renovação das instalações contaminadas pela bactéria, levando a perdas econômicas substanciais. No presente estudo, caracterizamos um bacteriófago em termos de especificidade do hospedeiro, cinética de crescimento e estabilidade sob diferentes condições ambientais, a fim de desenvolver um novo método de controle biológico para *S.* Gallinarum. O fago conseguiu infectar uma ampla gama de hospedeiros bacterianos e não mostrou atividade lítica contra a cepa vacinal 9R. Além disso, o fago demonstrou estabilidade em pH 3. A presença de clorofórmio não diminuiu significativamente seu título, e sua atividade não foi afetada por antibióticos ou compostos químicos comumente utilizados na avicultura. A aplicação de uma suspensão de fagos em superfícies contaminadas com *S.* Gallinarum resultou na redução significativa da carga bacteriana ($p < 0,05$). Estes resultados demonstram que bacteriófagos líticos, como o estudado aqui, representam alternativas inovadoras com potenciais aplicações para o controle de *S.* Gallinarum em granjas avícolas poedeiras.

Palavras-chave: Aves poedeiras; Bacteriófago; Biocontrole; Febre tifoide aviária; *Salmonella* Gallinarum.

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