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Phage therapy of colibacillosis in chickens

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Abstract:
Avian pathogenic *Escherichia coli* (APEC) strains are causative agents of colibacillosis, an infectious disease that inflicts substantial commercial losses on poultry farms worldwide. The growing resistance of *E. coli* to antibiotics necessitates the search for alternative approaches to treat and prevent avian colibacillosis, phage therapy being one of them.
This review covers 25 years of experimental studies (PubMed, Google Scholar) that examine various aspects of phage therapy with a particular focus on comparing different administration routes (with drinking water; injected orally, intratracheally, or intramuscularly; sprayed as aerosols). It also compares various techniques of introducing phage preparations to avian embryos (injections *in ovo*).
The review describes such aspects as the general efficacy of the administration routes and their specific advantages (e. g., convenience of the aerosol-spraying technique for treating large flocks). It focuses on the advantages of multi-phage cocktails over single-phage preparations, combined antibiotic/phage therapy during colibacillosis epidemics, and administering phage preparations in nanocapsules. The review also discusses the possible interference of the avian immune system with the delivery of phage particles to the infected organs and the importance of phage selection for the success of the therapy.

Keywords: Phage therapy, avian pathogenic *Escherichia coli*, chickens, antimicrobial resistance, coliphage, colibacillosis

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INTRODUCTION

Avian colibacillosis is an infectious disease caused by avian pathogenic *Escherichia coli* (APEC) strains [1, 2]. The disease affects multiple organ systems of a bird, with the mortality rate being quite high among both chicks and adults. The acute course of the disease leads to colisepticemia [3] and, eventually, death; in the subacute cases, the disease manifests itself in the forms of pericarditis, perihepatitis [4], airsacculitis [5], salpingitis [6], peritonitis, and internal lesions [7, 8]. Every year, many poultry farms suffer significant losses due to colibacillosis epidemics [9].

Colibacillosis can be prevented by interior disinfection and vaccination or cured with antibiotics [8, 10–12]. In recent years, interest in phage therapy has increased significantly, particularly due to the increasing resistance of *E. coli* strains to most known antibiotics, including β-lactams, tetracyclines, aminoglycosides, nitrofurans, quinolones, and sulfonamides [13–20].

Phage therapy relies on bacteriophages (viruses of bacteria) to destroy pathogenic microorganisms [21]. Phages are strain-specific towards their bacterial hosts, which makes them a very precise therapeutic tool: while targeting specific pathogenic strains of bacteria, phages will not affect the commensal bacterial strains of the treated organism [21–26]. Every year, scientists discover new bacteriophages that successfully lyse APEC *in vitro*. In 2024–2025 alone, dozens of new bacteriophages were reported to have efficacy against *E. coli* strains that cause avian colibacillosis [27–38].

Over the past decades, the capacity of phage therapy to combat colibacillosis in chickens has been thoroughly investigated *in vivo*. These experiments were controlled (induced infection) and uncontrolled (colibacillosis epidemics). To date, various phage cocktails have been tested, with their effects assessed in birds of different age groups (including chicken embryos). The experimental results show that the efficacy of the

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therapy depends on multiple factors: bacteriophage strain, concentration of phage particles in the cocktail, therapy duration, immune response to the cocktail administration, etc. From the practical standpoint, the method of phage administration is a particularly important factor. On the one hand, it should be technically simple, which is crucial for treating birds on large poultry farms. On the other hand, it should involve measures to preserve the activity of the preparation administered. The method of phage administration is the key to successful therapy, as demonstrated by studies that compared different routes of administration of the same preparations to experimental animals.

This review considers a number of phage cocktails tested for the treatment of colibacillosis in chickens: their composition, the administration routes, and the conditions of their applicability. The review also includes prospects of using phage therapy to treat colibacillosis in the foreseeable future. Tables 1 and 2 summarize the information about bacteriophages and their cocktails. Tables 3–8 structure the details of the *in vivo* experiments reviewed.

Table 1 A brief summary of *Caudoviricetes* bacteriophages used to treat colibacillosis in chickens

Genbank No.	Name	Genus	Family/subfamily	Host *Escherichia coli* strain	Test subjects	Isolation source	Reference
KY619305	vB_EcoS_ ESCO41	*Nouzillyvirus*	*Drexlerviridae*	APEC strain BEN3573 (serotype O78)	Chicken embryos	Content of avian caecum (France)	[46]
OM386652	vB_EcoS_ ESCO3	*Nonagvirus*	*Queuovirinae*	APEC strain Cp6Salp3 (serotype O1); APEC strains BEN2908, BEN3685, BEN4358 (serotype O2); DH5alpha; MG1655	Chicken embryos	Content of avian caecum	[46]
OM386654	vB_EcoM_ ESCO9	*Justusliebigvirus*	*Stephanstirmvirinae*	APEC strain BEN3685 (serotype O2); APEC strain BEN1487 (serotype O18); DH5alpha	Chicken embryos	Content of avian caecum	
OM386667	vB_EcoM_ REC	*Phapecoctavirus*	*Stephanstirmvirinae*	APEC strains BEN4234, BEN4311, Cp6Salp3 (serotype O1); APEC strains BEN2908, BEN3388, BEN3685, BEN4358, BEN5202 (serotype O2); APEC strains BEN88, BEN984 (serotype O5); APEC strains BEN3801, BEN3992 (serotype O18); APEC strain BEN3813 (serotype O78)	Chicken embryos	Laboratory	
OM386655	vB_EcoM_ ESCO10	*Dhakavirus*	*Tevenvirinae*	APEC strain BEN1274 (serotype O8); DH5alpha, MG1655	Chicken embryos	Content of avian caecum	
OM386662	vB_EcoM_ ESCO47	*Mosigvirus*	*Tevenvirinae*	APEC strain BEN4358 (serotype O2); DH5alpha; MG1655	Chicken embryos	Horse manure	
OM386666	vB_EcoM_ ESCO58	*Tequatrovirus*	*Tevenvirinae*	APEC strain BEN3843, strain Cp6Salp3 (serotype O1); APEC strains BEN2908, BEN4358 (serotype O2); APEC strain BEN2408 (serotype O5); APEC strains BEN1487, BEN3801, BEN3992 (serotype O18); APEC strains BEN536, BEN3328, BEN3573 (serotype O78)	Chicken embryos	Water	
OM386657	vB_EcoS_ ESCO30	*Tequintavirus*	*Markadamsvirinae*; *Demerecviridae*	DH5alpha, MG1655	Chicken embryos	Content of avian caecum	

Continuation of Table 1

Genbank No.	Name	Genus	Family/subfamily	Host *Escherichia coli* strain	Test subjects	Isolation source	Reference
KY295899.1	Phage R or Escherichia phage vB_EcoP_R	*Vectrevirus*	*Molineuxvirinae; Autographiviridae*	O18ac:K1:H7 ColV +	Chickens; chicken embryos	n.d.	[47–49]
KC206276.2	Escherichia phage EC1-UPM	*Gamaleyavirus* EC1UP	*Enquatrovirinae; Schitoviridae*	O78:K80	Chickens	Chicken fecal material (Malaysia)	[50, 51]
KF562341	Escherichia phage vB_EcoM_PhAPEC2	*Mosigvirus*	*Tevenvirinae; Straboviridae*	APEC strains of serotypes O45, O78, O2, O18, O83, K-12	Chickens	River water (Belgium)	[52]
KF192075.1	Escherichia phage vB_EcoP_PhAPEC5	*Gamaleyavirus*	*Enquatrovirinae; Schitoviridae*	APEC strains of serotype O78	Chickens	River water (Belgium)	[52]
KF562340	Escherichia phage vB_EcoP_PhAPEC7	*Gamaleyavirus*	*Enquatrovirinae; Schitoviridae*	APEC strains of serotype O78	Chickens	River water (Belgium)	[52]
MG867727.1	Escherichia phage vB_EcoM-G28 or G28	*Tequatrovirus* ecomg28	*Tevenvirinae; Straboviridae*	E28	Chickens	Manure (Germany)	[53, 54]
LC030304.1	Escherichia coli phage phiKAZ14	n.d.	n.d.	O1:K1:H7	Chickens	Chicken feces (Malaysia)	[55, 56]

n.d. – not detected

Table 2 Unidentified bacteriophages used to treat avian colibacillosis

Phage	Phage family (old classification based on particle morphology)	Host *Escherichia coli* strain	Test subjects	Isolation source	Reference
SPR02	*Myoviridae*	*Escherichia coli* (serotype 02)	Chickens	n.d.	[57]
DAF6	n.d.	*Escherichia coli* (serotype 02)	Chickens	n.d.	[58]
PhAPEC9	n.d.	APEC strain (serotype O78)	Chickens	River water (Belgium)	[52]
TM3	*Siphoviridae*	APEC strains O78:K80 and O2:K1; ExPEC strains O126:K71 and O86:K61	Quails, chickens	n.d.	[59, 60]
TM1	*Siphoviridae*	APEC strains O78:K80 and O2:K1; ExPEC strains O126:K71and O86:K61	Quails, chickens	n.d.	[59, 60]
TM2	*Siphoviridae*	APEC strains O78:K80 and O2:K1; ExPEC strains O126:K71 and O86:K61	Quails, chickens	n.d.	[59, 60]
TM4	*Siphoviridae*	APEC strains O78:K80 and O2:K1; ExPEC strains O126:K71 and O86:K61	Quails, chickens	n.d.	[59, 60]
Esc-A	*Siphoviridae*	*Escherichia coli* strain 3-1	Chickens	Sewage samples	[61]
A, C, D	*Siphoviridae*	*Escherichia coli* strain 3-1	Chickens	Soils, feces, and content of chicken intestinal tract during outbreak of chicken diarrhea (poultry farm, Jinan, Shandong province)	[62]
Phi F78E	*Myoviridae*	APEC strains of serotypes O78, O5, O103, O2, O88	Chickens	Poultry sewage	[63]
Phi F61E	*Myoviridae*	APEC strains of serotypes O 78, O5, O2, O88	Chickens	Poultry sewage	[63]
Phi F258E	*Siphoviridae*	APEC strains of serotypes O78, O5, O2	Chickens	Poultry sewage	[63]
O119	*Podoviridae*	APEC strains of serotype O119	Chickens	n.d.	[64]
KRA2, TB49, AB27, TriM, G28	*Myoviridae*	*Escherichia coli* E28	Chickens	n.d.	[53, 54]

n.d. – not detected

STUDY OBJECTS AND METHODS

In December of 2024, we systematically searched PubMed and Google Scholar for English papers using the following keywords and phrases: "phage therapy of (chickens OR birds OR broilers)"; "phage therapy of colibacillosis"; "phage against *E. coli in vivo* chickens"; "avian colibacillosis"; "phage against avian colibacillosis"; "phage therapy against APEC". The search was not refined by the publishing date, journal, its impact factor, authors, or their affiliations. The inclusion criteria for the articles to be reviewed were as follows: primary articles; articles published in English, regardless of the study design or publication date; and studies deemed most relevant for this review.

RESULTS AND DISCUSSION

Oral introduction of phages. Phages can be introduced orally. This route of administration primarily ensures the penetration of the phage into the gastrointestinal tract of the bird, where pathogenic *Escherichia coli* can first be localized. In this chapter, we consider the method of introducing the preparation with food and water or directly, as a phage cocktail. Encapsulated phage administration is considered separately. One of the simplest and most accessible ways to administer a phage cocktail to chickens is to add it to their food or drinking water. However, this method of administration makes it impossible to dose the medicine accurately. Usually, chickens are given free (*ad libitum*) access to water, which makes it difficult to control whether all the birds drink the same amount of it and thus receive a sufficient dose of the cocktail. In addition, phage titer in the food and water should be checked periodically, since phage particles can be unstable in an unbuffered and unsterile medium. Fluctuations of temperature and pH, as well as contamination with proteases and other enzymes are factors that can compromise the integrity of the phage capsid [39–43]. The resistance of bacteriophages to such stresses depends on their type [44]. For example, phages of the gastrointestinal tract are, as a rule, more resistant to low pH and digestive enzymes, although there are some exceptions [45].

Administration of phage cocktails as a drinking-water additive ("water route") was thoroughly studied by Huff *et al.* [57], who used a preparation containing bacteriophage SPR02 (Tables 2 and 3). According to the data of particle morphology and PCR tests, the phage was T4-related. The researchers simulated colibacillosis infection by injecting *E. coli* (serotype 02) into the bird's thoracic air sac. The birds injected with 10^3 CFU of *E. coli* were given water containing 10^3 or 10^6 PFU/mL of the phage (multiplicity of 1 and 1,000, respectively); the birds injected with 10^4 CFU of *E. coli* were given water with 10^4 or 10^8 PFU/mL of the phage (multiplicity of 1 and 10,000, respectively). The inoculation of birds with pathogenic *E. coli* took place during the first week of their life. Starting from the moment of their hatching, the chicks were given phage-supplemented water for a week. The experiment lasted three weeks.

The mortality of the phage-treated birds diminished, but not much: it was 25–30%, as compared with mortality of the untreated birds, which was in the range of 30–55%. The second experiment was similar to the first one, but with altered parameters of the therapy: the birds in the two experimental groups received phage-infused water for 14 days, starting from the day of hatching. The phage titer in the water was either 10^5 or 10^6 PFU/mL. At the age of one week, the birds were infected with 10^4 CFU/mL of *E. coli* into the thoracic air sac. By the end of the experiment, mortality in the two phage-treated experimental groups reached 60 and 90% respectively, whereas in the group of infected but untreated birds, it was at 75%. On week 3, the phage-treated chicken started to lose weight, as compared with the control birds (both uninfected and infected-untreated). Thus, administering a single phage with drinking water was obviously not enough to save the chickens or even to reduce the harmful consequences of the disease. The titer of the phage in water was low and could even become lower within the body, which may have affected the results of the experiment.

Other studies showed similar results [53]. Contrary to what was expected, phage therapy produced no positive effects. As compared to other routes, phage administration with drinking water proved less successful [52].

Instead of assessing mortality, Kittler *et al.* [53] monitored the dynamics of *E. coli* strain E28 and the total number of *E. coli* cells in chicken feces after administering phage preparation with drinking water. The research consisted of two experiments: 1) with elevated concentrations of *E. coli* and a reduced concentration of the phage cocktail; and 2) with reduced concentrations of *E. coli* and an elevated concentration of the phage cocktail. In experiment 1, the phage cocktail contained six phages (KRA2, EW2, TB49, AB27, TriM and G28). In experiment 2, two phages (KRA2 and TriM) were excluded from the cocktail (Tables 2 and 3). One-day-old chicks were divided into two groups (experimental and control), with the experimental group given access to phage-supplemented water ($\log_{10}$ 4.6 and $\log_{10}$ 6.7 PFU/mL in experiments 1 and 2, respectively). The water was changed daily, with phage titers regularly monitored. On day 6, the chicks were orally inoculated with *E. coli* E28 ($\log_{10}$ 8.9 and $\log_{10}$ 4.67 CFU in experiments 1 and 2, respectively).

To estimate the effect of phage therapy on *E. coli* in the infected chicks, their feces were collected and analyzed for *E. coli* count. The results showed that the *E. coli* count in the experimental group remained approximately at the same level, decreasing slightly over 3 h after infection. In the end, the content of *E. coli* E28 in the feces of the control birds dropped to approximately $\log_{10}$ 4 CFU/g; in the experimental group, it remained 1.5 log higher, at ~ $\log_{10}$ 6 CFU/g. The total fecal content of *E. coli* remained at the same level in both groups. The phage titers in the chick feces increased on days 6–7 and exceeded the overall dose that had been administered to them with the phage-supplemented

water. Experiment 2 partially confirmed the results of experiment 1: the fecal content of *E. coli* in the birds of the experimental group remained stable, not exceeding $\log_{10}$ 6 CFU/g; in the birds of the control group, it was slightly higher, varying from $\log_{10}$ 6 to $\log_{10}$ 7 CFU/g. When the birds were inoculated with *E. coli* E28, its content was higher in the experimental group. Two weeks after the hatching, the phage titer of their feces increased and exceeded the dose that the birds had received with water. Thus, these two *in vivo* experiments demonstrated that, judging by the fecal content of the orally introduced *E. coli* E28, administration of the six-phage cocktail as a water additive was ineffective in terms of preventing *E. coli* infection. However, the four-phage cocktail reduced the fecal content of *E. coli*. This finding was perplexing, since the analysis of isolated *E. coli* E28 cells showed that they remained sensitive to the cocktail phages (very few colonies were found to be immune to all of them). The authors supposed that, under specific environmental pressures of the avian gastrointestinal tract, the cocktail phages tended to infect other strains of *E. coli* rather than E28. The authors also hypothesized that the chicken immune system might have influenced the colonization of *E. coli* strains.

The data published by Kittler *et al.* [53] correlate with another study [65], which investigated the effect of a commercial phage cocktail on the microflora profile of the avian gastrointestinal tract. The cocktail (a product of C.J. Cheiljedang Corp.; Seoul, South Korea) was administered as a food additive; it was a mix of phages targeting *Salmonella gallinarum*, *Salmonella typhimurium*, *Salmonella enteritidis*, *E. coli* (each phage, at 10^8 PFU/g), and *Clostridium perfringens* (10^6 PFU/g). In the control group, which received a basic diet, the intestinal microbiome was mainly represented by *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*. In the birds treated with a low dose of the phage cocktail (0.05% per kg feed), the microbiome was dominated by *Firmicutes* and *Bacteroidetes* (in particular, representatives of *Lactobacillus* and *Prevotella*), with *Bifidobacteria* also growing in numbers. The dominance of these bacterial genera in the microbiome of broiler chickens is generally believed to have a positive effect on the function of their gastrointestinal tract, thereby improving their health. The birds that received a higher dose of the phage cocktail (0.1% per kg feed) also demonstrated a more abundant profile of *Proteobacteria* (which includes *E. coli*), whereas *Proteobacteria* became more diverse: 5.45% of total bacterial sequences vs. 1.38% in the untreated or 2.78% in the antibiotic-treated birds. Considering these data, the authors concluded that the phage cocktail used in the experiment might have been slightly overdosed. In addition, the analysis of feces of the phage-treated birds revealed no significant changes in the count of the pathogenic bacteria targeted by the cocktail (*E. coli*, *Salmonella* sp., *C. perfringens*), unlike the control groups (untreated or antibiotic-treated). However, the tests showed a slight linear increase in the *Lactobacillus* count, which was consistent with the results of the intestinal profiling. The addition of either phage cocktail or antibiotic to the basic diet had little effect on the absorption of nutrients. The authors attributed this result to the fact that the study was carried out on healthy, uninfected birds.

As part of feed or water, the oral introduction of phage cocktails cannot guarantee success. Yet, the major advantages of this administration method are its simplicity and accessibility. The literature data indicate that, under certain conditions, oral administration can be efficacious in treating colibacillosis in chickens.

An example is the oral administration of a phage preparation against *E. coli* O119 (Table 3), which is described in the study by Sorour *et al.* [64]. The researchers reported the results of two experiments that differed by the order of phage introduction and bacterial inoculation. In the group that received the phage preparation immediately after being infected with *E. coli* O119, no deaths were registered. In the group infected before receiving the phage preparation, mortality reached 10%. In the control group of infected-untreated birds, mortality was at 30%. By day 23, the cecum and heart/liver contents of *E. coli* O119 decreased in the experimental groups, as compared with the control group. The study revealed no statistically significant differences in these parameters between the two experimental groups. Thus, under certain conditions, the oral introduction of a phage cocktail could be efficacious. The concentration of the cocktail used in the study was rather low (10^2 PFU/mL), but the phage was administered on a weekly basis. Perhaps, the course-type administration of the medicine was the key to successful therapy.

The positive outcome of a course-type oral phage administration was also reported by Xie *et al.* [61]. They administered infected birds with a two-week course of either phage (10^5 PFU) or antibiotic (chloromycetin; 1 mg), with the control group receiving no treatment (Table 3). The experiments showed that the chickens of the phage-treated group had a slightly lower mortality (1.2% on week 1 and 2.8% on week 2), as compared with the antibiotic-treated birds (6 and 8%, respectively). The percentage of birds that developed symptoms of infection during week 1 was also slightly lower in the group receiving phage therapy than in the antibiotic-treated group, i. e., 26 and 36%, respectively. On week 2, the symptoms of infection completely disappeared in the phage-treated birds, contrary to what was observed in the other groups. Thus, phage therapy turned out to be quite efficacious in this case, being no inferior to antibiotic therapy. Unfortunately, the authors of the study did not provide data on the titer of inoculated *E. coli*, which complicated our comparative analysis.

In conclusion, neither one-time oral introduction of a phage preparation nor administering phages with drinking water seem to be efficacious enough. Overall, the analysis of *in vivo* experiments modelling *E. coli* infection in birds and the assessment of the efficacy of phage administration as a water/feed additive lead us to the conclusion that this route is not very good in terms

Table 3 Experiments *in vivo* on the treatment or prevention of avian colibacillosis using oral administration of phage therapy

Phages per cocktail	Administration	Concentration/dose of preparation	Duration, days	Mortality in phage-treated group, %	Mortality in untreated group, %	Concentration of inoculated *Escherichia coli*	Route and age of *Escherichia coli* introduction	Age at onset	Reference
6	With water; every day from day 1 of life	$\log_{10}(x) = 4.6$ PFU/mL	35	n.d.	n.d.	$\log_{10}(x) = 8.9$ CFU	Orally; day 6	Newly hatched	[53]
4	With water; every day from day 1 of life	$\log_{10}(x) = 6.7$ PFU/mL				$\log_{10}(x) = 4.67$ CFU/mL			–
4	With water; 2 h after infection	10^9 PFU	7	42	66 (for inactivated phage cocktail)	10^8 CFU	Intratracheally; 4 weeks	4 weeks	[52]
1	With water; for a week	10^3 PFU/mL	21	25	30	2.16×10^3 CFU	Injection into thoracic air sac; day 1	1 day	[57]
		10^6 PFU/mL		30					–
		10^4 PFU/mL		35	55	1.12×10^4 CFU			–
		10^6 PFU/mL		40					–
	With water; for two weeks	10^5 PFU/mL	21	60	75	2.29×10^4 CFU	Injection into thoracic air sac, week 1	1 day	–
		10^6 PFU/mL		90					–
1	Orally; 2 h after infection and on days 2, 7, 14, 21 of life	10^2 PFU/mL	23	0	30	10^9 CFU/mL	Orally; day 1	1 day	[64]
	Orally; 1 day before infection and on days 3, 7, 14, 21 of life			10	30				–
1	Orally; for two weeks	1×10^5 PFU	21	2.80	14.8	n.d.	n.d.	20 days	[61]
1	Orally; encapsulated (0.2 mL)	10^7 PFU/mL	21	16.7	58.0	10^9 CFU/mL	Intratracheally; day 7	1 day	[66]

n.d. – not detected

of reducing the colibacillosis-related mortality. However, a carefully chosen phage cocktail can have a positive effect on the function of the gastrointestinal tract in broiler chickens, improving the dynamics of their weight gain. Yet, oral administration of drugs remains the most accessible technique on large poultry farms. The low efficacy of phage therapy when the medicine is administered orally may result from susceptibility of phages to peptidases and instability of phage particles under the conditions of gastrointestinal tract with its acidic-to-alkaline gradients. The body's immune response may be another factor that affects the efficacy of phage therapy. It implies that the selection of phages should be done carefully. Otherwise, the number of phage particles reaching the intestine will be too low for effective therapy.

Nanocapsules offer a prospective biotechnological solution to the problem of phage delivery via the gastrointestinal tract. This approach was investigated in a number of studies [55, 56, 66]. The researchers designed and tested a nanoparticle-encapsulated preparation based on the T4-related bacteriophage ΦKAZ14, infecting pathogenic strains of *E. coli*, in particular *E. coli* O1:K1:H7 (Tables 1 and 4). The nanoparticles were obtained by adding a phage suspension (10^7 PFU/mL; 10 mL) to an equal volume of 1% (v/v) chitosan under gentle stirring. The resulting chitosan nanocapsules trapped up to 92% of phage particles, with an average diameter of 188 ± 7.4 and 176 ± 3.2 nm. The *in vitro* tests showed that the encapsulated phage was safe from peptidases and low pH. The nanocapsules were also assayed for cytotoxicity (*in vivo* tests on chick embryos). The embryos were inoculated (by the injection into the chorioallantoic membrane) with 0.5 mL of either phage-containing (C-ΦKAZ14-NP) or phage-free (CS-NP) nanoparticle suspension. After 24 h, the eggs were visually examined, and their images were qualitatively compared. The comparison revealed no toxic effects or vascular changes in all the eggs, regardless of the type of nanoparticles injected (CS-NP or C-ΦKAZ14-NP). The authors concluded that the administration of ΦKAZ14-containing nanocapsules to farm birds should be safe and carried out a series of experiments *in vivo* to estimate the efficacy of the encapsulated-phage preparation in comparison with the basic phage solution in buffer. In the experiments, seven-day-old chickens were injected intratracheally with *E. coli* O1:K1:H7 (10^9 CFU/mL). 2 h later, the birds were administered with the preparations (0.2 mL; orally): Group 1 received encapsulated phage (ΦKAZ14-NP; 10^7 PFU/mL). Group 2 received a phage solution in SM buffer (ΦKAZ14; 10^7 PFU/mL). Group 3 received a PBS buffer (placebo). As expected, the results confirmed a higher efficiency of the encapsulated preparation: the birds in Group 1 had fewer clinical symptoms of APEC infection and gained more weight by week 3. These birds also showed a lower mortality rate: 16.70% as compared with that in Group 2, where the mortality was almost as high as in the placebo group (50 and 58%, respectively). Assaying the bird feces for *E. coli* also revealed differences between the groups: on week 3, the fecal titer of *E. coli* in Group 1 was about 10^2 CFU (vs. 10^4 CFU in Group 2). Thus, the efficacy of the orally-introduced encapsulated-phage preparations can be comparable with the efficacy of the phage cocktails injected intramuscularly (see below).

Aerosol sprays against *Escherichia coli* infection in healthy birds. Another simple and accessible way to administer phage medicines on large poultry farms is to spray the flock with an aerosol. With this method of administration, phages can be delivered directly into the respiratory system, which is especially important for treating aerosacculitis, a complication of colibacillosis that develops when the infection is severe.

Administering phage cocktails in the form of sprays was mostly investigated by Huff *et al.* [67]. In their experiments, the phage cocktail consisted of two phages: SPRO2 and DAF6 (Tables 2 and 4). Healthy chickens were placed in a plexiglass chamber and sprayed with the cocktail using a special apparatus (10 mL/min for 1 min). After spraying, the birds were left in the chamber for 2 min. Following the spraying procedure, the chickens were infected with *E. coli* immediately after the procedure, 24 h after spraying, and 72 h after spraying. The experiment was repeated twice, each time with a different dose of the sprayed cocktail (either 10^7 or 10^8 PFU/mL). The lower dose of 10^7 PFU/mL was only moderately effective in preventing the immediate infection: the mortality dropped from 60 to 30%. Longer periods between treatment and infection gave no statistically significant differences between the groups. On the other hand, the higher dose of 10^8 PFU/mL provided a sufficient protection during the entire experiment (up to 3 days). The mortality rates were as follows: with no pause between treatment and infection, the mortality dropped from 63 to 7%; (a 24-h delay, the mortality was reduced by a factor of 1.5–2; and with a 72-h delay, the mortality went down from 40 to 3%. Thus, similar to what was observed in the first experiment, the best results were achieved when the treatment was done just before the exposure of the birds to the pathogen [67].

Another study conducted by Huff *et al.* [70] assessed the prospects of using phage sprays as a therapeutic, rather than preventive, measure: the sprays were used to treat a developing or already developed *E. coli* infection. The birds were sprayed with a phage cocktail containing SPRO2 (7.65×10^8 PFU/mL) and DAF6 (2.83×10^9 PFU/mL), with the treatment performed immediately after inoculation with *E. coli* or after a 24/48-h delay. The birds in the control group were sprayed with the same preparation, except that the phages were inactivated. The results showed that the immediate application of the medicine lowered the mortality rate to 20%, as compared with 50% in the control group. When the treatment was performed 24 h after infection, the rates were 18 vs. 30%, respectively. With the pause between infection and treatment increased to 48 h, the mortality rate in the experimental group was not much lower than that in the control group (37 and 47%, respectively).

Table 4 Experiments *in vivo* on the treatment or prevention of avian colibacillosis using aerosol sprays to administer phage therapy

Phages per cocktail	Administration	Concentration/dose of preparation	Duration, days	Mortality in phage-treated group, %	Mortality in untreated group, %	Concentration of inoculated *Escherichia coli*	Route and age of *Escherichia coli* introduction	Age at onset, days	Reference
1	Orally, with water and as spray (1 mL); immediately after infection	1.5×10^9 PFU	21	~ 50	~ 75	1.0×10^8 CFU	Injection into left thoracic air sac; day 10	10	[68]
1	Coarse spray in the face (~ 1 mL)	3.9×10^9 PFU	19	~ 40	~ 30	2.1×10^7 CFU	Intratracheally; day 1	1	[69]
	Fine spray in the face (~1 mL)	7×10^9 PFU	21	~ 65	~ 60	1.46×10^9 CFU			–
2	Aerosol spray; on day 7 of life (10 mL/min, for 1 min)	3.6×10^7 PFU/mL and 4.6×10^7 PFU/mL	21	30	60	5.6×10^4 CFU	Injection into air sac; day 7	1	[67]
				47	43		Injection into air sac; day 8		–
				38	39		Injection into air sac; day10		–
		2.6×10^8 PFU/mL and 2.35×10^9 PFU/mL		7	63	6.2×10^4 CFU	Injection into air sac; day 7		–
				27	40		Injection into air sac; day 8		–
				3	40		Injection into air sac; day 10		–
		1.88×10^8 PFU/mL and 3.60×10^8 PFU/mL		27	63	6.85×10^4 CFU	Injection into air sac; day 7		–
				37	73		Injection into air sac; day 8		–
				40	60		Injection into air sac; day 10		–
2	Aerosol spray; on day 7 of life (10 mL/min, for 1 min); 2 h after infection	7.65×10^8 PFU/mL and 2.83×10^9 PFU/mL	21	20	50	5.93×10^4 CFU (mortality without any cocktail injections, 43%; other rates refer to mortality in groups injected with inactivated cocktail)	Injection into air sac; day 7	1	[70]
	Aerosol spray on day 7 of life (10 mL/min, for 1 min); 24 h after infection			18	30				–
	Aerosol spray; (10 mL/min, for 1 min); 48 h after infection			37	47				–

Thus, the two studies by Huff *et al*. [67, 70] demonstrated that spraying birds with a phage cocktail could decrease the colibacillosis-associated mortality by a factor of 1.5–2. The technique was efficacious when the treatment and infection occurred on the same day. Apparently, this was related to a rapid decline of the phage titers in the bird bloodstream. Indeed, according to Huff's data, the initial titers were not particularly high (≤ 100 PFU/mL right after the treatment); a day later, the circulation of the phage particles in the bloodstream completely stopped. Perhaps, to make this method of phage administration more effective (as both therapeutic or preventive measure), the treatment is to be repeated several times, i. e., as a course.

In regard to the practical aspects of the spraying technique, it can be used preemptively on healthy birds to stop the spread of colibacillosis on the poultry farms where an outbreak of infection is registered. This idea was tested by Huff *et al*. [67]. The mortality rate in Control Groups 1 (untreated) and 2 (sprayed with inactivated phage preparation) was 20–27%. It was much higher than the rates registered in the previous experiments, as well as above the rate of 3% observed in Control Group 3 (treated with active cocktail but not infected with *E. coli*). The researchers assumed that the control birds were probably infected with some strain of *E. coli* at the beginning of the experiment and suggested that the timely treatment had a positive effect on the health of the birds in Control Group 3, substantially lowering their mortality.

Oliveira *et al*. [68] conducted an extensive study on the use of phage therapy (in particular; phage aerosols) on a poultry farm. The farm was experiencing an outbreak of APEC infection, with the mortality of seven-week-old chickens being high even after the treatment with antibiotics. In that situation, the researchers tried a cocktail of three phages: phi F78E, phi F258E, and phi F61E, each at the concentration of 5×10^7 PFU/mL (Tables 2 and 4). The birds received the cocktail either with drinking water or as aerosol spray. In one week, mortality was taken under control in 5 out of 11 flocks. By week 3, control was established over 10 of them. A flock totaled 5,000–10,000 birds, with the situation considered under control when mortality was ≤ 0.5% of the flock size. In one flock, the mortality rate remained at 0.68%. Thus, the results were quite satisfactory: a single treatment with a relatively low dose of the phage cocktail substantially reduced the colibacillosis-related mortality in chickens. The authors also tested the effect of that phage preparation (a suspension of phi F78E) against a simulated *E. coli* infection. Ten-day-old chickens were injected with the APEC strain H839E (5×10^8 CFU/mL; 200 μL; into the left thoracic air sac), which was followed by the administration of the phage preparation (1.5×10^9 PFU/mL; 1 mL; administered orally as a spray). As a result, the morbidity and mortality of the birds were reduced by 41.7 and 25% respectively, as compared with the untreated chickens.

However, the successful application of aerosol sprays depends on the technical details of the procedure. This problem was examined in another study by Huff *et al*. [69]. The authors experimented with an SPR02-based spray, analogous to the preparation used previously (Tables 2 and 4). The preparation was applied either externally (sprayed at the bird's face) or internally (injected into the trachea). In experiment 1, the authors tested a coarsely dispersed spray ("coarse spray"; 3.9×10^9 PFU when applied externally and 3.9×10^8 PFU when injected intratracheally; the concentration of *E. coli* was 2.1×10^7 CFU). In experiment 2, the spray was finely dispersed; it was administered using a special system which produced finer droplets ("fine spray"; 7×10^9 PFU when administered externally and 7×10^8 PFU when introduced intratracheally; the concentration of *E. coli* was 1.46×10^9 CFU). The results showed a high efficacy of the intratracheal method as compared with external spraying. In both experiments, the mortality of the birds receiving the medicine by intratracheal injections remained below 10%, whereas external spraying proved ineffective in both cases. The mortality rate of the externally-sprayed birds even exceeded that of the untreated (control) chickens (40–70 vs. 25–60%). Huff's team had already tested that system for fine dispersion of aerosols, demonstrating its high effectiveness. The exposure time and volume of the cocktail sprayed were increased; besides, the birds were placed in a special chamber. As noted by the authors, this may have affected the dose of the cocktail received by the birds, determining the positive outcome of the therapy.

Intratracheal administration of phage preparations against APEC-associated aerosacculitis. The intratracheal method of administration of phage cocktails have been investigated in multiple studies. This route of administration can reduce the degree of lung infection with *E. coli* and alleviate the symptoms of aerosacculitis developing against the background of colibacillosis, thereby decreasing mortality. Indeed, model experiments with simulated colibacillosis proved that intratracheal administration of phage cocktails could reduce the mortality rate in the course of developing infection to 0–15% [51, 69, 71, 72].

Huff *et al*. [69] reported that intratracheal introduction of a single-phage preparation (3.9×10^8 PFU or 7×10^8 PFU) 2 h after the infection of birds with *E. coli* (2.1×10^7 or 1.46×10^9 CFU; intratracheally) reduced their mortality from ~ 30 to ~ 3% in the first case and from 50 to 5% in the second case. When compared with aerosol spraying (see above), intratracheal administration was much more effective.

Eid *et al*. [71] compared the efficacy of therapy with intratracheally-introduced phage cocktail, antibiotic (doxycycline), and their combination. The birds were infected with *E. coli* strains of serogroup O78 (intratracheally; 10^8 CFU/mL). The phage cocktail was introduced once, at the concentration of 10^8 PFU/mL. It contained a lytic bacteriophage of the family Siphoviridae (ICTV classification adopted until 2022 [73]), which

was obtained from the feces of birds infected with APEC strains (Tables 2 and 5). The authors monitored visual appearance of the birds and manifestations of the disease symptoms. According to their observations, the phage-treated chickens tolerated colibacillosis better than the birds of the positive-control and antibiotic-treated groups. Moreover, no deaths were registered in the group of phage-treated chickens – in contrast to what was observed in the other groups. These observations were also consistent with the weight-gain data: in all the groups that received treatment (phage, antibiotic, or their combination), the chickens improved their average weight as compared with the birds in the untreated group. Notably, the highest gains were observed in the phage-treated group. The paper also provided data on the abundance of *E. coli* in the chicken lungs. As expected, the birds treated with antibiotic or phage showed a more pronounced decline in the viable bacterial cell count as compared with the other groups. The authors concluded that the success of phage therapy was associated with the method of phage administration (intratracheal), as well as the high concentration of the cocktail introduced (10^8 PFU/mL).

Similar results were obtained by Lau *et al.* [51]. They tested phage EC1-UPM, capable of lysing *E. coli* O78:K80 (Tables 1 and 5). One-day-old chicks were infected with 200 μL of *E. coli* O78:K80 (10^9 CFU/mL); 2 h later, they were injected with 200 μL of a phage preparation (10^{11} PFU/mL). The introduction of both *E. coli* and the phage was carried out intratracheally, using a special needle designed for feeding chicks on a farm. The researchers evaluated the chicken weight, the degree of damage of their organs, and the bacterial contamination of the organ tissues. The experiment was carried out over 3 weeks. In the end, the mortality rate in the phage-treated group was significantly lower (13.3%) than that in the infected-untreated one (83.3%). Within two weeks after the beginning of the therapy, the chickens of the phage-treated group gained weight and became statistically indistinguishable from the uninfected (control) birds in regard to this parameter. Overall, the condition of the phage-treated birds that survived the infection was more satisfactory than that of the untreated birds. As shown by the tests conducted 2 h after the treatment, the number of *E. coli* cells in the lungs of the phage-treated chicks decreased, as compared with that in the lungs of the untreated birds. In addition, the internal organs of the phage-treated birds (heart, liver, and spleen) were not enlarged, unlike those of the untreated chickens. The symptoms of aerosacculitis were still observed in the birds of both groups; however, the symptoms of pericarditis were less common in the phage-treated chickens, and they also showed no symptoms of perihepatitis. Also, no *E. coli* was detected in the blood of the phage-treated birds during the experiment, in contrast to what was observed in the untreated chickens. Thus, phage EC1-UPM proved to be a good choice for treating chickens. The method of its administration and a relatively high dose of the preparation must have contributed to the success of the therapy as well.

In the two studies considered above [71, 51], the infection in the birds with *E. coli* decreased only by one order of magnitude by the end of the experiments. Evidently, suppressing the growth of pathogenic bacteria at early stages of infection plays a decisive role in controlling the course of the disease.

A rather interesting result was obtained by Tawakol *et al.* [72]. They tested the ability of a single-phage cocktail to prevent the development of avian colibacillosis as a complication of viral bronchitis. The cocktail contained phages specific for APEC O78, which

Table 5 Experiments *in vivo* on the treatment or prevention of avian colibacillosis using intratracheal administration of phage therapy

Phages per cocktail	Administration	Concentration/ dose of preparation	Duration, days	Mortality in phage-treated group, %	Mortality in untreated group, %	Concentration of inoculated *Escherichia coli*	Route and age of *Escherichia coli* introduction	Age at onset, days	Reference
1	Coarse spray in the face (~ 1 mL)	3.9×10^9 PFU	19	~ 40	~ 30	2.1×10^7 CFU	Intratracheally; day 1	1	[69]
	Intratracheally	3.9×10^8 PFU		~ 3					–
	Intratracheally	7×10^8 PFU		~ 5					–
1	Intratracheally; 2 h after infection	10^{10} PFU	21	13.30	83.3	10^8 CFU	Intratracheally; day 1	1	[51]
1	Intratracheally	10^8 PFU/mL	21	0	26.7	10^8 CFU/mL	Intratracheally; day 1	1	[71]
1	Intratracheally; on days 1, 5, 8, 13 of life	10^8 PFU	15	0	16	10^8 CFU	Intratracheally; day 2	1	[72]
	Intratracheally; on days 1, 5, 8, 13 of life; infected with either *Escherichia coli* or *Escherichia coli* + IBV			0	29	10^8 CFU and additionally infected with IBV			–

were isolated from the poultry-farm wastewater. They were not sequenced, so their taxonomic affiliation was unknown (Table 5). The experiment was performed on healthy one-day-old chicks, which were divided into several groups. A half of the birds (experimental groups) received a course of phage therapy (injected intratracheally with the phage cocktail; 10^8 PFU; on days 1, 5, 8, and 13 of life). The other half (control groups) remained untreated. During the next step, the chicks were infected with either APEC O78 (10^8 CFU, injected on the day 2); or IBV (10^6 EID_{50}, injected on day 5); or both pathogens (same doses and injection timings). The therapy delayed the onset of clinical symptoms of the bacterial and mixed infections and reduced the severity of their course. The symptoms of the viral infection were also alleviated, but its onset was not delayed. As expected, the phage therapy significantly reduced the abundance of APEC in the lungs of both the APEC- and APEC/IBV-infected birds, as compared with the untreated chicks. The titer of the pathogenic bacteria in the lung tissue gradually declined over time. The authors also discovered an interesting effect of the phage preparation on the course of the mixed bacterial/viral infection: in addition to lowering the titer of APEC O78, the preparation also decreased the titer of IBV on disease day 15. However, no statistically significant effect of the therapy on the IBV titer was registered when the chicks were only infected with the virus. Hence, the phage cocktail was demonstrated to alleviate the symptoms of both the APEC infection and the APEC-complicated IBV infection. The authors attributed the positive outcome of the therapy to the method of the cocktail administration, as well as the fact that the cocktail was administered repeatedly and frequently.

Thus, if administered intratracheally, a single-phage cocktail can effectively protect birds from colibacillosis. In all these studies, however, the phage titer of the preparations was fairly high, which may have a substantial impact on the outcome of the therapy. In the experiments reviewed, the infection of birds with *E. coli* also occurred via the intratracheal route, and the phage cocktail was administered on the same day. Therefore, the high efficacy of the treatment could be due to the fact that the medicine was rapidly delivered directly to the infected organs (lungs). It is not clear how efficacious the therapy would be if the birds received the cocktail with a delay, when the disease was already developed. Thus, a cautiously optimistic conclusion from the results of the studies considered in this section would be as follows: a timely intratracheal treatment of APEC-infected chickens with a high-titer phage cocktail can help reduce their mortality rate.

Intramuscular injection of phage cocktails: the best results against colibacillosis? Intramuscular injection is arguably the most effective route, by which phages can be delivered to chickens [44]. Indeed, with a good choice of the phage and a high dose of the cocktail injected intramuscularly, chickens can be reliably protected from colibacillosis – as demonstrated, for example, in the above-cited study, in which zero mortality was observed in the experimental groups. Another important feature of this method of administration is that injections can be carried out at the stage of avian embryos, which was shown to be very effective. Table 6 summarizes some examples of this technique.

The first experiments on the intramuscular injection of phages belonged to Barrow *et al.* [47]. They tested a preparation of phage R for its potency against colibacillosis in newly hatched chicks and three-week-old chickens (Tables 1 and 6). The phage preparation was injected intramuscularly (50 μL; into the right thigh); the infection of birds with *E. coli* was also performed through injections: either intramuscularly (into the left thigh) or intracranially. The intracranial injections were made in order to imitate meningitis. The titers of the introduced phage and *E. coli* were experimental variables.

In Barrow's experiments, the best results were obtained when the chickens were given equal doses of phage R and *E. coli* (10^6 PFU and 10^6 CFU). In that case, no disease symptoms or deaths were observed in both newly hatched and three-week-old birds. When *E. coli* was injected intramuscularly, the birds could actually be protected with a lower dose of the phage preparation (10^4 PFU). The infection caused by the intracranial injection of the pathogen (10^3 CFU) turned out to be more serious and difficult to treat: the three-week-old birds survived only upon administering a high dose of the phage preparation (10^8 PFU), with the situation in the group of the newly hatched chicks being even worse. The researchers also collected interesting data on the dynamics of the phage titer in the internal organs of the birds during *E. coli* infection. The phage preparation (10^8 PFU) was administered intramuscularly 8 h after the development of the infection, and its titer in the spleen remained high even after 120 h. The phage was found in the blood as well; however, its blood titer dropped significantly within 24 h after administration. At that point, phage particles were also registered in the brain at a relatively high titer ($\log_{10}$ PFU/g = 7.2), which decreased only after 120 h. Considering these fluctuations, one can assume the development of a phage infection. Parallel measurements of the *E. coli* titer (10^3 CFU, introduced intracranially) revealed that it gradually declined, reaching the minimum ($\log_{10}$ CFU/g < 2) either in 12 h (blood and spleen) or 24 h (brain). It was not clear how the phage was able to pass through the blood-brain barrier; the authors, though, offered some possible explanations. For example, the barrier could have been weakened because of a negative impact of the inflammatory processes accompanying the infection. All the phage-treated birds that survived (7 out of 10) recovered in 24 h, whereas all the untreated birds had died by that time. The same team conducted another experiment with the same parameters, except the order of the phage/pathogen administration: the birds were infected with *E. coli* a few days after the phage injection. The mortality of the phage-treated chickens was also significantly lower than that of the untreated birds.

Table 6 Experiments *in vivo* on the treatment or prevention of avian colibacillosis using intramuscular phage injection

Phages per cocktail	Administration	Concentration/ dose of preparation	Duration	Mortality in phage-treated group, %	Mortality in untreated group, %	Concentration of inoculated *Escherichia coli*	Route and age of *Escherichia coli* introduction	Age at onset	Reference
1	Injection into thoracic air sac	10^3 PFU	21 days	25	80	2.16×10^3 CFU	Injection into thoracic air sac; day 3	3 days	[57]
		10^6 PFU		5					–
		10^4 PFU		35	85	1.12×10^4 CFU			–
		10^8 PFU		0					–
2	Intramuscularly (into left thigh); day 7 of life; immediately after infection	1.88×10^8 and 6.35×10^7 PFU	21 days	17	53	6.27×10^4 CFU (mortality without any cocktail injections, 53%; other rates refer to mortality in groups injected with inactivated cocktail)	Injection into air sac; day 7	1 day	[70]
	Intramuscularly (into left thigh); day 8 of life; 24 h after infection			10	46				–
	Intramuscularly (into left thigh); day 9 of life; 48 h after infection			20	44				–
2	Intramuscularly; day 7 of life	1.34×10^9 PFU and 8.45×10^8 PFU	21 days	13	~ 43	6.0×10^4 CFU (mortality without any cocktail injections, 57%; other rates refer to mortality in groups injected with inactivated cocktail)	Injection into air sac; day 7	1 day	[58]
	Intramuscularly; days 7–9 of life; daily (3 injections in total)			0	~ 42				–
	Intramuscularly; day 8 of life			10	~ 55				–
	Intramuscularly; days 8–10 of life; daily (3 injections in total)			10	~ 42				–
	Intramuscularly; day 9 of life			28	~ 50				–
	Intramuscularly; days 10–12 of life; daily (3 injections in total)			27	~ 50				–
2	Intramuscularly; once on day 7 of life	3.7×10^8 PFU and 9.3×10^9 PFU	21 days	15	68	6.0×10^4 CFU	Injection into left thoracic air sac; day 7	1 day	[74]
	Intramuscularly; once on day 7 of life; + antibiotic added to water			0	68				–
1	Intramuscularly (into left thigh); day 7 of life; with SPRO2	4×10^8 PFU	21 days	7	48	6.0×10^4 CFU	Injection into left thoracic air sac; day 7	1 day	[75]
		4×10^6 PFU		24					–
		4×10^4 PFU		10					–
		4×10^2 PFU		28					–
		3×10^8 PFU		7	47	9.0×10^4 CFU			–
		3×10^6 PFU		13					–
		3×10^4 PFU		50					–
		3×10^2 PFU		53					–
1	Intramuscularly (into left thigh); day 7 of life; with DAF6	4×10^8 PFU	21 days	20	48	6.0×10^4 CFU	Injection into left thoracic air sac; day 7	1 day	–
		4×10^6 PFU		7					–
		4×10^4 PFU		23					–
		4×10^2 PFU		23					–

Continuation of Table 6

Phages per cocktail	Administration	Concentration/ dose of preparation	Duration	Mortality in phage-treated group, %	Mortality in untreated group, %	Concentration of inoculated *Escherichia coli*	Route and age of *Escherichia coli* introduction	Age at onset	Reference
1	Intramuscularly (into left thigh); day 7 of life; with DAF6	3×10^8 PFU	21 days	40	47	9.0×10^4 CFU	Injection into left thoracic air sac; day 7	1 day	–
		3×10^6 PFU		47					–
		3×10^4 PFU		47					
		3×10^2 PFU		37					–
1	Intramuscularly; day 17 of life (into left thigh)	6.8×10^8 PFU	17 days	8	55	10^6 CFU	Injection into left thoracic air sac; day 17	1 day	[76]
	Intramuscularly; day 10 (into left thigh) and day 17 of life (into right thigh; immediately after infection)			33	55				–
1	Intramuscularly (into right thigh)	1×10^6 PFU	120 h	0	100	10^6 CFU	Intramuscularly; day 21	21 days	[47]
		1×10^4 PFU		0	100				–
		1×10^2 PFU		40	100				–
	Intramuscularly (into right thigh)	1×10^8 PFU		0	60	10^3 CFU	Intracranially; day 21	21 days	–
		1×10^6 PFU		0	100	10^6 CFU	Intramuscularly; day 1	Newly hatched	–
		1×10^8 PFU		20	100	10^3 CFU	Intracranially; day 1		–
		1×10^6 PFU		60	100	10^3 CFU	Intracranially; day 1		–

Table 7 Experiments *in vivo* on the treatment or prevention of avian colibacillosis in chicken embryos using injection of phage therapy

Number of phages per cocktail	Administration	Concentration /dose of preparation	Duration, days	Mortality in phage-treated group, %	Mortality in untreated group, %	Concentration of inoculated *Escherichia coli*	Route and age of *Escherichia coli* introduction	Age at onset	Reference
8	Injection; 2 h after infection	4×10^3 PFU	6	10	100	2×10^2 CFU	Injection; embryonic day 12	Embryos	[46]
4	Injection; on embryonic day 16; once	5.2×10^7 PFU	7 (after hatching)	27.80	29.40	2.5×10^3 CFU	Intramuscularly; into chest under right wing	Embryos, one-day-old chicks	[78]
1	Injection	2,000 PFU	6	100	30	100 CFU	Injection	Embryos	[79]

Apparently, the persistence of the phage in the internal organs of chickens observed by Barrow *et al*. [47] was phage-specific: it depended on the nature of the phage itself and the characteristics of its spread through the bird organs. Huff *et al*. [70] conducted similar experiments with a cocktail of SPRO2 and DAF6 (injected intramuscularly; 3.75×10^8 and 1.68×10^9 PFU/mL, respectively) (Tables 4 and 6). The researchers monitored the phage titers in the blood, which was sampled by a cardiac puncture. According to the data collected, the titers remained at a stable level (10^4 PFU/mL) within 6 h after the phage administration. A day later, they diminished to 70 PFU/mL. These results were consistent with Barrow's data, except that in Huff's experiments, the titers declined significantly faster (the initial dose of the preparation was the same in the two studies; see [70] for comparison). The mortality of the phage-treated chicken in Huff's study went down by at least 50% in comparison with that of the birds injected with the heat-inactivated cocktail (17 vs. 53% right after *E. coli* inoculation, 10 vs. 46% in 24 h, 20 vs. 44% in 48 h). The researchers also experimented with phage sprays to find that the mortality of the intramuscularly-injected chickens was lower than that of the aerosol-sprayed birds (Tables 4 and 6).

The intramuscular injections of phage cocktails were further investigated by Huff *et al*. [58, 76]. They tested the administration of the cocktails as a course (multi-shot) therapy against the induced colibacillosis in chickens and studied the response of the avian immune system to the intramuscular introduction of phages. In these 2 studies, the chickens were administered intramuscular injections of a DAF6/SPR02 cocktail (1.34×10^{10}/8.45×10^9 PFU/mL, respectively), either once or multiple times over three days (Tables 3 and 7). The injections were carried out immediately after the infection of the birds with *E. coli* (day 7 of life) or after a delay (24 or 72 h). The mortality in the group that received inactivated cocktail ranged from 40 to 60%, which was not much different from what was observed in the control group of infected but untreated chickens (57% mortality). A significant drop in mortality was only observed in the groups receiving active phage preparation. More specifically, the mortality rate of the birds treated on the day of infection was 13%, and the subsequent injections of active phages (within 3 days, on days 7–9 of life) reduced the mortality rate to 0%. In the birds that received active phage cocktail once or thrice (on days 8–10 of life), the mortality was about 10%. When the treatment began on day 2, the mortality rate of the birds that received a dose once or thrice (on days 9–11 of infection) was 8–27%. Thus, a course of intramuscular phage injections could be quite efficacious, saving up to 90% of the flock, even when the treatment was started at a rather late stage of the disease outbreak.

In their experiments, Huff *et al*. [75] also evaluated the permissible titers of intramuscularly-injected bacteriophages. As expected, the best results belonged to the preparations that had the highest concentration of phage particles and a high MOI (in particular; with titers of 10^6 PFU/mL and higher). However, the results were highly dependent on the phage used.

In another study by Huff *et al*. [57], a single-phage cocktail was injected directly into the thoracic air sac of chickens (Tables 2 and 6). The cocktail contained SPR02, a phage capable of lysing serotype-02 *E. coli*. The experiments were performed on three-day-old chicks, with the experimental and control groups arranged as follows. In 2 groups, the birds were injected twice with 100 µL of *E. coli* (10^3 and 10^4 CFU/mL Groups 1 and 2, respectively). In 4 groups, chicks were administered a mix of SPR02 and *E. coli* prepared a day before the administration. The mix contained: 1) 10^3 CFU *E. coli* + 10^3 PFU SPR02 (phage multiplicity: 1); 2) 10^3 CFU *E. coli* + 10^6 PFU SPR02 (multiplicity: 1,000); 3) 10^4 CFU *E. coli* + 10^4 PFU SPR02 (multiplicity: 10); and 4) 10^4 CFU *E. coli* + 10^8 PFU SPR02 (multiplicity: 10,000). The titer of *E. coli* stored for 24 h in a refrigerator remained the same. During the first two weeks after the treatment, the infected and uninfected-untreated (control) chickens started to show statistically significant differences in body weight; the differences between the infected-treated (10^4 CFU + 10^4 PFU) and control chickens became apparent on week 2. The mortality rates were 0% in the control groups, 5–10% in the groups of uninfected-treated chickens, and 80% in the groups of infected but untreated birds. The mortality in the infected-treated groups varied depending on the dose of the injected bacterium and phage. The best result (0% mortality) was achieved in the group that received 10^4 CFU *E. coli* and 10^8 PFU SPR02. In the group that received 10^3 CFU *E. coli* and 10^6 PFU SPR02, the mortality was at 5%. In the groups that received an equal dose of bacteria and phage (10^3 CFU + 10^3 PFU or 10^4 CFU + 10^4 PFU), the mortality reached 25 and 35%, respectively. Thus, the phage multiplicity seemed to be an important factor affecting efficacy of the treatment. The relation of mortality to the *E. coli* infection was confirmed by weighing the internal organs, which showed no changes in healthy chickens but demonstrated characteristic pathological changes in the organs of the birds that died from the disease.

Another study by Huff *et al*. [74] compared the antibacterial activity of a phage cocktail administered intramuscularly with that of an antibiotic (enrofloxacin). As in their earlier experiments [70], the authors used a cocktail containing two phages: DAF6 (3.7×10^9 PFU/mL) and SPR02 (9.3×10^{10} PFU/mL) (Tables 2 and 6). The cocktail (100 µL) was injected into the left thigh of seven-day-old chicks. Entrofloxacin was administered with drinking water (50 ppm, for a week, starting from day 7 of life). The experiment involved three variants of treatment: phage cocktail, phage cocktail + antibiotic, and antibiotic. Two groups of chickens were assigned for each treatment variant: one group remained uninfected (control) and the other group (experiment) was inoculated with *E. coli* (6×10^5 CFU/mL, 100 µL, injected into the left thoracic air sac on day 7 of life). On top of this, the experiment included two general-control groups: one

receiving neither pathogen nor medicine (uninfected-untreated) and the other one receiving only pathogen (infected-untreated). At the age of three weeks, the birds were assessed for mortality, weight, and condition of their internal organs. The group of infected-untreated chickens demonstrated 68% mortality; in the group of antibiotic-treated chickens, in was 3%; in the group of phage-treated birds, it was 15%. All the birds survived in the group that received a combined antibiotic/phage therapy. The antibiotic-treated chickens suffered the least amount of the internal organ damage (3% of the birds affected). In the chickens treated with a combination of phage and antibiotic, the damage was slightly higher (5%). In the phage-treated birds, it was 15%. All these values were lower than the percentage of affected birds in the infected-untreated group (38%). Thus, in terms of the colibacillosis-related mortality, entrofloxacin turned out to be a more potent medicine than the phage cocktail – yet the best result (no deaths at all) was achieved in the group receiving the combined therapy. However, the condition of the internal organs of the antibiotic-treated chickens was slightly better than that of the birds treated with both antibiotic and phage. The authors concluded that the combined antibiotic/phage medicine was the most efficacious therapeutic option.

Thus, we can conclude that the administration of phage cocktails to chickens by intramuscular injections is arguably the most efficacious route in regard to both prevention and treatment of colibacillosis. We have found no reports of this administration route being completely unsuccessful – giving that the phage and its dose are chosen correctly. This result is quite different from other routes of phage-cocktail administration (see the examples below). The high efficacy of injections is probably related to the fact that the injected phage particles are getting directly into the bloodstream, quickly spreading all over the organism. The timely delivery of the medicine to the lesions of bacterial infection is evidently the key factor to suppress the progression of the disease.

Oliveira *et al*. [63, 77] studied the persistence of phages in the internal organs of birds depending on the route of the phage-cocktail administration. The authors examined three phages, capable of infecting a large number of APEC strains: phi F78E (*Myoviridae*), phi F258E (*Siphoviridae*), and phi F61E (*Myoviridae*) (Table 2). The phages were administered either orally, or in the form of a spray, or by intramuscular injection. After 3, 10, and 24 h, the birds of the corresponding experimental groups were euthanized, and their organs were assayed for the phage titer. The phage cocktails (1 mL) were administered at the concentrations of 10^6, 10^7, and 10^8 PFU/mL. The results of the experiment indicated that there was a relationship between the type of the phage, the method of its administration, the phage dose, and the titer of phage particles in the internal organs. The best results were obtained when the phages were administered intramuscularly. Being introduced by this route, they were detected in all the organs analyzed (3 h after injection; 10^8 and 10^7 PFU/mL). The highest titers were registered in the spleen (phi F78E and phi F258E) and liver (phi F61E). Being sprayed or administered orally, the phages spread worse. They were detected in the internal organs 3–10 h after the infection, and only if their administration dose was high (10^7–10^8 PFU/mL). Moreover, only phi F788E was detected in all the examined organs; the spread of phi F258E and phi F61E was limited to the lungs. In terms of persistence of the phages in the organ tissues, the best results were obtained for phi F78E: regardless of the dose and the route of administration, it was detected more frequently and at a higher titer than the other phages. This phenomenon might be related to the presence of commensal phi F78E-sensitive *E. coli* strains in the bird microbiome. Probably, phi F788E could also get into the bloodstream after its absorption in the intestine, as evidenced by the data on the spread of orally-introduced phi F788E into the liver, spleen, and colon. The penetration of orally-administered phages into the internal organs depends on the dose of the phage cocktail [44].

Thus, the studies reviewed indicate that intramuscular injections are the best method of phage administration, ensuring a fast delivery of phage particles into the internal organs. The data on the drop of phage titers in the chicken body within a day suggest that, perhaps, the treatment should be carried out as a course of multiple injections. At the same time, intramuscular injections of phage cocktails may trigger a quick immune response. To avoid the immune response, the parameters should be selected with special care (phage concentration, cocktail dose etc.; see the paragraph below).

Injection of phage cocktails into avian embryos to prevent and treat colibacillosis. To prevent outbreaks of colibacillosis on poultry farms with phage therapy, chickens are to be treated in a timely manner, e. g., by administering phage cocktails as early as at the stage of embryogenesis. Early injections are effective on farms which have recently gone through an outbreak of the disease. In this section, we review the technique of injecting a phage preparation into the chicken embryos (administration *in ovo*).

As reported by Nicolas *et al*. [78], phages introduced into the chicken embryos on development day 17 persisted and spread into the organs of the newly hatched chicks. The phage cocktail tested by Nicolas *et al*. [78] consisted of four phages (REC, ESCO3, ESCO47, ESCO58); it was injected at the dose of 100 μL (4.3×10^7 PFU/mL) (Tables 1 and 7). The yolk sacs and caeca of the newly hatched chicks were assayed for the phage titers. The results of the yolk-sac assays showed that the phages were present in 88% of the newly hatched chicks with an average titer of 1.15×10^5 PFU/g. The caecum tests showed 72 and 7.48×10^4 PFU/g. The 4 phages tested were distributed heterogeneously: e. g., ESCO3 was present in 100% (yolk sac) and 77% (caecum) of the chicks, and ESCO58 was discovered in 77% (yolk sac) and 28% (caecum). In four birds, phages were found in the

yolk sac, but not in the cecum. In the next experiment, the researchers tested whether administration *in ovo* would help prevent the development of colibacillosis in chicks. On embryonic day 16, the embryos were inoculated with a cocktail similar to that used in the previous experiment (5.2×10^8 PFU/mL or pure buffer for control). The newly hatched chicks were infected with 200 µL of *E. coli* BEN4358 (2.5×10^3 CFU, injected subcutaneously into the chest area under the right wing), and the infected birds were observed for 7 days. The mortality rates on day 1 of life was not much lower in the phage-treated birds than in the control group: the difference was within the limits of statistical error (27.8 vs. 29.4%, respectively). The authors explained the absence of a positive effect by insufficient concentration of phage particles (too low to lyse a large number of bacterial cells). On the other hand, the internal organs of the survived chickens showed no damage in the phage-treated chicks and characteristic lesions in seven out of twelve chicks of the control group. In addition, the abundance of the pathogenic *E. coli* strain BEN4358 in the cecum of the phage-treated chicks was reduced as compared with that in the control birds. The titer of the phages in the cecum was 9.44×10^6 PFU/g, with 2 of the 4 phages (ESCO47 and ESCO58) not detected in any of the survived birds. In the control group of uninfected-treated chicks, none of the phages were detected in the cecum on day 7 of life. The authors also tested clones of *E. coli* BEN4358 isolated from the organs of the colibacillosis-affected birds for the sensitivity to the phages in the cocktail. The tests showed that only one out of 14 clones remained sensitive to all the four phages, with the sensitivity of other clones being moderate at best. One clone was found to be completely resistant to all the phages; luckily, it was also the least virulent (20% embryonic mortality) in comparison with the wild strain BEN4358 (70% embryonic mortality).

Summing up the results of Nicolas's study [78], we can conclude that the technique of phage administration *in ovo* proved rather effective. The phages were detected in the bird bodies immediately after the infection, with their titers being sufficient to limit the rate of the bacterial growth and reduce the severity of the internal organ damage upon progression of the disease. At the same time, the parameters of the phage-cocktail administration tested in Nicolas *et al*. [78] did not seem to be strong enough to reduce the mortality. Therefore, one might consider an adjustment of the cocktail dose or continuation of phage administration after the birds' hatching. The latter seems reasonable in view of the authors' data that no phage particles were detected in chicks on day 7 of life (meaning that, in the absence of the host bacterium, the phages were eliminated from the body in two weeks).

Trotereau & Schouler [79] tested phage administration *in ovo* for its potency to protect already infected chicken embryos. The embryos were inoculated with *E. coli* BEN5202 (100 CFU) and injected with ESCO5 (2,000 PFU) 2 h later. The experiment was successful: the introduction of the phage helped all the embryos survive the infection (for comparison, the survival rate of the infected-untreated embryos was only 30%).

Another study reported that a cocktail of eight phages, representing different bacteriophage genera, protected up to 90% of chicken embryos from induced colibacillosis [46]. The embryos were infected with APEC BEN 4358 (serogroup O2), and the cocktail contained three types of phages: plaque-forming phages (REC, ESCO3, ESCO47 and ESCO58); phages which lyse bacterial cells without formation of plaques (ESCO9, ESCO10, ESCO30), and ESCO41, a phage uncapable to lyse the selected *E. coli* strain (Tables 1 and 2). The pathogen was introduced on embryonic day 12; the infected embryos received the medicine 2 h later: a phage cocktail (100 µL; total phage concentration, 4×10^4 PFU/mL); ceftiofur, a third-generation cephalosporin (100 µL; 1 mg/mL); and nothing (control group). A survey conducted on infection day 6 showed that all the antibiotic-treated embryos were alive, and 90% of the phage-treated embryos survived as well. In contrast, all the untreated embryos in the control group died by day 4 of infection. Thus, the use of phage therapy at the stage of embryotic development is advisable. In the studies reviewed, the efficacy was quite high, almost comparable with antibiotic therapy.

An example of failed phage therapy. The cases of phage therapy considered in the previous sections were mostly successful; however, the picture would not be complete if we did not analyze a case when the therapy failed. Failures happen regardless of the route of phage(s) administration, their doses, and the *in vitro* potency of the phage cocktails.

Tsonos *et al*. [52] examined a multi-phage cocktail, lysing several APEC strains (mostly belonging to serogroup O78). The phages included in the cocktail were vB_EcoM_PhAPEC2, vB_EcoP_PhAPEC5, vB_EcoP_PhAPEC7, and PhAPEC9 (Tables 1, 2, and 8). One of the phages (vB_EcoM_PhAPEC2) was able to lyse a rather wide range of bacteria, including several APEC strains of serogroups O45, O78, O2, O18, and O83. In the experiment, four-week-old chickens were infected with APEC CH2 (serogroup O78; 500 µL of bacterial culture; 10^8 CFU; injected intratracheally). After 2 h, the experimental groups received the phage cocktail: Group 1 – intratracheally; Group 2 – intraesophageally; and Group 3 – with drinking water. The total dose of the administered phages was 10^9 PFU. The effect of the therapy was assessed on days 1–7 of the infection. The analyses showed no statistically significant differences between the treated and untreated birds in regard to the parameters assessed, i. e., the number of chickens died, their average weight, the total lesion score, and the lesion score per organ. The differences were statistically significant only between the uninfected-untreated chickens and the birds of the experimental groups.

Discussing the possible causes of the failure, the authors hypothesized that the conditions for the propagation of the cocktail phages inside the avian body

Table 8 A failed experiment *in vivo* on the treatment or prevention of avian colibacillosis using phage therapy

Phages per cocktail	Administration	Concentration/ dose of preparation	Duration, days	Mortality in phage-treated group, %	Mortality in untreated group	Concentration of inoculated *Escherichia coli*	Route and age of *Escherichia coli* introduction	Age at onset, weeks	Reference
4	Intratracheally; 2 h after infection	10^9 PFU	7	50	50 and 66% (for inactivated phage cocktail)	10^8 CFU	Intratracheally; 4 weeks	4	[52]
	Intraesophageally; 2 h after infection			50					–
	With water; 2 h after infection			42					–

might be suboptimal. However, checking the tissues of the internal organs for the phage titers showed that the phages were present there (in small amounts) even 4 days after the infection. After 24 h, all the phages were successfully isolated from the lung tissue of the birds in every phage-treated group. Therefore, the cocktail phages were spreading within the bird bodies, and the therapy should have failed because of something else. Apparently, the medicine was introduced too late, when the harm had already been done; this is indicated by the severe damage to the internal organs, as well as the high titers of the pathogenic bacteria within the chicken bodies. The degree of bacterial contamination was not much different in the treated and untreated birds, with all but 2 of the isolated bacterial colonies remaining sensitive to the phages of the cocktail. Another possible cause of the therapy failure, also discussed by the authors, was the response of the immune system, which might have perceived phages as pathogens. In conclusion, the authors recommended to use phage cocktails as a preventive, rather than therapeutic, measure.

Comparing this case to the examples of successful phage therapy, we would like to note that the birds used in the experiment were quite mature; in other studies, intratracheal administration of phages, for example, was usually tested on one-day-old chickens. Apparently, the bird age is an essential factor, which should be taken into account in the experimental design.

Immune response to the phage cocktail as a probable cause of failed therapy. As noted above, administering phage cocktails multiple times, as a course therapy, increases the chances of birds to survive and recover, regardless of the method of administration. However, when the titer of the phage in blood is high, the avian immune system may react to this, nullifying the effect of the therapy. In this section, we shall consider two studies, in which colibacillosis-afflicted chickens were administered a course of intramuscular injections of a phage preparation.

In the first study [76], the researchers measured the blood level of IgG in the chickens treated with a SPRO2-based preparation. The preparation was injected into the left (day 10 of life) or right (day 17 of life) thigh (6.8×10^8 PFU); *E. coli* was injected into the left thoracic air sac (1.0×10^6 CFU) on day 17 of life. The experiment involved five groups: 1) a control group of uninfected-untreated chickens; 2) a group of uninfected birds treated twice (on days 10 and 17); 3) a group of infected-untreated birds; 4) a group of infected birds treated on day 17 of life; and 5) a group of infected birds treated twice (on days 10 and 17 of life). Blood samples were collected on day 17 of life. The results showed that the blood level of IgG_{phage} in the twice-treated chickens was higher than in the control birds. The researchers also noted that the inhibited activity of the phage in the serum of the birds that were treated twice and infected. This explained a relatively high mortality rate in Group 5 (> 30%). For comparison, the mortality rates of the infected-untreated (Group 3) and treated-once-and-infected (Group 4) chickens were 55 and 10%, respectively. Thus, the data of this study indicate that the acquired immunity to the phage can limit its further use.

The immunogenicity of phages varies, and so does the intensity of the body immune response [80, 81]. The response intensity depends on the route of the phage administration, its dose, and the structure of the phage capsid. Phage capsids are formed by various proteins, some of which are more immunogenic than others [82–84]. For example, the most immunogenic proteins of phage T4 are Hoc and gp23 [84]. Furthermore, the antibodies induced by one phage can react to other phages carrying similar epitopes in their capsid: the so-called cross-immune response [84]. For example, antibodies to gp23 of phage T4 can interact with other T4-related phages, since they carry a capsid protein homologous to gp23.

The response of the avian immune system (including cross-immune reactions) to the multiple high-dose injections of a phage under the conditions of a simulated *E. coli* infection was investigated by Naghizadeh *et al*. [59]. The study involved seven groups of chickens: 2 negative-control groups and 5 experimental groups. The birds in Groups 1, 2, and 3 were preimmunized with phage TM1 (which, according to the authors, was the most immunogenic); the birds in Groups 4 and 5 were left unimmunized (native birds). A week after pre-immunization, all the groups were infected with *E. coli* O78:K80 (10^8 CFU/mL). 8 h later, the birds were injected intramuscularly with either TM1 (Groups 2 and 4) or TM3 (Groups 3 and 5), with the birds in Group 1 receiving no phages. The results of the experiment were as follows. In Group 1 (TM1-preimmunized, infected, untreated), the mortality averaged 43.3%. In Group 2 (TM1-preimmunized, infected, TM1-treated), it was

36.6%. Both these rates were higher than the rate observed in Group 4 (native, infected, TM1-treated), in which 80% of chickens survived. The treatment with TM3 was more efficacious: mortality in Group 5 (native, infected, TM3-treated) was only 10%. In Group 3 (TM1-preimmunized, infected, TM3-treated), the mortality also was relatively low. The *E. coli* count in the lungs of the birds in Groups 3 and 5 was significantly lower, as compared with that in the other groups. Additionally, the TM3-treated native chickens (Group 5) showed the lowest incidence of pericarditis and perihepatitis ($P < 0.05$, infection day 7). Overall, as compared with the TM1-treated birds, the TM3-treated native birds (Group 5) lost less weight and had fewer lesions in their internal organs. The researchers attributed these results to the fact that TM3 induced a weaker immune response in comparison with other phages, especially TM1. The exact mechanism underlying the low immunogenicity of TM3 is unknown.

The 2 studies considered in this section indicate that the selection of phage cocktails for avian therapy should be done with 2 considerations in mind: 1) preferences should be given to the phages with low immunogenicity; and 2) if the therapy is administered as a course, it should be a successive use of different phages without similar immunogenic capsid structures – to minimize the risk of cross-immunization.

CONCLUSION

Summing up the data considered in the review, we can conclude that the success of phage therapy in the treatment of avian colibacillosis depends on multiple factors, starting with the selection of phages suitable for the therapy. The selection of phages is a major challenge for researchers: the prospective candidates must satisfy multiple criteria. They should be effective against the targeted pathogenic strain of *Escherichia coli*, lytic, and non-transducible (or, at least, the rate of their transduction should be very low) [85–88]. In addition, they should not cause a strong immune response of the body and be resistant to the changes of pH, as well as certain agents actively produced by the body upon inflammation [44, 89].

Arguably, intramuscular injections are the most optimal and universal method of administering a phage cocktail to the chickens, as demonstrated in multiple experiments, including those discussed above and reviewed earlier [44]. Other administration routes are less universal but have their specific advantages. For example, the intratracheal route, which ensures a fast delivery of phages into the respiratory tract, is good for controlling *E. coli* infection in lungs and, thus, is generally considered to be more efficacious than oral administration [44]. On the other hand, nanocapsules offer a promising technique of oral phage administration. Encapsulation shields phages from the environmental pressures of the gastrointestinal tract and may also protect them from the immune response of the body.

Assessing the prospects of phage therapy in poultry, we believe it can be a good supplement to antibiotic therapy. Indeed, according to the works reviewed, this combination yielded the best results in terms of preserving the flock. Another option worthy of consideration is using phage therapy – with some caution – as a prophylactic measure. The therapeutic doses of the phage, as well as timing of the treatment should be chosen carefully, keeping in mind the possibility of the undesirable response of the avian immune system. An untimely introduction of the phage can cause harm, and the extensive use of the same cocktail can make it ineffective. Perhaps, the optimal approach for the preventive use of phage preparations on poultry farms should imply switching between different phages when the therapy is arranged as a course of multiple treatments. Finally, phage therapy can be used in case of emergency or as a “last resort” option, e. g., when farmers are trying everything they can to save as much flock as possible, with antibiotics not working. In such a situation, a single oral/spray administration of a phage cocktail can, as demonstrated in [68], reduce mortality of the birds to acceptable levels.

In conclusion, phage therapy seems to be a promising approach to combat avian colibacillosis. The problems and challenges of the approach are solvable, and it has its niche.

CONTRIBUTION

A.N. Nikulina, N.A. Nikulin, and A.A. Zimin developed the research concept; A.N. Nikulina designed the methodology, provided validation and data curation, performed the investigation, and wrote the original draft; N.A. Nikulin was responsible for software, visualization, and formal analysis; A.N. Nikulina, N.A. Nikulin, and A.A. Zimin wrote the review and proofread the final version of the manuscript; A.A. Zimin supervised the project. The authors reviewed and approved of the final version of the manuscript.

CONFLICT OF INTEREST

The authors declared no conflict of interest regarding the publication of this article.

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