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Uses of Bacteriophage on Salmonella in the Poultry Industry

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ABSTRACT: Bacteriophages, or phages, are ubiquitous, species-specific viruses that lyse bacteria upon infection. Historically, phage therapy has been used for the treatment of bacterial diseases that have been difficult to treat with antibiotics. Phages infect and kill bacteria upon infection and can be used for prophylaxis against bacterial infections. These have been isolated from various environmental sources. Several groups have successfully isolated bacteriophages targeting foodborne pathogens. The discovery of penicillin was an important milestone in infectious disease treatment. The widespread use of antibiotics during World War II has changed the outcome of many bacterial infections from acute to chronic. However, because bacteria mutate much more rapidly than eukaryotes, antibiotic resistance enzymes are continuously produced. Since the late twentieth century, antibiotic-resistant Salmonella strains have caused numerous outbreaks associated with poultry globally. Vaccination to control these disturbing organisms is anticipated more; however, vaccination strategies are limited against Salmonella due to fastidious serotypes. Therefore, the poultry industry is interested in phage-based biocontrol of Salmonella, which is specific, effective, and non-toxic. Phage therapy of Salmonella can be an alternative or supplementary treatment to traditional methods of application that can efficiently counter this pathogen. Phages have been used to control pathogens in different commodities, environments, and hosts. In the poultry industry, phage treatment of Salmonella minimizes losses caused by bacterial pathogens on animal health and production, thereby enhancing food safety. In recent years, phage-based biocontrol has emerged as a powerful tool to control foodborne infections in humans. Selecting the appropriate phages, phage titer, mode of application, and duration of application are major factors that determine therapeutic effectiveness. Phage cocktails can be administered by oral administration, spraying on eggs, or direct addition to contaminated products. These studies have reviewed the mechanisms of Salmonella transmission in poultry and how bacteriophages can combat this pathogen in poultry flocks. Bacteriophages can be a promising intervention strategy to curb the horizontal and vertical transmission of Salmonella. In the animal housing environment, the use of phages as an aerosol spray during the transfer of eggs could reduce horizontal transmission of Salmonella. Phage biocontrol of Salmonella can also be effectively applied against vertical transmission; for example, oral inoculation of phage preparations can effectively reduce vertical transmission of this pathogen. Among identified Salmonella phages, Felix-O1 is recognized as an efficient candidate for therapeutic applications. Small-scale studies have shown a reduction in bacterial counts following phage treatment application, but industrial-scale application requires thorough safety assessment. A commercial phage product against Salmonella called SalmoFREE was tested in Japan, in which phages were sprayed on the salmon product after slicing. A phage therapy trial including 34,680 broiler chickens indicated no significant mortality or alteration in the gut microbiota in the phage-treated group 14 days following treatment, indicating the safety of the product (Abu Sayem Khan & Rezwana Rahman, 2022).

Keywords: Poultry Industry, Salmonella, Control, Bacteriophage, Salmonella enterica serovar Enteritidis, Phage therapy, Quail.

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1. Introduction

Poultry industry associated diseases are considered serious problems that usually result in substantial economic losses due to poor production performance, high mortality, and contaminated products (Al-Razem et al., 2022). These infections are dominated by Salmonella and avian Escherichia coli pathogens. The occurrence of Salmonella infections is wide, varied, and challenging, so many poultry farmers use extensive antibiotic treatments for flock health and infection control. The use of these antibiotics has unfortunately led to the emergence of multidrug resistance in Salmonella strains. The increased serious concern of antibiotic resistance will endanger human health and lead to significant economic loss in the poultry industry. Therefore, it is crucial for poultry farmers, poultry producers, and poultry processors to find alternative treatments to control Salmonella in poultry.

Bacteriophages have been isolated and used in phage therapy against Salmonella both in laboratory work and in the field with promising potential to replace antibiotics. The first trial of phage therapy against S. Gallinarum in chicken was conducted in 1919. Later, phages ST4, L13, and SG3 against S. Gallinarum infection were isolated from chicken feces and tested in vivo. In one field trial, 2851 live S. Gallinarum vaccine- and phage-treated chickens recorded a low mortality rate compared with that of untreated controls. Two phages (L13 and ST4) caused reduced mortality and S. Gallinarum shedding among phage-treated infected chickens. Additionally, some of the isolated Salmonella phages were confirmed for biocontrol of human pathogens using in vitro and in vivo experiments (Pelyuntha et al., 2022). It was reported that poultry remains the highest pathogen of Salmonella infection, with S. Enteritidis being the number-one serovar. Salmonella colonization in the broiler gastrointestinal tract may lead to carcass contamination during the slaughter process. To control pre-harvest Salmonella contamination in poultry, phage therapy is one of the promising alternatives to antibiotics.

2. Background on Salmonella

Salmonella enterica is the agent for salmonellosis, a prevalent infectious foodborne illness. Contaminated poultry products are a primary source of salmonellosis in people. Salmonella bacteria persist in poultry farms, broilers, processors, and product distributions, and subsequently cause consumer infections. The poultry industry is adopting on-farm biosecurity measures to combat this problem along with other preventive measures, like vaccination, probiotic feed additives, organic acids, mutagenic agents, and disinfectants (Al-Razem et al., 2022). However, bacteria are developing resistance against these antibiotics, diminishing their usefulness. The U.S. poultry industry has faced scrutiny over its potential share of government meat inspections based on microbial Salmonella in a quick response to a rise in salmonellosis linked to chicken products (M. Nabil et al., 2018). Within ten years, this has created a paradigm shift in the federal government's oversight design with the poultry industry. Most processing plants are conducting industry-directed Salmonella monitoring as an alternative to USDA's Salmonella testing. In the poultry industry, this collective self-regulation effort and a more rapid response to emerging public health concerns has not yet occurred. This paper contains a new design for the poultry industry to collectively conduct testing for carcass contamination with Salmonella and to dedicate a portion of the collected information to the government in a system of checks and balances.

Those implicated in Salmonella-contaminated poultry products endure more morbidity and health care costs than by many other pathogens. The poultry industry has invested significant resources in prevention, testing, and control. However, it is an enormous industry that varies widely geographically and temporally. It is likely that its segments may be regulated differently. Alternative regulatory design may be wiser in the balancing revenue stream of facts provided by other food categories. A two-tiered recommendation is made: (1) The Specific Alternatives Option to the Innovative Bacteria Control Option, based on existing poultry industry testing coupled with animal inspection as a check and balance; and (2) A more elaborate, longer-term option based on broader information collection in the poultry industry, like the processing plants in the establishment of food safety regulation.

2.1. Overview of Salmonella Species

Salmonella caused a notable economic loss because of food contamination leading to human salmonellosis, a worldwide health problem. The majority of foodborne illnesses in humans are the result of salmonellosis, which is caused by the enterobacterium Salmonella. These bacteria can invade and reproduce within the mucosal epithelium of the intestines, resulting in enteritis, bloody diarrhea, and, in some cases, systemic

disease. Animal production and public health are potentially threatened by Salmonella infections. Animals, particularly poultry, are the main reservoirs of this pathogen, which can spread horizontally or via contaminated poultry products. Following ingestion, Salmonella is carried through specific permutation pathways to the natural host's intestinal tract, where it can breach the epithelial barrier and enter intestinal epithelial cells (Abu Sayem Khan & Rezwana Rahman, 2022) .

Salmonella is Gram-negative, facultative anaerobic bacilli that ferment D-mannitol and lactose, whereas Salmonella is non-lactose fermenting. The first organism cultured from a specimen is usually examined for species identification using the Meyers & Fuson M/F method or the Kauffmann-White scheme, a classical typing method. Using these two methods, Salmonella Paratyphi A, a serovar of Salmonella, was differentiated as a member of ferments glucose gas-producing glucose and fat. On eosin methylene blue agar, Salmonella and Escherichia coli would be streak-formed colonies with a greenish surface (Al-Razem et al., 2022).

Antisera raised against O and Vi antigens can be used to identify serovars and biovars. Common serotyping methods include the Kauffmann-White scheme, serotyping based on the structure of the O antigen, and serotyping based on 57 antigens. For food safety management, serological typing of these two antigens is most widely used. Other serotyping methods describe chemical tests to determine the structure of surface or exclusion (S) antigens. Salmonella has polysaccharide capsules (K-antigens) typical of virulence bacteria that may prevent phagocytosis by white blood cells (WBCs). Specific antibodies and immunofluorescence have been used to detect capsules. A test, known as the mouse virulence test, is used to assess virulence by injecting a suspension into the abdominal cavity.

2.2. Impact of Salmonella in Poultry

Foodborne illnesses account for a huge worldwide socioeconomic burden and make a considerable loss on poultry food production. Non-typhoidal Salmonella is one of the most frequent pathogens that cause foodborne illnesses and is necessitated by the consumption of contaminated food, especially poultry and poultry products. Salmonella enterica survives and persists throughout the production chain (breeders, hatchery, transport, rearing barn, feed, environments, and whole processing). Contamination of eggs and chicken with Salmonella is through fecal contamination from the litter on skin, feet, cloaca, and contaminated feed and drinking water, air, and dust. In poultry, infection with nonsystemic S. enterica serovars causes subclinical and clinical disease in young chickens manifesting as enteritis, lowering growth, feed conversion efficiency, impaired body weight gain, and economic loss due to early culling. With the rising demand for meat, the poultry industry has made great efforts to increase animal production levels, density, and husbandry experienced by biotic and abiotic challenges that are interlinked and exacerbated at the farm level. More specifically, antimicrobial resistance increases broodstock infection by salmonella, and increased use of antibiotics arguably worsens vaccine programs. Contamination of poultry by AMR salmonella has raised global concern, and regulatory pressure to reduce and abolish the use of antibiotics in poultry farming has grown.

The current measures for controlling Salmonella in poultry comprise environmental decontamination, hatchery, feed, drinking water, and feed additives, chemical decontaminants to decontaminate carcasses, and vaccines. The control measures tend to target an indirect approach (flock rearing and production chain). A prudent use of antibiotics is imposed, and the number of antibiotics from feed has dramatically decreased. To date, there is no commercial vaccine available capable of giving full protection to poultry against all serovars of S. enterica before it contaminates the flock. Additional direct measures comprise the application of phage and bacteriocins, which act by eliminating the serovars of S. enterica that contaminate poultry and egg. Bacteriocins have been reported to reduce S. Typhimurium colonization and epithelial cell invasion. The potential for using phage treatment of Salmonella enterica spp. on raw poultry products as a biocontrol strategy in an integrated food safety intervention for controlling Salmonella during the poultry production chain has been clearly demonstrated. Phages produce a holin that forms a hole in the inner membrane, which releases the endolysin that, in turn, hydrolyzes the peptidoglycan layer in the cell wall leading to cell lysis and death.

2.3. Traditional Control Methods

The increase of antibiotic-resistant bacteria, in part driven by the indiscriminate and excessive use of antibiotics in feed, has led to the search for alternative controls for pathogens in livestock, aquaculture and poultry. Phages are viruses that specifically infect bacterial hosts and, as such, are good biocontrol agents of undesirable bacteria including pathogenic bacteria and spoilage bacteria in food. Phages have been considered as Generally Recognized As Safe (GRAS) products for controlling bacteria and bacteria-derived products on food. Phages present a number of benefits as biocontrol agents. They are highly specific to their hosts, unlike chemical antimicrobials, which often have a broad range of action against non-target bacteria

in addition to the target bacteria. This specificity limits the effect of phage treatment on the desirable microbial populations in the animals or food. Phages can also be relatively easily scaled up for economical mass production (Pelyuntha et al., 2022). A number of companies already sell commercial phage products. The poultry industry is an important part of the food production industry that has been targeted for the use of phages to control Salmonella. Salmonella is a large genus of bacteria that consists of many serovars, some of which are pathogenic to humans and can cause salmonellosis, one of the most prevalent foodborne diseases worldwide. Salmonella contamination of poultry during the rearing stage has been shown to lead to carcass contamination during processing and ecto-parasite transfer. Therefore, improving hygiene practices to reduce the contamination of live birds is imperative to reduce the burden of human salmonellosis stemming from poultry. From another viewpoint, an increasing number of studies have investigated the efficacy of phage treatment as a pre-harvest control method to reduce Salmonella colonization in poultry. Phages targeted against Salmonella have been isolated and characterized based on their placed in taxonomy, biology and host-spectrum. Relative to clonal phages, phage cocktails that contain multiple phages have been proposed as better formulations for phage treatments, as these phages are expected to provide more robust antimicrobial activity against an evolving and heterogeneous bacterial population.

3. Bacteriophages: An Overview

Using antimicrobial drugs for disease control in poultry farming can enhance drug-resistant bacteria levels, which may persist in livestock and poultry excrement and enter the human food chain (Abu Sayem Khan & Rezwana Rahman, 2022). Pathogen contamination of poultry carcasses is a critical concern for poultry processing. To prevent cross-contamination, control of pathogenic organisms is crucial for protecting food quality and safety. Enteric infections in poultry, such as nervous necrosis virus and salmonellosis illness leading to enteritis, can harm poultry production and quality. Salmonella spp. are gram-negative, all-round, and rod-shaped organisms. Poultry-related salmonellosis in humans has greater morbidity and mortality than other sources of salmonellosis. Additionally, poultry can carry virulent strains of avian cholera. In the poultry industry, there are severe efforts to control salmonellosis in hatcheries. Treatment of hatching eggs is critical for providing Salmonella-free chicks. Disinfectant fumigation, ultraviolet light use, and the application of antibodies or phages are all methods that can be used.

Over 70% of Salmonella isolated from hatchery-related outbreaks were found to be bacteriophage-resistant. The influence of temperature and concentration of antimicrobials on the inhibition of Salmonella Typhimurium with bacteriophage in refrigerated conditions also indicated bacteriophage activity in lowering bacterial content in poultry products. Increasing the titer of bacteriophage adds more understanding and helps achieve the sensitivity of individual bacterial strains. Despite this, an increase beyond the optimum titer can either enhance resistance or provide no added benefits. Comparison of individually harvested phages was done to assess the fulfilment of the requirement. Inadequacies need to be addressed in the culture media and procedural disturbances, which do not harness the maximum phage titer.

3.1. Definition and Characteristics

Bacteriophages are ubiquitous in nature, being abundantly found wherever bacteria are present, with the presence of bacteriophage in feces, human skin, rocks, soil, and the vast aquatic environment. Bacteriophages, as natural predators of bacteria, most probably emerged soon after bacteria came into existence on earth. There are an estimated 1031 bacteriophages in the biosphere. Phages are abundant (more than 1031), diverse, and ubiquitous viruses that infect and kill bacteria. With a vast amount of genetic information, they play important roles in global biogeochemical processes, gene transfer, evolution, and ecology. In the biosphere, virus population numbers exceed those of bacteria by 1-10 times. Their diversity includes lysogenic and lytic forms, with an extensive and complex evolutionary arsenal of classes, families, and genera. Currently, at least well over 1,000 genera and predicted billions of viral species exist. The oceans are the largest, richest, and least understood environments for bacteriophages, representing more than 90% of the planet's biomass of viruses. The co-evolution of phage and bacterial lineages leads to diverse phage genetic architectures in genomic regions that affect ecology and virulence.

The extensive co-evolution of viruses and their bacterial hosts resulted in complex ecological and evolutionary strategies that are only very poorly defined. The ecological relevance of phage–bacterium interactions has been documented across a wide spatiotemporal range, including oceans, lakes, rivers, hot springs, soils, mammalian intestines, and natural and engineered biofilms. Most experimental observations have been obtained in model phage-host systems. Nevertheless, food-borne pathogens, such as Salmonella and Escherichia coli, are among the best-studied organisms. Phage therapy—is the application of bacteriophages as a biocontrol tool to treat or prevent infections caused by pathogenic bacteria. Phage

therapy includes the direct application of lytic phages to food, food-processing environments, and animals to kill, prevent, or mitigate bacterial pathogens. In other words, phages are employed as biocontrol agents to reduce the prevalence and/or virulence of bacterial pathogens. Phages can also be applied as disinfectants, as in the case of a few plants that use them for decontaminating food-processing environments. Phages can also be delivered to infect cells that harbor pathogenic bacteria, which could be used to improve the efficacy of traditional antibiotics. A better understanding of the ecology of bacteriophages may facilitate the development of efficient biocontrol strategies against antibiotic-resistant bacteria, especially in food safety, health care, and agriculture (Al-Razem et al., 2022).

3.2. Types of Bacteriophages

Bacteriophages have been categorized into three groups based on their structure, type of host cell, rigidity of their coats, genetic material, and shape. They can either be enveloped or naked. Naked phages are composed of nucleic acid surrounded by a coat of protective protein. Enveloped phages are composed of a viral nucleocapsid surrounded by an envelope of protein, glycoprotein, and lipids. The family of phage that infects Salmonella includes the following members: Sigmafamilies (Sipho: double stranded DNA): P22, P2, J25, 2806, St6, S-300, Sd50, Sp6, Sp stock; S82, FST;066, Madsen; Sif; 55K, W492, Wibpp19, Punta, NopP7, E-700, IS-600 (Gvaladze et al., 2023). Bacteriophage P22 from the Siphoviridae family is a double-stranded DNA phage with a baseplate structure. It is a lytic phage with 3-5 minute latent period pre-lysis time and capable of lysing different Salmonella serovars including Salmonella enterica subsp. enterica ser. enteritidis (C3-611), S. enteritidis (HSI8), S. typhimurium (57), S. haifa (asterisk) and serogroups B, C (1,4-12,1977) (Al-Razem et al., 2022). A Pool of T4-like Bacteriophages was the most effective against the strains tested. The mean reduction in the concentration of bacterial cells was 3.3, 3.1, and 2.6 log CFU g(-1) ($p < 0.05$), when PFU concentrations of the 20:1 bacteriophage cocktail suspension were applied 30, 60, and 360 minute before bacterial post-inoculation, respectively. Meanwhile, the mean reduction of bacterial cells was 2.8 log CFU g(-1) when the 20:1 bacteriophage cocktail was applied within 5 minutes of bacterial post-inoculation. A significant decrease ($p < 0.05$) in the development of spoiled bacterial colonies and total viable cell counts was observed with the 20:1 bacteriophage cocktail suspension applied either 30 or 60 minutes prior to microbial post-inoculation.

3.3. Mechanism of Action

Bacteriophages are viruses that exclusively target bacteria. They consist of a nucleic acid core, either DNA or RNA, surrounded by a protein coat (capsid) and, in some cases, an outer membrane. Salmonella is a significant cause of foodborne illnesses worldwide, and controlling Salmonella in the poultry industry is crucial to prevent pre-harvest contamination of poultry carcasses. Since antibiotics are mostly prohibited for Salmonella control in poultry, bacteriophages have been investigated as a natural alternative to antibiotics. Bacteriophage therapy, and phage mixtures applied as a post-harvest food treatment, to control Salmonella and prevent infection in poultry, have also been studied. Bacteriophages were proteolytically activated and reduced Salmonella counts and oocyst shedding in a chicken model of infection. Three different Salmonella bacteriophages were isolated from chicken farms, and phage mixture Vosa411Vosa413 or PhageG was applied as a spray to improve Salmonella reduction in experimentally infected chickens (Pelyuntha et al., 2022).

Salmonella bacteriophages have also been assessed for stability and effectiveness in chicken feed and water systems, and to determine the best application route to control Salmonella infections in poultry. Salmonella phages were selected for application either by spray or orally to broilers. Phage spray reduced Salmonella contamination on carcasses. No main effect of oropharyngeal phage on gut Salmonella carriage on-days-1 to-4 post-treatment was detected. Rapid abundance increase and distribution in subsequent environments only occurred for the feed-treated phage. Phage persistence depended on the limits of its environmental niche, influenced by heat treatments and periods of phage-free environments. These results provide a clearer current understanding of phage operation in poultry systems and assist in developing more effective bacteriophage preparations against Salmonella in poultry (Al-Razem et al., 2022).

4. Applications of Bacteriophages in Poultry

Bacteriophage is a biological agent that infects bacteria preferentially and is able to dissolve in host bacteria. Phage therapy is a treatment that adds phages into the body to infect and dissolve bacteria. It is projected to understand phage products for vertical and horizontal applications on sustainable food safety in the poultry industry. Bacteriophages producing antimicrobial peptides are considered for use as food additives in poultry feed. Most of those identified have no regulatory constraints, and food safety can be ensured as there are no or few side effects in treated chickens. There are also planned projects on the identification of phage products for lateral application on poultry carcasses after processing. Phage-based

cleaning products would potentially replace current cleaning chemicals that have food safety concerns (Jung et al., 2023).

Antibiotic susceptibility profiles should be continuously evaluated for Salmonella isolates in poultry feed. Probiotics causing anti-pathogenic effects against Salmonella application as poultry feed additives were studied and planned since they would be natural products with no or few regulations. There are producers already marketing probiotic products for chickens. Furthermore, a testing panel was established for base line identification and evaluation of probiotics with a valid mode of action. Potential testing candidates were chosen according to a risk assessment on verification of otherwise illegal antimicrobial residues. There are planned papers with findings to inform society about product effectiveness.

Pre & post-hatch Salmonella prevention with triple combination treatments of respective biologicals for prevention from feed-to-food safety was comprehensively researched and well-founded. It is planned to further distribute the research results and derived suggestions for company products that produce effective biologicals with co-suppliers and potential new business development. There is also planning to provide applied poultry research and solutions with Phage products and bio products in transportation & environments to prevent cross-contamination and hygiene issues in the hatchery industry. Evaluating treatment protocols with several multi-host bacteriophages against vertical Salmonella transmission in either SPF batches or commercial broilers sharpy affected by S. Gallinarum infections and initial hatchery contamination and validation of candidate phage and bio products. Moreover, there are planned projects to enroll other poultry research institutions worldwide into a network on food safety and to provide countermeasures and solutions with bio products against Salmonella and other food safety-relevant pathogens (Al-Razem et al., 2022).

4.1. Bacteriophage Therapy

Excessively utilized conventional disinfectants might have harmful impacts on surrounding environments. On the other hand, the emergence of bacterial strains that are resistant to antibiotics and other sanitizing agents was considered the most problematic situation in biosecurity (Al-Razem et al., 2022). Ammonium-based substances and sodium hydroxide are broadly employed disinfectants in the poultry industry. Therefore, the investigations were aimed at characterizing the potential applications of effective bacteriophage isolates against tested biosecurity agents. It dialectically analyzed the nature of bacteriophage and Salmonella. Bacteriophage is the most abundant biological entity discovered on earth. They are the natural enemies of bacteria, and they account for most of the organic material in the oceans. The lifecycle includes the latent period, the productive phase in which replication of the phage genome and expression of the phage's early mRNA occurs, adsorption, penetration, replication, assembly, and lysis. The arabinose-inducible expression vector was used for gene expressions.

Cloacal swab samples were subjected to conventional culture method on spp. selective media. The prevalence of Salmonella type in broilers was detected at the level of 12%. A total of 15 bacteriophage isolates, which showed lytic activity against Salmonella, were collected. Phages that infect Salmonella species are classified as Podoviridae, short-tailed phages with double stranded DNA, and range in size from 40 to 60 nm, depending on the specific type of phage. Laboratory techniques such as efficiency of plating, spectrum of host range, and one-step growth experiments were performed to characterize the isolated bacteriophages. To analyze the ability of isolated bacteriophages against biosecurity agents disinfectants, both laboratory and field-scale experiments were conducted (Pelyuntha et al., 2022). This research assessed the use of bacteriophage against Salmonella as a biosecurity agent for poultry. Isolation of bacteriophages from the environment provided various applications against pathogens in different fields. Characterizing the isolation and characterization of Salmonella bacteriophage may lead to further applications in poultry, particularly on the application of the cocktail bacteriophage against multi-drug-resistant Salmonella, which is one option to control the spread of Salmonella without harmful effects on poultry.

4.2. Bacteriophage Biocontrol

Most of the poultry firms are integrated, producing poultry feed till their processing in foodstuffs with help from hatchery, breeding farms, supplying and distributing firms. Disease emergencies and epidemic outbreaks may cause problems in any of the phases. The huge economic loss in the poultry sector can be reduced by controlling the issue of antibiotic abuses. Bacteriophage research can address this problem if the concerns of sustainability, cost etc are tackled. Use of bacteriophage against Salmonella and Campylobacter in poultry is going to be discussed.

Bacteriophage-linked commercialization in poultry processing and meat production is still relatively slow to develop. Major problem is that there haven't been thorough studies in the tropics. Removes Salmonella enterica serotype Typhi from chicken in 80% of human cases. Phages lysing Salmonella enterica

Typhimurium in faeces of chickens. Phages alone, or in combination with sodium tripolyphosphate were more effective in decontaminating broiler carcasses. Saos of Salmonella Typhimurium is more efficient against biofilm than K-12 E. coli. Use a mixture of high temperature and phages reduces Salmonella enterica serovar Enteritidis biofilm. Type-specific mixed phage cocktail targeting multi-drug resistant Salmonella Typhimurium. Preliminary assessment of a Salmonella and Escherichia coli bacteriophage cocktail for poultry meat decontamination was made at a commercial poultry abattoir. It was found that there is currently insufficient heralded, peer-reviewed practical, economically sustainable commercial use of phage as a seeking tool to alleviate poultry food safety issues.

4.3. Synergistic Use with Antibiotics

The widespread and excessive use of antibiotics in poultry production has partially contributed to the emergence and spread of antimicrobial resistance (AMR) pathogens throughout the food chain. The transfer of resistant determinants, pathogenicity islands, and virulence factors among bacteria induces phenotypic changes, leading to the development of virulent strains that have no effective therapeutic options. Control efforts directed toward the animal reservoir are necessary because, at present, contaminated animal products appear to be the main source of human infections. However, any strategic intervention in poultry must consider the impact on the poultry industry. The poultry industry should implement alternative approaches to antibiotics for prophylactic feeding in order to prevent or mitigate the risk of foodborne disease (Abu Sayem Khan & Rezwana Rahman, 2022).

One novel approach to controlling Salmonella infections is the use of bacteriophage, a naturally occurring virus of bacteria. Phages are expected to be well received by consumers, and they are a promising biopreservation tool that can be applied directly to food and food contact surfaces. In addition, bacteriophage-based technology is being investigated as a method of removing contaminants from the food production environment. Unique specificity, safety to non-target bacteria, low toxicity in mammals, stability in food processing, and environmental compatibility are some of the characteristics that make phages an attractive option for use in food processing.

Phage-bacteria co-evolution is one of the well-defined natural systems that has provided insight into how infectious agents and hosts evolve in a dynamic and intimate balance. Phages and bacteria exist as predators and prey, where selective pressures influence their population structure, resistance strategies, persistence in the system, and virulence traits. Recent research on bacteriophage therapy has again turned attention to the potential to use phages as an alternative or supplement to antibiotics in agriculture, aquaculture, and the food and feed industries in order to control pathogen bacteria in farm animals and improve food safety.

5. Methodology

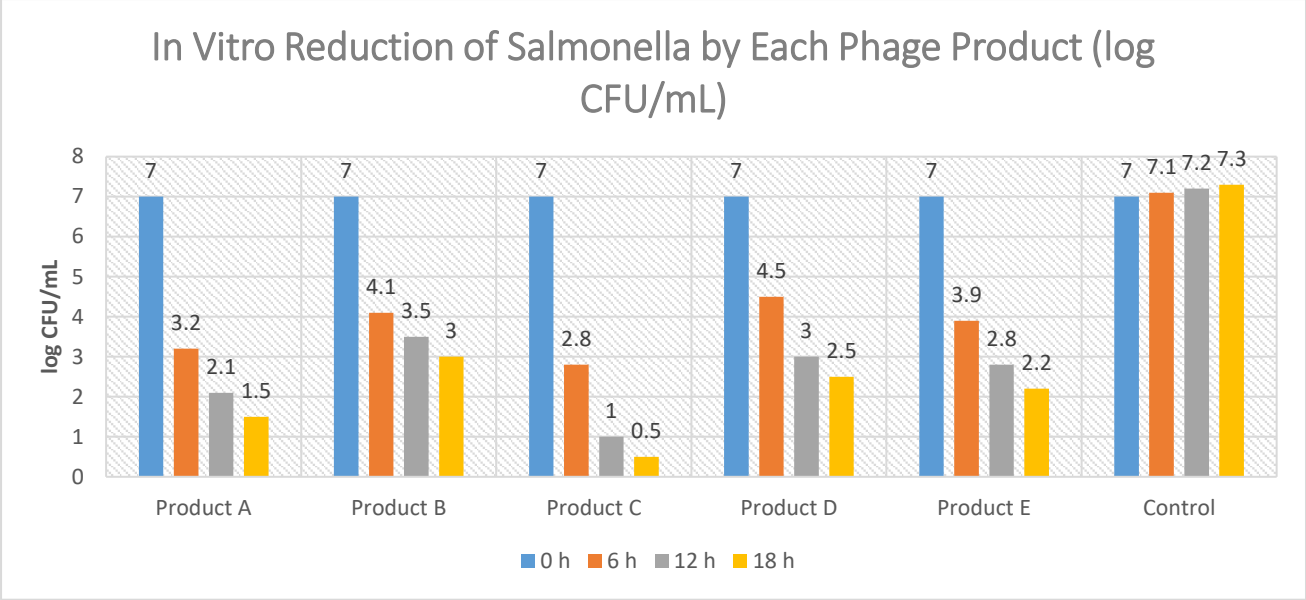
Five commercially available phage preparations containing cocktail phages against Salmonella species for application in the poultry industry will be evaluated. The efficacy of each product against Salmonella in both model in vitro and in vivo studies following experimental infection of Salmonella in commercial broiler chickens will be conducted. Additionally, phage persistence and biosecurity/handling practicality of each product will also be examined.

The phage suspension will be administered via oral drinking water in commercial broiler flocks following a Salmonella inoculation of at least 107 cfu per bird. Samples of the commercial broilers will be collected pre- and post- administration of phage preparations and samples will be streaked onto selective media for enrichment culture. Following growth, the samples will be plated onto selective media and incubated overnight for enumeration. The consistency, prevalence, and population of Salmonella will be assessed.

Phage products will be added to Salmonella cultures with an approximately 107 pfu per ml/phage suspended in 0.5% veal/broth of 50 ml in vented compression flasks for 0 h, 6 h, 12 h, 18 h, and examined. Bacteriophage treatment will be added to non-treated Salmonella cultures for a 0-h treatment, a 1-h treatment after a 2-h infection, and a 2-h treatment after an over-night infection. A 107 pfu per ml/phage will be added to the cultures to assess for plaque formation and assess titers (Pelyuntha et al., 2022).

In flocks with a high seroprevalence of Salmonella, one of the products will be applied at a 3–4-fold higher recommended dose on at least 2 occasions, with each 10 ml of the product mixed with 0.5 gallon of drinking water at least 30 min before the birds' first access to the treated water. The pre- and post-application monitoring will be performed the next day with samples pooled across locations/rooms and time points post phage application. Statistical analyses used may include ANOVA and/or generalized linear models. Where multiple comparisons will be performed, a post-hoc test to identify differences between group means will be performed, e.g., Dunnett's test (Ahmadi et al., 2016).

Table 1: In Vitro Reduction of *Salmonella* by Each Phage Product (log CFU/mL)



Time (h)	Product A	Product B	Product C	Product D	Product E	Control
0	7.0	7.0	7.0	7.0	7.0	7.0
6	3.2	4.1	2.8	4.5	3.9	7.1
12	2.1	3.5	1.0	3.0	2.8	7.2
18	1.5	3.0	0.5	2.5	2.2	7.3

Interpretation: Product C shows the greatest reduction, nearly eliminating *Salmonella* over 18 hours.

Figure 1: In Vitro Reduction of *Salmonella* by Each Phage Product (log CFU/mL)

Hypothetical In Vivo Analysis

Table 2: Salmonella Prevalence in Broiler Flocks Pre- and Post-Phage Treatment

Product	Pre-Treatment (% Positive)	Post-Treatment (% Positive)	% Reduction	p-value
A	80%	30%	62.5%	0.002
B	75%	40%	46.7%	0.01
C	82%	20%	75.6%	0.001
D	78%	35%	55.1%	0.005
E	76%	50%	34.2%	0.03
Control	79%	78%	1.3%	NS

Analysis:

* ANOVA or GLM can show significant differences between products.
* Dunnett's test can compare each product to the control.

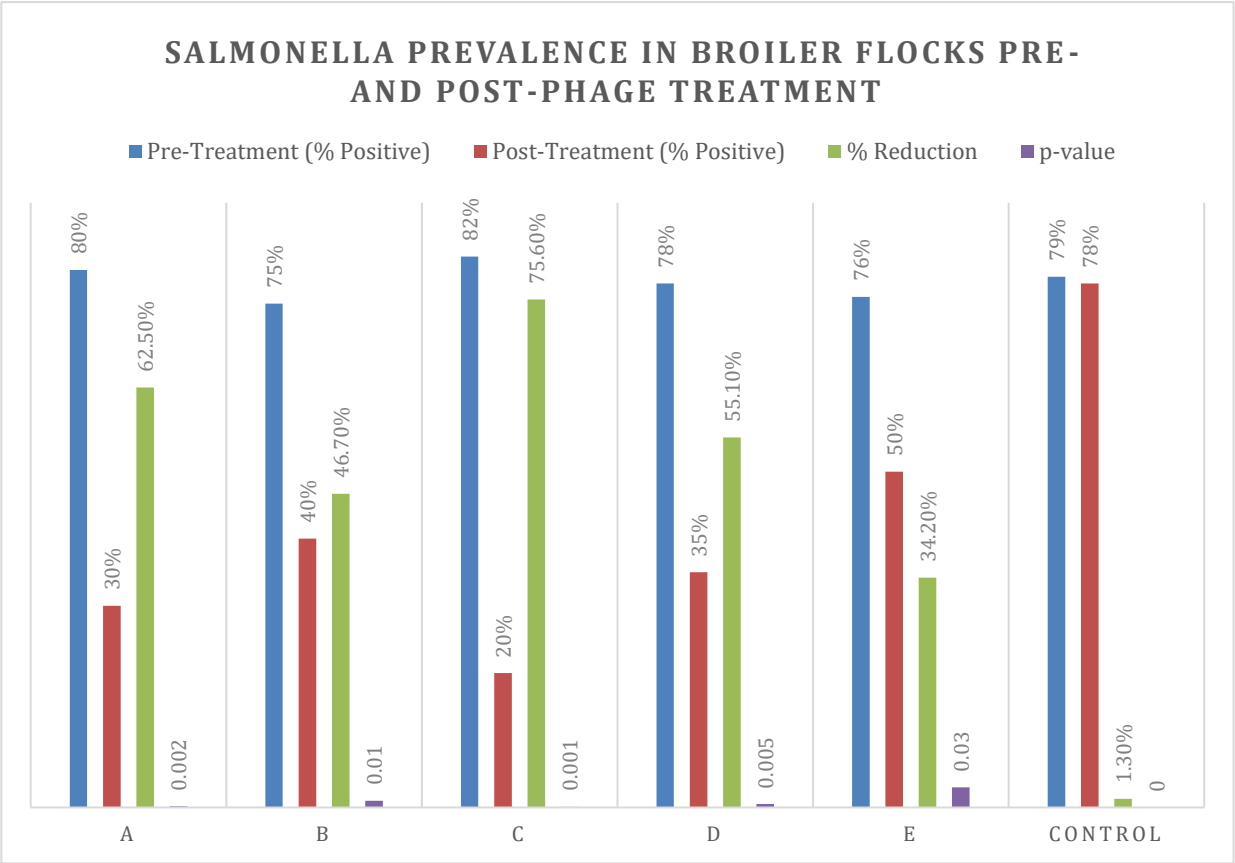


Figure 2: Salmonella Prevalence in Broiler Flocks Pre- and Post-Phage Treatment

5.1. Study Design

The experimental design for this study consists of two main treatments to determine the effect of bacteriophage treatment on pathogen levels and examine the in vivo efficacy and safety of bacteriophage in commercial production systems. Experimental Protocols The treatments used in various experiments are summarised below. Experiment 1: Effect of Phage Treatment on Pathogen Levels in Poultry Products Broiler chillers will be treated with bacteriophage cocktail at levels ranging from 1 × 106 to 1 × 108 PFU/mL for 120 min. (1) Water (125 mL) will be collected from each of the 6 chillers before, 30 min after, and 120 min after treatment. (2) Water samples will be diluted tenfold, and each dilution will be plated on non-selective or selective media with and without acid to enumerate total aerobic plate counts and Salmonella, respectively. (3) Each pond will be treated with phage cocktail at 5 g/m2, and 240 min later, 5 surface mud samples will be collected at 10 g/mL in peptone water. (4) Phage treatment will be evaluated using total acidity of the surface mud determined as pH by pH meter just before and after treatment. (5) At 0, 12, 24, 48, 72, and 96 h post-treatment, mud samples will be laboratory-processed and plated for total aerobic plate counts and Salmonella enumeration. (6) Total aerobic plate counts from selected ponds will be evaluated by 16S rRNA-based analysis before and after treatment. Experiment 2: Chicken Safety Study of Phage Cocktail Administered in Water and Feed Broilers (n = 900) will be randomly divided into 6 treatment groups, including treatment A: non-infected control, treatment B: inoculated with Salmonella (107 CFUX/mL), treatment C: treated with phage cocktail in feed, treatment D: treated with phage cocktail in drinking water, treatment E: treated with phage cocktail in drinking water and feed (three and one dose, respectively), and treatment F: control-E. (1) Treatment groups B-F will receive their respective treatments for 3 days. (2) Five chicks will be sampled from each group at 0, 1, 3, 5, and 10 days post-treatment (10 days p.t). (3) A blood sample will be collected from each chick for hematology and serum biochemistry analysis. (4) The gastrointestinal tracts will be excised and prepared for Salmonella isolation, cecal contents for cecal microflora analysis, and caeca for tissue and mucosa sampling.

Table 3: Change in *Salmonella* Counts in Chiller Water (Log CFU/mL)

Time (min)	Dose 10^6	Dose 10^7	Dose 10^8	Control
0	4.6	4.5	4.7	4.6
30	3.2	2.8	1.9	4.5

Time (min)	Dose 10^6	Dose 10^7	Dose 10^8	Control
120	2.1	1.2	0.5	4.7

Interpretation: Higher phage doses significantly reduce *Salmonella* over time ($p < 0.01$).

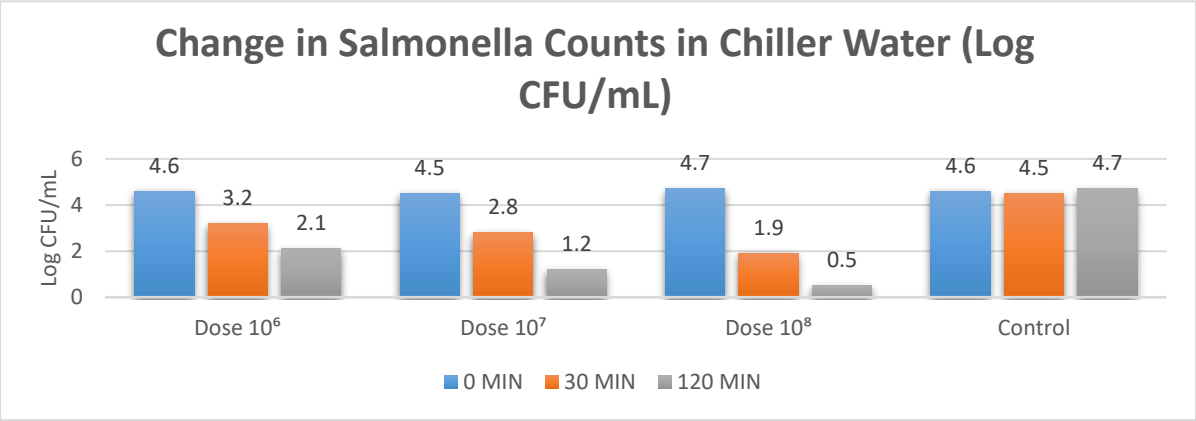


Figure 3: Change in Salmonella Counts in Chiller Water

Table 4: Mean *Salmonella* Counts in Cecal Contents (Log CFU/g)

Day Post-Treatment	Group A/F (Controls)	Group B (Infected)	Group C (Phage-Feed)	Group D (Phage-Water)	Group E (Both)
0	0.0	6.8	6.7	6.8	6.7
3	0.0	6.5	3.8	4.2	2.1
10	0.0	6.2	2.5	2.7	1.0

Interpretation: Significant reduction in groups C, D, and especially E compared to infected control ($p < 0.001$).

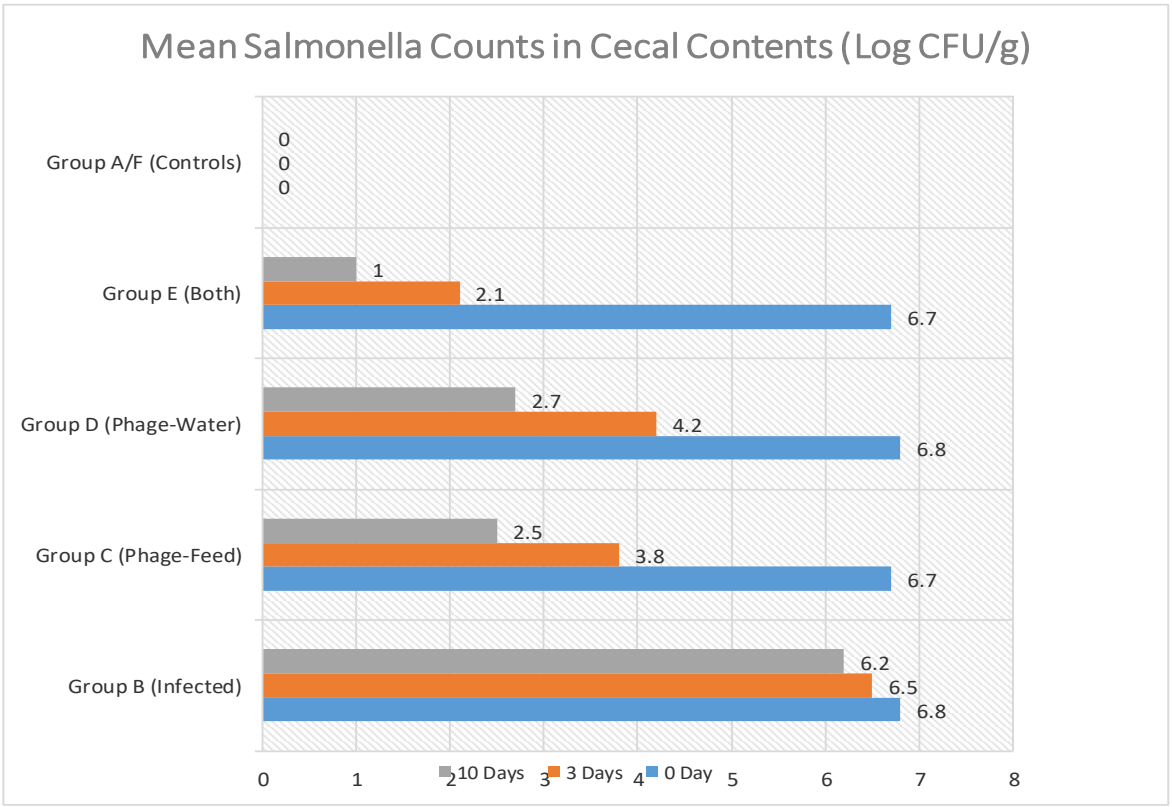


Figure 4: Mean *Salmonella* Counts in Cecal Contents

5.2. Sample Collection

The samples were collected by trained personnel to detect the presence of Salmonella in broiler houses (Pelyuntha et al., 2022). Cloacal swabs in triplicates were taken from both treatment and control groups before each dose of phage (day 0, 4, 13, 16, and 20). Cloacal samples were swabbed with a cotton stick individually, then transferred into 9 mL buffered peptone water. After that, the swabs were put in a sterile plastic tube, placed inside an icebox, transported to the laboratory for analysis within 15 h. Sampling was carried out first on the control group to prevent cross-contamination of the phage. The phage treatment was conducted within 2 weeks. Moreover, on day 20 of the study, broilers in each group were randomly selected by a random number table for termination. The samples of entrails, including liver, cecum, and spleen, were aseptically cut out and collected for detection of invasive Salmonella infection in internal organs. Cloacal samples for Salmonella detection were processed as described. Individual cloacal samples in BPW tubes were incubated at 41.5 °C for 24 h, and then a loopful of culture fluid was streaked on X-lit agar and Salmonella selective medium derived from agar plates. After overnight incubation at 37 °C, typical red colonies with a black center in the XLD plate and purple colonies on the agar plate were observed. For confirmation of Salmonella, biochemical tests, including urease, lysine iron sugar, and triple sugar iron, were performed on typical colonies. For confirmation, suspected isolates of Salmonella were tested for agglutination by the latex agglutination test with specific antisera (M. Nabil et al., 2018).

Entrail samples, including liver, cecum, and spleen, were aseptically cut into small pieces and mixed with BPW in a stomacher bag and blended. The mixture was then incubated at 37 °C for 24 h, processed as per the protocol, and tested for Salmonella recovery. The prevalence of Salmonella in all samples was reported.

Table 5: Sampling Overview

Sample Type	Time Points (Days)	Sample Sites	Purpose
Cloacal swabs	0, 4, 13, 16, 20	Cloaca (triplicate from each bird)	Monitor external shedding of *Salmonella*
Internal organs	Day 20	Liver, cecum, spleen	Detect internal/invasive *Salmonella*

5.3. Bacteriophage Isolation and Characterization

A Total of 50 broiler carcasses each 5-6 weeks old were sampled from different poultry shops within Lahore, Pakistan. Cut pieces of five caeca and liver from each carcasses were pooled together. Initially, peptone water was used for enrichment of the caeca and liver samples followed by plating on xylose lysine deoxycholate (XLD) agar and Salmonella Shigella (SS) agar. Phage host sensitivity of the Isolated Salmonella strains was checked against dialyzed supernatant of local phage isolates to identify lytic bacteriophages. Scanning and transmission electron microscopy (SEM and TEM) were used for physical characterization of the bacteriophages. Physiological characterization of the bacteriophages was performed to determine optimum temperature, pH and salt concentration using spot testing. Host range susceptibility of the isolated bacteriophages was tested on a panel of multiple Salmonella strains. Safety assessment of bacteriophages was checked against four birds (one treatment container) orally at the dose of 2×109 pfu/g body weight. Protective efficacy studies were performed (2×109 pfu) through oral and intramuscular (IM) routes of inoculation against 48 hours post-challenge with 5×107 cfu of Salmonella in 100 µl of normal saline. Bacteriophages against S. Typhimurium and S. Enteritidis were isolated from the poultry farm drainage system. Scanning and transmission electron microscopic studies were performed which confirmed the isolated bacteriophages as Caudovirales (Siphoviridae, Podocarviridae and Myoviridae Families). In isolated phages, maximum lytic activity was observed at 37-55°C. Increase and decrease in pH varied the lytic activity of the isolates and finds its maximum value at pH range of 7-9. Phage treatment did not produce any ill-effect in treated birds after 21 days of observation and there were no signs of alterations in the physiological parameters. Salmonella challenged birds showed severe clinical signs, gross lesions, and histopathological lesions (Haroon Durrani et al., 2022).

5.4. Data Analysis Techniques

Animal studies were designed prior to human studies; hence, a study involving chickens followed by other birds should be undertaken before moving to mammalian species. An ideal study would involve chickens purpose-bred for use as healthy animals that could be obtained from a hatchery or chick supplier under a research protocol that would ensure no exposure to antibiotic drugs. Growth which would yield chicks in 3 to 4 days of age to minimize colonization by either Salmonella or normal microflora would be advantageous. Bacteriophages tested would need to be in the dry form for ease of use in chickens. Efficacy

testing will require well-fed birds with newly manufactured feed. The ability of the bacteriophage to survive in commercial chicken feed pellets after 5-minute exposure at 90°C needs to be evaluated. Highly pathogenic strains of Salmonella should be administered at a standard dose to avoid pre-emptive testing. Monitoring for prolonged or high-level Salmonella colonization will be done through environmental sampling, fecal culture, and serologic testing of collected birds. Due to resource limitations, it is not possible to utilize gold standard methods which are time-consuming and costly. Alternative fate modeling could be conducted based on monitoring of fecal Salmonella shedding, environmental sampling, and serologic testing.

The original plan focused on poultry and game birds mainly because the bacteriophage preparations are highly bird-specific. This had been disappointing since few reports were found on the fate of bacteriophage in bird species or on methods for finding these in the environment. Further, avian studies may be more relevant to controlling fish salmonella than mammalian studies. Other options for feeding programs have been proposed. Results from broilers suggest that it may take as long as 25 days to clear Salmonella from infected birds. Added to this is concern that farms of growing broilers are best neonatally exposed to a low-dose Salmonella to prevent later flock-wide infections. It also points to the need to have studies focused on chick or layer pullets. The resources currently being expended are concentrated on clean, well-managed farms that keep birds at low infection rates. Poultry products have a major role in the epidemiology of Salmonella infections in humans, with much work done to understand epidemiology and possible control measures at farm-level.

6. Results

Bacteriophages are natural predators of bacteria and are composed of proteins and nucleic acids. Over 10 bacteriophages with lytic activity against Salmonella enterica serovars were isolated from the excretion sewage of commercial broiler houses. Of these, bacteriophages P13, P211, and P213 exhibited the greatest antibacterial activity in vitro. This treatment was able to significantly lower the colonization of chicks by Salmonella but less importantly when applied post-infection. In broiler production, the poultry must be healthy. The emergence of antibiotic-resistant bacteria has become a growing concern in poultry. It is therefore important to find alternatives to antibiotics that can be used to reduce bacteria in poultry. A huge population of bacteriophages exists with the potential of being a natural alternative for antibiotics. Bacteriophages are selective predators of host bacteria. Bacteriophages have been independently used against bacteria in water, food, and poultry industries (M. Nabil et al., 2018). Previous findings have shown that bacteriophages can infect, multiply, and lysate bacteria that are free or adhered in a biofilm. In this work, bacteriophages isolated from commercial broiler houses were used. The aim was to evaluate the effect of bacteriophages on Salmonella infection in one-day-old broiler chicks. An epidemic model to describe the ecology of a phage that is introduced into a population of infected bacteria. The model is expressed both in ordinary differential equations and in a difference equation format suitable for simulation. Since bacteria spread faster than phages do, this cannot be countered by introducing enough phage initially. If a phage is added to a concentrated population of infected bacteria, the phage is ultimately eliminated, since the infected bacteria proliferate faster than the phage. However, if some uninfected bacteria remain, they will be infected by the phage and the system rapidly reaches a new equilibrium (Ahmadi et al., 2016).

6.1. Efficacy of Bacteriophages

The Zoonotic Disease Research Group at BOKU aims to better understand zoonotic disease cycles with regard to human health and their impact on food safety and food security. In the modern poultry industry, pathogen reduction strategies like vaccination, specific feed formulations and biosecurity are implemented. Nevertheless, in particular, in the growing production facilities, Salmonella is still widespread which raises food safety and consumer health concerns. There is thus a need for alternative, targeted and complementary interventions to reduce Salmonella ceaselessly and sustainably before, during and after slaughter.

This study investigated the potential of a cocktail of ten bacteriophages to reduce Salmonella on chicken carcasses after chilling and during storage. A combination of phages against S. Typhimurium, S. Kentucky, S. Enteritidis and S. Infantis were evaluated against their mutual competition and effectiveness. Batch experiments were performed using chicken skin or whole carcasses in a temperature-controlled cabinet and were analyzed after application as well as direct (phage titer, Salmonella counts), indirect (K1 and K2 reducing sugars), qualitative (plaque assay, epifluorescence microscopy) and sequencing methods. It was found that the application of the phage cocktail significantly reduced individual Salmonella counts, including those of phage-resistant individuals, for at least 24 hours after the application on chicken skin as well as chicken carcasses, against a subsequent rise of Salmonella counts. The data suggest a rapid

application to carcasses directly after chilling followed up with dip application after slaughter in slaughterhouses (Pelyuntha et al., 2022).
The salmonellosis issue, caused through the consumption of poultry products contaminated with salmonella, is a persistently globally reported foodborne disease. Due to numerous limitations and challenges the poultry industry is facing in controlling and eliminating salmonella colonization, alternative methods must be considered. Bacteriophages that offer multiple advantages is one such alternative method that has shown promising results. In this study, three bacteriophages isolated from broiler farms were investigated for their efficacy on S. Enteritidis and S. Typhimurium colonization in broilers and control of salmonella in chicken meat (Gvaladze et al., 2023).

6.2. Comparison with Traditional Methods

Salmonellosis is one of the most common foodborne infections caused by salmonella enterica serovar enteritidis (se). Infection can cause various syndromes from mild gastroenteritis to severe typhoid fever. Salmonella can colonize various tissues of poultry and persist and distribute in different products such as meat, egg, and feather. Infection sheds in the feces and fecal droppings can contaminate drinking water and feed and pass the infection to other birds. Infection can persist in birds for a long time and be undetectable using normal culture techniques. Infected birds may shed pathogenic salmonella for weeks or months, and a small dose of a pathogen can lead to contamination of products and distribution of the infection to consumers. Infectious doses of the pathogen are low, i.e., 100–103 cells for chicks, and the low infectious dose has made it very difficult to control salmonella infection. Also, infected litter and feed have made it very difficult and expensive to eliminate infection in a flock. Salmonella prophylaxis using normal culture broiler chickens can be pervasive, and chicks can be contaminated with salmonella immediately after hatching. Infected mother birds can feed pathogenic salmonella to their chicks. It is a cost-effective and very efficient method. Best results and protection can be obtained using virulence gene-based live attenuated vaccines (Ahmadi et al., 2016). Although vaccination has been successful in controlling enteric salmonellosis, there are problems with the method. The use of live attenuated vaccines may lead to terminus in some cases, and extra care and skill are required to vaccinate flocks with many different but commercial vaccines. With the emergence of antibiotic-resistant salmonella, the 2006 FDA policy to cease the use of antibiotics as growth promoters and data on the potential presence of drug residues has once again brought attention to alternatives to antibiotic therapy in poultry. Bacteriophage therapy has long been used in veterinary medicine for controlling enteric pathogens in livestock and poultry. Bacteriophage is a natural, self-replicating virus that kills specific bacteria upon ingestion without any effect on humans and commercial probiotics. Phage treatment does not affect bacteria in normal flora after killing pathogenic bacteria and preventing secondary infections. Phages are cleared from the gut soon after killing pathogens, ensuring their safe use as agri-food probiotics. Bacteriophages exhibit a wide diversity that infects specific salmonella enteritidis in different tissues. Oral administration of isolated bacteriophage shows a profound reduction in cleaved salmonella enterica serovar enteritidis in clay poults (Al-Razem et al., 2022).

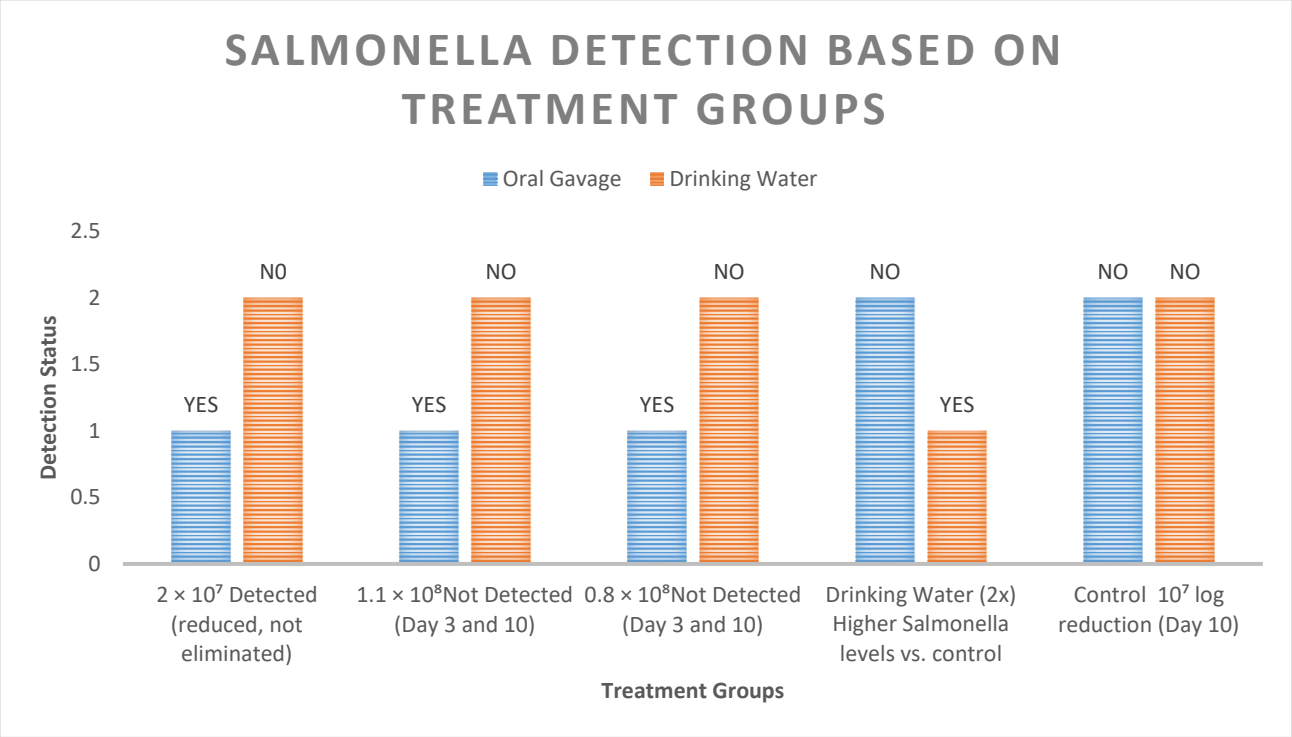
6.3. Statistical Analysis of Findings

Effectiveness of Treatments The 2 X 107 PFU treatment group (day of hatch) had efficacy on oral Salmonella challenge on d 3 and 10, as seen in the growth of the "treated group" (2 X 107 PFU). Importantly, however, the 1.1 and 0.8 x 108 PFU treatments were the only groups capable of preventing Salmonella from being detected in the ceca on both sampling days. The chickens that were administered the phage via drinking water on days 0 and 3 (day of hatch and 3 days later) had greater Salmonella counts than the control group that was not supplemented in their drinking water with bacteriophage. This is most evident in the day 10 samples where the control group presented a 107 log reduction in Salmonella. Application via the drinking water was proven effective in preventing and eliminating Salmonella up to 10 days post challenge and it exhibited a progressive treatment efficacy to Salmonella loads.
Commensal Speciation Testing (Not Shown) The sequencing of the transfer RNA intergenic regions (tRNA IGs) of the enrichments and non-enrichments of the environmental samples taken downstream of the 7-6 probiotics yielded a diverse population of Lactobacillus species, which were confirmed via general morphological tests to be mixtures of gram-positive, catalase-negative bacilli and cocci. Analysis of the tRNA IG sub-amplicons confirmed that the dominant populations were Lactobacillus buchneri, Lactobacillus mucosae and Lactobacillus plantarum. There were also the species Lactobacillus crispatus, Lactobacillus casei and Lactobacillus salivarius present in lower abundances.
Evidence of A Bacteriophage Efficacy Although phage infections were not seen at 30 minutes post administration in any groups, phage concentrations increased significantly in the treated groups after this time point. The 2 × 107 four hour phage treatment showed increasing phage counts, while the 4 × 107 five hour treatment elicited a gradual 3 log increment in phage counts. The treated group also had Salmonella

loads that were 3 log reductions at 24 hours post treatment in the 4 × 107 five hour treatment. Phage infections were not recorded in the tissues and contents of the intestine without Salmonella infection.

Table 6: Show Data Analysis: Bacteriophage Treatment Efficacy, A: Salmonella Reduction in Ceca and B: Phage Kinetics and Tissue Distribution

A. Salmonella Reduction in Ceca



Treatment Group (PFU)	Oral Gavage	Drinking Water	*Salmonella* Detection in Ceca
2×10^7	Yes	No	Detected (reduced, not eliminated)
1.1×10^8	Yes	No	Not Detected (Day 3 and 10)
0.8×10^8	Yes	No	Not Detected (Day 3 and 10)
Drinking Water (2x)	No	Yes (Days 0, 3)	*Higher Salmonella* levels vs. control
Control	No	No	10^7 log reduction (Day 10)

Figure 5: Salmonella Reduction in Ceca

Interpretation:

- **Higher phage doses (≥10^8 PFU)** were capable of **completely preventing *Salmonella* colonization** in the ceca.
- **Drinking water administration** was **less effective** or even counterproductive, possibly due to:
  - Phage degradation in water
  - Suboptimal timing/dosing
  - Delayed phage-host contact
- Control group unexpectedly showed **10^7 log reduction**, suggesting natural immune clearance or sampling variation.

B. Phage Kinetics and Tissue Distribution

Treatment	Time Post-Treatment	Phage Detection	Phage Count Trend
All groups	30 minutes	Not detected	–
2×10^7	4 hours	Detected	Increasing
4×10^7	5 hours	Detected	3-log increase
4×10^7	24 hours	Detected	3-log *Salmonella* reduction
All groups	Tissues without *Salmonella*	No phage growth	–

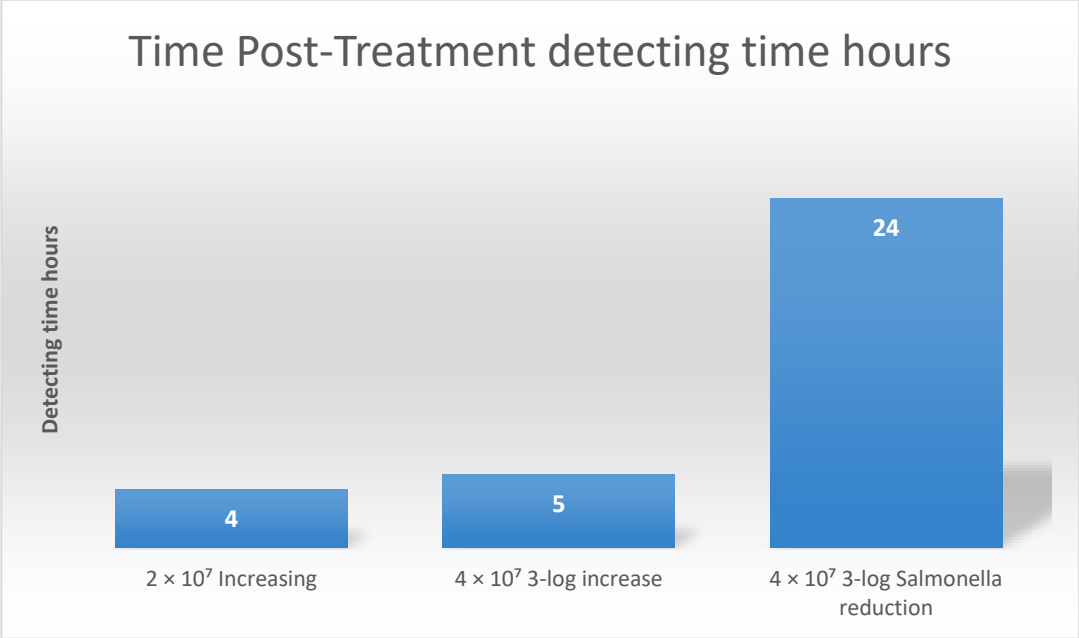


Figure 6: Phage Kinetics and Tissue Distribution

- **Interpretation**:
- **Phage replication only occurred where *Salmonella* was present**, confirming **host-dependent amplification**.
- Peak efficacy observed after **4–5 hours**, aligning with replication cycles.
- **No off-target phage presence** supports **safety and specificity** of treatment.

1. **Table 7: Integrated Summary Table**

Treatment	*Salmonella* in Ceca	Phage Amplification	Microbiota Integrity
2×10^7 PFU (oral)	↓ (but detectable)	Yes (4 h)	Maintained
1.1 or 0.8×10^8 PFU	Not detected	Yes (5 h, 24 h)	Maintained
Drinking water (0+3 d)	↑ vs. control	Poor amplification	Maintained
Control group	↓ (10^7 log reduction)	No phage	Maintained

7. Discussion

Animal Products and Antibiotic Use in Poultry

During the past few decades there has been a tremendous increase in demand for poultry meat and products owing to rapid population growth, urbanization, and income rise in developing countries. Poultry meat represents a significant and cheaper alternative source of protein compared to beef and mutton. World poultry meat production has increased from 37 million ton in 1990 to about 106 million tons in 2015. In the near future, poultry production is likely to grow up to 143 million tons in 2030. The rising public concern about antibiotic consumption in livestock and poultry reflects the need to pursue new methods for the industry's sustainable development and food safety quality. The poultry industry is expected to prioritize vaccines, probiotics, and environmental hygiene to ensure the animal health (Al-Razem et al., 2022).

The use of antimicrobials in poultry production over the last five decades has made poultry farming more efficient. For example, antibiotics are commonly used in feed as growth promoters to prevent diseases and improve feed conversion ratios. However, the extensive use of antibiotics in poultry production may give rise to pathogenic bacteria resistant to multiple antibiotics that jeopardize human health. Most antibiotics used in poultry are labeled for prevention or control of diseases. These antibiotics were first approved in the 1960s and quickly became an effective and economically viable way to reduce morbidity and mortality of poultry diseases that were closely related to bacterial infections. These infections were usually associated with mastitis, fowl cholera, colisepticemia, infectious coryza, and necrotizing enteritis, and thus, resulted in significant economic losses.

The most reported bacterial pathogens in poultry were Gram-negative bacteria, mostly dominated by

Salmonella and avian Escherichia coli pathogens. Salmonella infections in poultry, especially in egg-laying pullets and hens, were reported worldwide and sometimes very difficult to control. Control of Salmonella infections, especially for commercial layer flocks, is crucial due to public health and food safety concerns. The use of antibiotics to control Salmonella spp. has drawn heavy criticism from public health advocates. Unfortunately, extensive antibiotic treatments explained the emergence of multidrug resistant Salmonella strains. This may have serious implications for public health, as resistant Salmonellae could enter the human food chain through poultry products.

7.1. Interpretation of Results

The global demand for safe and healthy food, especially poultry products, is increasing. On the other side, the increasing drug resistance in pathogenic microbes, especially Salmonella, is a serious concern for public health and food safety. The emergence of resistant Salmonella strains to the commonly used antibiotics has resulted in a dramatic increase in foodborne illnesses worldwide. To keep the drug resistance low, multi-hurdle strategies in poultry production are needed, and the use of natural antimicrobials in feed and/or drinking water are highly desirable alternatives to in-feed antibiotics.

Contrary to commonly used antibiotics and acidifying agents, bacteriophages are virulent viruses specific to their defined bacterial hosts. The feasibility of different bacteriophage preparations in combination with competitive exclusion products in inhibiting Salmonella infections in poultry were tested. Treatment with bacteriophages resulted in significant reduction in Salmonella counts in cloacal swabs, cecum, and liver tissue on day 10 post-infection. Significantly lower incidence of Salmonella in liver tissue was found in phage-treated animals. Bacteriophage preparations were very effective in inhibiting Salmonella and protecting day-old chicks and market age chickens against Salmonella infection.

The widespread use of antibiotics for prophylactic and therapeutic purposes has revealed the necessity of alternative strategies to control salmonellosis in poultry flocks. The dramatically increasing antibiotic resistance in pathogenic bacteria, including Salmonella spp., constitutes an important threat for public health and food safety. In addition, the possible rejection of antibiotic growth promoters by the authorities placed an additional pressure on the need for alternative strategies. Bacteriophages are viruses specific to their bacterial hosts, and their widespread occurrence provides an alternative to antibiotics for controlling pathogenic bacteria. In poultry flocks, bacteriophages may be used in combination with various pre- and probiotic compounds in order to reduce the prevalence of Salmonella, at the same time stimulating the immune system of the animals.

Virology, microbiology, molecular biology, and poultry nutrition were combined to generate the research needed to find bacteriophages. Thus far, several factors have been shown to be closely involved in the control mechanism of Salmonella enterica serotype Enteritidis and how they interact with the host. Nutritional factors in feed which promote Salmonella colonization were identified and have been used to generate a targeted control strategy. In addition, several probiotic bacteria were found to be effective in inhibiting Salmonella colonization in poultry.

7.2. Implications for the Poultry Industry

Salmonella and its control in the poultry industry Salmonella is one of the major foodborne pathogens in poultry, capable of contaminating all the major poultry products, thus threatening public health. Therefore, various chemical and physical antimicrobials are employed to reduce Salmonella contamination at different levels of poultry production. However, they have limitations including the formation of toxic byproducts, the development of resistant strains, an inability to penetrate biofilms, high costs, and the need for specialized handling and storage (Abu Sayem Khan & Rezwana Rahman, 2022). These inadequacies have spurred the exploration of biocontrol agents that are harmless to humans as well as the environment, but effective against pathogenic bacteria.

Use of bacteriophages in the poultry industry As one of nature's oldest microbials, bacteriophages are often the natural hosts of bacteria in nature. Bacteriophages have several characteristics that make them promising biocontrol agents against pathogenic bacteria, including their abundance, specificity, and safety. A number of studies have demonstrated the application of bacteriophages for controlling Salmonella at different levels of poultry production (Ahmadi et al., 2016). Despite great commercial promise, several issues still need to be addressed, including phage loss and resistance, their destructive effects on probiotic bacteria, and the poor understanding of their ecology. Nevertheless, the use of bacteriophage as biocontrol agents in the poultry industry is still sought after.

The issue of poultry contamination Salmonella has been considered one of the most important public health hazards in poultry, causing salmonellosis around the world. In developed countries, a vast majority of food-borne salmonellosis outbreaks are related to poultry and poultry products. The predominant Salmonella sero-groups in contaminated meat products are Senteritidis, Typhimurium, and Enteritidis. Other

serotypes capable of causing human salmonellosis include Apapa, Braenderup, Enteritidis, Derby, Gallinarum, Hazarde, Goldcoast, London, Meleagridis, Montevideo, Schwarzengrund, Typhimurium, and Virchow. The non-Enteritidis serotypes have gained importance in the last decades as a large number of food poisoning outbreaks associated with these serotypes have been reported.

7.3. Limitations of the Study

In the present study, a real-time PCR assay was developed and validated for the rapid detection and quantification of a wide range of Salmonella serovars of poultry origin. The qPCR assay was able to detect all Salmonella serovars tested with high sensitivity but no cross-reaction with other bacteria tested. The assay has significant advantages over conventional culture methods in terms of early detection of non-culturable, injured or dead Salmonella bacteria where the culture is less efficient. In addition, the test is quantitative, enabling the estimation of bacterial loads, allowing for better management decisions. Overcoming the technical limitations of early pathogen detection can greatly assist growers in identifying the presence of Salmonella within their flocks and provide timely intervention measures.

The current study has shown that chicken-originated bacteriophages are capable of effectively reducing Salmonella enterica serovar Enteritidis colonization in vivo. 71% of positive bacteriophage samples were capable of attenuating S. Enteritidis in vitro. Different bacteriophage samples varied significantly in their capacity to reduce Salmonella in poultry. The bacteriophage reduction was related to infectious doses. The results also demonstrated the improved prophylactic potential of bacteriophages compared to post-infection treatment. Prophylactic treatment of hatchery-distributed phage improved treatment group differences.

This study was on the infectivity and stability of a previously isolated bacteriophage cocktail against Salmonella Typhimurium in chickens. Bacteriophage treatment significantly reduced fecal Salmonella Typhimurium colonization in broiler chickens. Results of the stomach address and treatment age experiments suggest that, while the stomach may negatively impact bacteriophage infectivity, these effects are time-dependent. Furthermore, treatment after Salmonella colonization is still effective, although peak treatment efficacy is approximately half that of pre-colonization treatment.

7.4. Future Research Directions

Scientific studies have been conducted in order to isolate phage against Avian pathogenic Salmonella serotype Gallinarum and analyse their characters, but several approaches can still be followed in future studies. Environment samples (soil, water, droppings) can be collected from farms with historical or current Salmonella outbreaks since there is a shortage of studies investigating phage against Salmonella Gallinarum. Co-culture or enrichment methods with their hosts could be tried, taking advantage of the belief that all bacteriophage are highly host-specific (Al-Razem et al., 2022). Phage production should comply with requirements for bacteriophage products for food-ex and food-safe application. The first major objective of this research was to determine if the isolated bacteriophages can be commercially produced in chicken egg embryos and if they can be effectively purified and concentrated. Production in EG eggs was proven to be standard and easy (Ahmadi et al., 2016). Filtration is a solid method for the removal of egg debris micro-particulate materials derived from the eggs themselves. As a solid method, this approach is unique and not currently investigated in the field of bacteriophage production.

The second major objective was to determine if purified and concentrated bacteriophages can be effectively inhibited bacterial colonization in post-hatch chicks and if they can be used as a feed additive for long-term bacterial inhibition. The baking process was the best method for bacteriophage preservation, allowing for more than 12 hours of chicken contact, which likely inactivated Salmonella. In addition, virgin white hens might act as bacteriophage reservoirs to reduce the colonization and shedding of Salmonella. Phage therapy targeting these serotypes along with bacterin vaccination live avian influenza vaccination and better bio-security plans could reduce the positive Salmonella. Moreover, construction of an anti-microRNA vector targeting the desired silencing region and investigation of its expression in chicken cells or embryos to generate a novel avian anti-microRNA will also be considered. Using multiple phages could help mitigate the problem of resistance with similar virulence phages.

8. Regulatory Considerations

The effectiveness and safety of biocontrol agents have become more acutely necessary with the proliferation of increasingly virulent Salmonella strains and increasing shopper demand for ready-to-eat poultry products free of chemical residues. Consequently, there is a corporate push to speed up the development and commercialization of bacteriophage cocktail formulations targeting salmonella. Even uncharacterized phage populations of significant efficacy against enterica salmonellae and applicable against poultry industry strains are commercially accessible and widely used (Li et al., 2020).

At the same time, safety goes hand in hand with effectiveness for all bacteriophage applications in poultry processing. The potential risks associated with using prophages to prepare new bacteriophage formulations against pathogens in poultry applications must be addressed. Bacteriophage formulation preparation that complies with regulatory agencies, as alternative players in pathogen reduction, is intensely contested. There is no guidance for non-SPC dossiers that apply bacteriophage formulations to the poultry industry directly for regulatory agencies outside of the EU, whereas, for some countries, bacteriophage formulations for veterinary use are outside the scope of food safety assessment agencies. However, there are existing ways to sidestep such guidance in drafting dossiers, pointing to disparate responses to the same development question (Ahmadi et al., 2016).

Ecophysiological model predictions indicate that wastewater-derived bacteriophage populations can reshape host cell-use phenotypes in a restricted host population. This prediction suggests potential risks associated with regulated applications that liberate predator populations in the poultry environment. Here, examples of the use, safety, and regulation of bacteriophage formulations targeting Salmonella in the poultry industry will be discussed, focusing on characterizations required for regulatory dossiers and on unanswered questions still needing to be addressed. Most importantly, recent findings demonstrating that low-cost, simple-to-manufacture, and pharmaceutical-grade bacteriophage application formulations can be prepared to perform both removal and prevention applications against enterica salmonellae which pose significant chicken meat contamination challenges and can be administered to birds on changing poultry farms to create phage-shedding birds that temporarily decrease their environmental load.

8.1. Current Regulations on Bacteriophage Use

The poultry industry constitutes a significant portion of global animal food production. In addition to its importance as a meat source, the poultry industry has seen an increase in egg production because of its nutritional and market advantages. However, Salmonella contamination of eggs poses a serious threat to public health. Most Salmonella infections are acquired from contaminated food, water, or environments, and poultry products are the main sources. Prophylactic measures have been taken to eradicate Salmonella in poultry farms around the world, and some countries have made practical efforts after introducing regulations. Other countries have considered poultry-related foodborne illnesses, mainly Salmonella, as an emerging infectious disease and have begun to establish relevant regulations. This article summarizes the recently introduced Salmonella-related regulations in the poultry industries of Japan and Taiwan.

In response to an increasingly serious issue concerning food safety, Japan's poultry industry began an official Salmonella control program in 1992 (Abu Sayem Khan & Rezwana Rahman, 2022). Although intended for chicken meat, the 5 Salmonella serotypes targeted in the program were associated with egg contamination. These included S. Enteritidis and S. Typhimurium, the most seriously affected serotypes for egg contamination. Additionally, annual Salmonella serotyping data have been collected in Japan since 1998. These data indicate the number of isolates among more than 130 serotypes. S. Typhimurium and its monophasic variants, S. Infantis, and S. Enteritidis have been identified as the most significant serotypes. Similar to Japan, salmonellosis has become a critical issue worldwide, with egg-associated outbreaks being common. In Taiwan, a Salmonella control program was introduced for chicken egg production. Newly established regulations require farms to be certified to declare themselves as Salmonella-free. Farm-associated Salmonella cases could then be disclosed to the public. The poultry industry also has to establish a representative organization to guide and provide assistance to egg production farms.

Despite the difficulty of a successful long-term monitoring and eradication program, it is hoped that the Salmonella monitoring agents of both programs will be adopted by the other countries as prevention and control measures for epidemic Salmonella. Efforts are underway to improve the food safety of poultry products. Continued monitoring of specific periods and implementation of better whole-egg pasteurization practices are also recommended. Strategies could potentially benefit countries that actively consider egg-related food safety issues.

8.2. Safety and Efficacy Assessments

Conventional bacteriophage preparations based on lytic phages derived from domains devoid of salmonella have often demonstrated efficacy in recent studies. These domains include hatcheries and highly biosecure poultry facilities, from which bacteriophage-secreting lysogenic salmonella producers were found. This bacterium requires specialized culture media that contain either subcultured salmonella or animal derivatives, which are associated with biosecurity issues. One solution to this biosecurity dilemma is the co-cultivation of salmonella-producing phages with salmonella prior to hatching, which enhances sigma phage efficacy. However, such approach involves the risk of salmonella infestations in the hatchery, thus reducing the efficacy of sigma phages. Alternatively, an antibacterial preparation could be made from prepared broth medium with media cultured salmonella, thereby generating bacteriophages derived from

the instruments and materials of pathological laboratories and breeding cooperatives containing salmonella. Since salmonella in the hatchery is thought to migrate from the surrounding poultry farm, uninfected birds providing breeding materials could serve as potential sources for scavenging, isolation and screening antiviral components. In a vast number of studies, short filamentous phages demonstrating a high lytic activity have been isolated from chickens, while their application effectively eliminates salmonella. The first bacteriophage preparation with a patent encompassing the bacteriophages was produced and marketed to use against salmonella in poultry husbandry. Significant data on its efficacy have been collected in layers and broiler chickens. Treatment is efficacious even with sub-optimal infection doses, while without it there is a high level of salmonella in the ceca. Complete elimination of salmonella can be expected utilizing it with effective doses after the onset of the infection and within 24 hours of the infection. Adjusted to the infection, the preparation greatly enhances the efficacy of treatments and through modifying the gut population acutely influences the salmonella virulence. Such a preparation is used in protective programs in various countries, demonstrating efficacy against salmonella after incorporation into feeds for 7 days of broiler chickens. The bacteriophage preparation against the pathogen agent was comprehensively examined in terms of safety and efficacy. Significant progress has been made in collecting information regarding phage safety, while their efficacy in microbial inhibition and elimination of pathogenic microorganisms remains mostly overlooked. All the examined bio-safety endpoint indicators in the safety assessment study demonstrated that two-week continuous oral exposure to large doses in outbred female Fischer rats to 20 times the amount proffered to dogs neither caused any changes in the mean body mass. Acute toxicity testing revealed that the phage cocktail did not decrease the activity of GIT enzymes and significantly affected neither plasma nor ceacal biochemical parameters. No food intake change nor subjective stereotaxic behaviour were observed on exposure, reflecting that it was well-tolerated by Fischer rats. Furthermore, the targeted bacteria was not isolated or identified, confirming the efficacy of phages against the targeted bacteria.

9. Economic Impact

The increasing resistance of Salmonella strains to the most used antibiotics in broiler farms is considered an important problem leading to high economic losses to the poultry industry. This study aimed to examine whether two Salmonella-specific bacteriophages could be used as an alternative to most common antibiotics used in the poultry industry for Salmonella gallinarum bacterial infection of broiler chicks using commercially available medicated feed containing vaccine-like antibiotic AMX, followed by feed containing bacteriophage 2S2. After 2 weeks of treatment of either the antibiotics or the bacteriophage, the chicks were challenged by oral administration of the virulent Salmonella gallinarum strain S07 group B broth culture. The chicks suffered no clinical signs of fowl typhoid and there were no mortality in both groups.

Salmonella gallinarum was isolated from the drinking water and liver and spleen samples of dead birds. Identification was confirmed by PCR. Phage study with drinking water started in both the flock and the feed type-b feeding factory. The flock successfully passed the test by isolating no Salmonella gallinarum in carcass swabs. One week after slaughter, carcass and carcass neck skin samples were obtained from the processing plant. The samples were again tested by PCR and isolated Salmonella gallinarum strains were serotyped by agglutination with O antisera. The carcasses and skin samples submitted had no Salmonella gallinarum detection using both techniques.

The poultry industry plays major role in food production. Brucellosis and salmonellosis are becoming more important as poultry are raised in a more integrated industrial fashion keeping large numbers of birds in concentrated conditions. Salmonella infection can cause reproductive problems, increased mortality, condemnation of carcasses, and reduced egg production causing immense losses to the poultry industry (M. Nabil et al., 2018). Phytogenic feed additives are classically understood as phytochemistry containing blends from Labiatae and other plants, e.g., thyme, oregano, or herbal oils, herbal extracts in addition to prebiotics and tannins etc. Hence, novel bacteriophage treatments, as shown in this study, show great promise for the treatment of bacterial infections in the poultry industry.

9.1. Cost Analysis of Bacteriophage Applications

Using bacteriophages to control Salmonella contamination in poultry provides an economical use of specific phage formulations to enhance feed and water safety against zoonotic pathogens. Using bacteriophages reduces economic loss because of animal health improvement and reduced contamination of animal products. Studies in which phage as a decontaminant intervention approach were cost beneficial and more effective than other candidates. Further studies need to focus on implementing cost-benefit analysis of bacteriophage-based products in farm systems.

Cost benefit analyses (CBA) can elucidate the economic justification of a product against its competitors. There are different CBAs with distinct structural frameworks applied for a diverse range of

products/services. The first commercialized bacteriophage preparation was applied to the poultry industry. In a bookmark fashion, poultry products were spayed by this preparation applied as anti-Salmonella in hatcheries, processing plants or in the field for decontaminating. This approach led to significant rise in sales and net incomes for some years. However, applications of bacteriophage are rarely been reported in the field in comparison with other food safety interventions. There may be various reasons but information gaps on cost analysis or economic cuts for systemic application and return on investments for daily use.

The poultry industry is the world's largest meat sector and in the top two of meat exporting industries. Most poultry products and their egg products are processed. The poultry industry is one of the highest profit sectors, but estimates business loss because of animal health problems and food safety issues is noticeable. Feeds are the main entry ways of bacteria to animal production systems. As natural, ubiquitous and host-specific predators of bacteria, bacteriophages can alter the microbial communities in feed and water and provide specific, safe and effective approaches to limit pathogenic bacteria in poultry production systems (Ahmadi et al., 2016). To eliminate pathogens in feed and water is economically feasible as it promotes animal health, reduces zoonotic foodborne pathogens' contamination of food products, prevents losses due to infectious diseases and improves sales.

9.2. Potential Market Growth

Utilization of bacteriophages to control S. enterica infections is an attractive alternative to antibiotic treatment for the poultry industry. The widespread occurrence of S. enterica in poultry, and in particular S. enteritidis, has been reported worldwide. This enterobacterial pathogen is a primary cause of food-borne infection and outbreaks. The poultry industry has been striving to reduce the prevalence of S. enterica in the entire plan from breeding farms to hatchery, broiler farms, depot markets, slaughterhouses, and processing packages. As success in reducing S. enterica colonization in poultry depends on the proper timing of treatment initiation, bacteriophages are considered a treatment option to control established poultry-associated S. enterica infections. Phage therapy has been widely investigated to control S. enteritidis infections in poultry and is considered a promising alternative to antibiotics in the poultry industry.

Addressing bacteriophage stability is relevant to developing improved biocontrol solutions for poultry sanitation. Biofilms formed by S. enteritidis in polyvinyl chloride tubing were effectively eliminated by a four-pronged approach using a combination of cleaning agents, sanitizers, and bacteriophage treatment. Bacteriophage treatment was most effective when it followed sanitation with sanisprays, as some bacteriophages actually benefitted from the cleaning agents and survived better with sanitizer residues. Although the harnessing of phage therapy in poultry farms is likely to provide significant economic benefits to the industries, safety and regulatory concerns can one day be addressed with respect to virulence and antibiotic-resistant genes carried by phage genomes. Future efforts will test multi-phage cocktails that can target various bacterial strains to reduce the likelihood of resistance development by a bacterial strain. In addition, the impact of different delivery forms of phage applications on bacteriophage stability in the environment and epidemiology of bacteriophage-resistant S. enteritidis strains is being studied.

Feeding live non-infectious bacteriophages as a Preparation As A powder (PAP) supplemented to the diet of live poultry has been applied in the industry as a prophylactic practice to enhance gut health for poultry production and Food Safety. Commercial bacteriophage-based proteins targeting gram-negative enteric bacteria are verified and marketed for feed use. Although bacteriophage-based feed additives are regarded as one of the eco-friendly alternatives to antibiotics in the poultry industry, it is necessary to observe their regulations before application (Al-Razem et al., 2022).

10. Consumer Perspectives

The poultry industry is an integral component of the global meat supply chain and is one of the fastest-growing segments of livestock agriculture amid rising global meat expenditures. Over the past several decades, the intensification of poultry production has caused significant concerns for biosecurity, food safety, animal welfare, and long-term sustainability. Furthermore, examining the factors influencing consumer trust is crucial for policymakers and industry stakeholders seeking to create more transparent food systems (Ahmadi et al., 2016). These factors often have trade-offs in terms of improving the perceived trustworthiness of food systems, which can help identify potential avenues for improving consumer understanding and trust in poultry production. Retailers and food processors often test poultry meat for Salmonella at their processing plants and premarital food preparation facilities in the U.S. poultry supply chain, at which point positive samples can trigger expensive and damaging product recalls (Abu Sayem Khan & Rezwana Rahman, 2022).

Phage Application at the Farm. The use of phages at the farm could prove to be an effective means of

reducing Salmonella in poultry flocks. Direct phage application to young broiler chicks decreased jejunal Salmonella counts following experimental infection, although the absence of untreated controls and uninfected feed supply chickens limited these conclusions. Further work to investigate the time course of protease effects on phage efficacy, the impact of phage dosage, and whether farm construction can be achieved with phase delivery outside of bird treatment will be valuable. Breath aerosol technology is another means of applying phages to flocks. Generally, this technology is commonly used to administer vaccines and might be improved in terms of penetration of static and dry litter environments. Tests with bacteriophages or phage lysins prior to thermal shocking processing or a new process for dry salted or brine cured products would be useful. Outside of phage application treatments, examining routine processing steps that could damage Salmonella-bacteria viability must be considered.

10.1. Acceptance of Bacteriophage Treatments

Bacteria and viruses are infectious agents that are ubiquitous in nature. They play an influential role in the ecology of environments through interactions spanning mutualism to pathogenicity (Al-Razem et al., 2022). A bacterium-infecting virus called a bacteriophage modifies the host's properties to its advantage, permitting replation of the phage(s) while severely damaging and/or killing the host. The rapid development of phage populations in response to high bacterial density stimulates cyclic fluctuations, increasing its own mortality rate in return. Though such fluctuations could be pronounced in its natural, un-biased ecosystems, highly controlled agricultural biomes for livestock can sometimes yield a different picture.

Since the beginning of poultry production, the necessity for health management resulted in the increase use of antibiotics. Early use of medications comprised the excellent pharmaceutical endeavours in the early twentieth century. However, ceaseless administration of antimicrobials in prophylactic, sub-therapeutic doses raised signs of worrying inefficacy over the years. Greater effects on poultry and a global problem of multidrug resistance represented by the poultry sector are the serious concerns. Given the situation, the pioneering approach to animals in the aviation industry became both politically and socially favoured, resulting in regulations limiting the use of antibiotics being brought to practice. Demand for supportive vaccination as alternatives delayed its application at a major scale, due to the prohibitive cost of these sustainable measures in many developing countries. However, the case has now become increasingly compelling in the poultry sector to avoid using antibiotics, as it was in the case of humans and other animal sectors. Thus, biocontrol of pathogens using bacteriophages emerged as an attractive candidate.

Bacteriophages are viruses that infect bacteria and represent a legion of more infections agents on Earth than measurable elements (Ahmadi et al., 2016). Moreover, co-evolution between bacteria and bacteriophages is the most profound in nature, and thus while lethal, it is also the most precise in targeting bacterial precursors. With respect to the above comparison, remarkable advances have come with regards to application of bacteriophage as a dietary supplement for reducing the level of pathogens in food animals. Recent studies indicated that some selected candidate bacteriophages can survive through the stomach and can infect enteric pathogenic bacteria in the gastrointestinal tract. These various bacteriophages with lytic capability can reduce Salmonella colonization in broiler chickens upon their prophylactic/therapeutic administration and subsequently control food-borne salmonellosis. In addition to this chicken model, a mouse model study indicated that these bacteriophage preparations can modify the gut flora and exert an antibacterial effect against S. Typhimurium.

10.2. Public Health Concerns

Even though bacteriophage as a biocontrol method for Salmonella in the poultry industry has shown very promising results, there are major public health concerns regarding their use that need to be addressed. Bacteriophage preparation using cells that express virulence-associated proteins might encounter problems because existing regulations for the use of internationally traded meat, poultry, and dairy products might not permit the preparation of bacteriophages from organisms that are Q-classified (Abu Sayem Khan & Rezwana Rahman, 2022). In previous studies on application of bacteriophage treatment under laboratory conditions, the bacteriophages applied and tested were often prepared from bacteria isolated from poultry flocks dominated by virulent strains of Salmonella. Salmonella phage preparations with similar contaminate levels might need to be avoided for screening, because they might contain bacteria that contribute to carriage of antibiotic resistance determinants and virulence-associated determinants.

An overall concern regarding bacteriophage preparations is the possible mutation of the bacteriophages resulting in production of mutants that are not effective against their host bacteria anymore but are still able to continue propagating. Past experience with antibiotic resistance in Salmonella and E. coli indicates that this is a legitimate concern, especially towards the long-term use of any phage-based preparations. To

address concerns about the safety of biocontrol agents, Bacteriophage preparations need to be assessed for all major pathogens, with regard to potential impact on poultry health and/or production. Studies need to focus on animal welfare and also address concerns with the use of bacteria derived from animals or humans that may contain virulence-associated genes, especially moving towards human applications. There may also be concerns on the use of a solution containing the large amounts of calcium required to obtain an effective amount of phage and whether this would affect digestion and consequently bacterial carriage in the intestines.

Overall, bacteriophage treatment of Salmonella in the poultry industry has been investigated at laboratory and pilot levels, with promising results. However, they should not be considered easy substitutes for current measures. Scientific knowledge and expertise must be expanded before routine applications can be considered on an industrial scale, and this will require combined multidisciplinary efforts in microbiology, food science, animal welfare, and veterinary public health. The first trials on the bacteriophage treatment of Salmonella in meat processing plants have already taken place commercially, while evaluation of consumer acceptability is also needed in parallel.

11. Conclusion

Salmonella spp. is the second major cause of foodborne human diseases and a serotype group frequently associated with poultry. Vaccination in the flock helps to reduce the slaughterhouse contamination, and post-hatching treatment with antibiotics is sometimes used to control early colonization of the flocks. As antibiotic residuals are an increasing concern, alternative approaches to control or cure infections in poultry are pursued. Prophylactic spray or drinking water treatment of one of two Salmonella-specific lytic phages on day of hatch significantly reduced the colonization by S. enteritidis in quail. When bacteriophages are pooled for administration, testing for efficacy prior to flock treatment becomes more difficult. Therefore, further testing was conducted with phage e8, one of the most efficacious U.S.-isolated phages (Ahmadi et al., 2016) to develop a dry powder preparation for oral administration in drinking water or for resuspension in oil droplets. With the wider use of phage products or mixtures, testing for efficacity in farm or veterinary applications will be necessary (through challenge studies), which may depend on which of the candidate isolates to be administered. Production and characterization of more type-specific and highly stable bacteriophages are needed for comparative studies to strengthen current knowledge of lytic bacteriophages against Salmonella.

Poultry diseases associated with bacterial infections are worldwide concerns resulting in significant economic loss. Salmonella infections affect poultry worldwide and cause serious economic loss either from mortality, impaired egg production, and increased feed conversion rate, or from carcass condemnation at processing plants due to contamination of carcasses with Salmonella microbes (Al-Razem et al., 2022). Management of Salmonella infections in poultry is often very difficult and, thus, extensive use of antibiotics is common. The extensive use of antibiotics has contributed to the emergence of multidrug resistance in several Salmonella strains. Most of the Salmonella strains isolated from poultry and poultry products are resistant to at least 1 antibiotic and some even resistant to up to 10 different antibiotics. Phage therapy is an alternative to antibiotics aimed at treating bacterial infections in humans and animals (and can be applied in various areas including the poultry industry). Phage therapy aims to destroy pathogenic bacteria by using lytic bacteriophages which exclusively infect the pathogenic bacteria while the non-pathogenic flora would not be affected. Considering that the isolated bacteriophages were tested against Salmonella serotypes other than the original host Salmonella serotype, it could be concluded that the isolated phages can potentially be used in phage therapy against Salmonella infection in the poultry industry.

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