Research Article

Effects of Bacteriophage Cocktail to Prevent *Salmonella enteritidis* in Native Broiler Chickens

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Abstract | The study aimed to evaluate the effects of a bacteriophage cocktail (BC) against *Salmonella enteritidis* (SE) in native broiler chickens. Totally, 320 native chicks were divided into two experiments using a completely randomized design consisting of 4 treatments and 4 replicates of 10 chicks per experiment. In both experiments, BC was used at a dose of 10^9 PFU/mL, and SE was used at $10^{8.2}$ CFU/mL. In the protective experiment, 1-day-old chicks were arranged in CD) control diet; BC+S) CD + 1 mL BC (gavaged at days 1, 2, 3, 4, 5) + 1 mL SE (gavaged at day 3); S) CD + 1 mL SE (gavaged at day 3); S+A) CD + 1 mL SE (gavaged at day 3) + gentamicin at 1 g/4 L water (gavaged at days 1, 2, 3, 4, 5). In therapeutic experiment, 2-days-old chicks were arranged in CD; BC+S) CD + 1 mL BC (gavaged at days 2, 3, 6, 8, 10, 13, 15) + 1 mL SE (gavaged at day 2); S) CD + 1 mL SE (gavaged at day 2); S+A) CD + 1 mL SE (gavaged at day 2) + gentamicin at 1g/4L water (gavaged at days 2, 3, 6, 8, 10, 13, 15). The results showed that the mortality rate and the number of bacteria in the internal organs of chicks were dramatically decreased in BC+S group compared to S group in both treatments. Moreover, supplementing BC tended to improve the weight gain of chicks.

Keywords | Bacteriophage, Mortality, Native chicken, Protection, Treatment efficiency

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INTRODUCTION

In Vietnam, poultry production has a significant role in the economy of small households (a typical livestock model of Vietnam), constituting approximately 19% of total household income (Desvaux et al., 2008). In recent years, the poultry industry has become increasingly important to small households across the whole country due to the adverse effects of the emerging infectious pandemics in larger livestock, such as African swine fever in pigs and lumpy skin disease in cattle (Nguyen-Thi et al., 2021; Tran et al., 2021; Trinh et al., 2022; Hien et al., 2022; 2023). Besides the advantages, numerous challenges exist in the poultry industry, including product consumption, livestock quality improvement, feed source, and diseases (Desvaux et al., 2008). Therefore, improving the quality of the poultry industry and reducing infection is paramount in Vietnam. Salmonellosis has been a worldwide pandemic not only in the poultry industry but also in public health (Berchieri et al., 2001; Ferrari et al., 2019; Ruvalcaba-Gómez et al., 2022). Poultry products have been considered as top food-borne sources of *Salmonella* spp. When poultry products contaminated with *Salmonella* spp. have been exported, pathogens have also been unintentionally spread worldwide (Torres-Acosta et al., 2019). Indeed, 118/326 (36.2%) fresh chicken meat samples collected from the wet markets

in upper northeastern Thailand were contaminated with *Salmonella* spp. (Vidayanti et al., 2021). Noticeably, the multidrug resistance of *Salmonella* spp. has been highlighted in several popular antibiotics in humans, and it could be linked to multidrug resistance in public health (Chotinan and Tadee, 2015; Vidayanti et al., 2021). In Vietnam, *Salmonella* has been reported as zoonotic foodborne bacteria related to the poultry industry. Unfortunately, the multidrug resistance of *Salmonella* spp. has emerged in nearby decades, directly affecting customer health (Nga et al., 2019), thus necessitating *Salmonella* control in the poultry industry of Vietnam.

Several strategies have been suggested to reduce *Salmonella* in the poultry industry, such as using antibiotics for treatment or administering probiotics and prebiotics to enhance intestinal immunity, and/or using bacteriophages to be specifically controlled (Nam et al., 2022; Ngu et al., 2022). Amongst them, bacteriophages have been considered as a promising alternative to antibiotics in the poultry industry (Lim et al., 2011; Kim et al., 2013; Noor et al., 2020; Ngu et al., 2022), resulting in reducing of *Salmonella* load in food products. The effectiveness and safety of bacteriophage therapy in comparison to antibiotics have been partially due to the specificity of bacteriophages for particular bacteria, manifested as the ability to infect only one species, serotype, or strain. Besides, bacteriophage could not bind and replicate in eukaryotic cells which may correlate with the reduction of pathogenic agents in the host. Moreover, bacteriophage has been known as a non-toxic agent mainly composed of proteins and nucleic acids (Loc-Carrillo and Abedon, 2011). Due to the promising benefits of bacteriophage in controlling bacteria, the current study aimed to investigate the protective and therapeutic effects of bacteriophage cocktail (BC) isolated from local broiler households to prevent *Salmonella enteritidis* (*S. enteritidis)* in native broiler chickens of Vietnam. The results of this study illustrated the role of BC in preventing *S. enteritidis* in native broiler chickens and may contribute to reducing the effect of *Salmonella* in the poultry industry of Vietnam.

MATERIALS AND METHODS

Ethical approval

This study was accorded to the Animal Husbandry Law of Vietnam (32/2018/QH14). The experimental procedure followed the guideline of the approved version by the Council for Science and Education, College of Agriculture (A10-02-2019/KNN), Can Tho University.

Bacteriophage isolation and bacteria preparation

Three bacteriophages (G3D03, L1R06, and L1N01) were isolated and screened from chicken feces, chicken intestines; soil, and water nearby local broiler households in Tra Vinh province of Vietnam. The samples were cultured in Tryptic Soy Broth (TSB) (Merck, Germany) at 37°C, 150 rpm for 24 hours. Following, 2 mL of the samples were centrifuged at 12,000 rpm for 10 minutes and the supernatants were transferred into the Eppendorf including chloroform at 4°C for 10 minutes. The bacteriophages were collected after centrifuging at 6,000 rpm for 5 minutes. After collection, the bacteriophages were diluted into a concentration of 10^{-5} PFU/mL (Ngu et al., 2022).

In the current study, a commercial product of *S. enteritidis* (ATCC® 49223™) was cultured in Tryptone Soya Agar (TSA) (Merck, Germany) for 24 hours and used at a concentration of 10^8 CFU/mL. A total of 0.1 mL diluted bacteriophages were mixed with fresh log-phase *S. enteritidis* and cultured on 0.6% agar in TSB, incubated overnight at 37°C. The bacteriophages were collected from a clear plaque and were diluted into a concentration of 10^{-5} PFU/mL. The previous steps were replicated until a plaque was homogenous in shape and size (Poxleitner et al., 2017).

The pure bacteriophages were incubated with fresh supernatant *S. enteritidis* in TSB at 37°C for 24 hours. Following that, 0.1 mL of chloroform was added, vortexed, and incubated for 2 hours. After incubation, the samples were centrifuged at 4°C, 6,000 rpm for 15 minutes. Next, 0.1 mL of the supernatant was collected into the Eppendorf, tested for pH tolerance (Verma et al., 2009), and stored at 4°C until used (Phuong et al., 2022). The BC was chosen according to the host spectrum, pH tolerance, and capacity to lyse *S. enteritidis* of the previous experimental infection on hens.

Chick management and diet

The one-day-old native broiler chicks were utilized in this study. All the chicks were immunized against Marek disease (day 1st), Newcastle disease (days 1st and 14th), Fowlpox disease (days 7th), Gumboro (days 7th and 14th), and H5N1 (days 21st). A commercial feed without antibiotics was used as a basal diet and was equally provided for individual pens at 7.00 am and 2.00 pm over the experiment. The chemical compositions of a basal diet were presented in Table 1.

Lethal dose (LD) 50 determination

A total of 90 one-day-old native broilers were randomly divided into 5 treatments and 3 replicates of 6 chicks based on a completely randomized design. The treatment groups were as follows: i) chicks gavaged with *S. enteritidis* at a concentration of 10^5 CFU/mL (S.5), ii) chicks gavaged with *S. enteritidis* at a concentration of 10^6 CFU/mL (S.6), iii) chicks gavaged with *S. enteritidis* at a concentration of 10^7 CFU/mL (S.7), iv) chicks gavaged with *S. enteritidis* at a concentration of 10^8 CFU/mL (S.8), and v) chicks

gavaged with *S. enteritidis* at a concentration of 10^9 CFU/mL (S.9). During 5 days of the experiment, all chicks were carefully checked for health conditions. All dead chicks were immediately necropsied and harvested internal organs (heart, liver, spleen, cecum). The samples were then cultured and *S. enteritidis* was isolated in Xylose lysine deoxycholate (XLD) agar (Merck, Germany) to confirm. LD50 was calculated according to the formulae of Reed and Muench (1938).

Table 1: The chemical compositions of a basal diet for 1-21-day olds chicks in the current study.

Item	1-21-day olds period
Metabolisable energy (Kcal/kg) (min)	3,050
Crude protein (%) (min)	21
Crude fiber (%) (max)	5
Digestible lysine (%) (min)	1.2
Digestible methionine+cysteine (%) (min)	0.9
Calcium (%) (min-max)	0.7-1.4
Available phosphorus (%) (min-max)	0.5-1

Evaluation of the protective effect of BC to prevent *S. enteritidis* in native broiler chicks

A completely randomized design was performed on 160 one-day-old native broilers with 4 treatments and 4 replicates of 10 chicks. Dietary treatment groups were as follows: i) control diet (CD) (basal diet without any antibiotics), ii) CD + 1 mL BC at a concentration of 10^9 PFU/mL (oral administration at days 1, 2, 3, 4, 5) + 1 mL *S. enteritidis* at a concentration of LD50 (oral administration at day 3) (BC+S), iii) CD + 1 mL *S. enteritidis* at a concentration of LD50 (oral administration at day 3) (S), iv) CD + 1 mL *S. enteritidis* at a concentration of LD50 (oral administration at day 3) + gentamicin at a concentration of 1 g/4 L water (oral administration at days 1, 2, 3, 4, 5) (S+A). The body weight of chicks was recorded weekly for each replicate until the end of the experiment (day 21). Moreover, a total of 4 chicks (1 chick/replicate*4 replicates) through 4 treatments were randomly chosen for sacrifice on days 5, 11, and 21 of the experiment. After necropsy, the internal organs (heart, liver, spleen, cecum) of chicks were collected and weighed. The data were recorded to evaluate the protective effect of BC to prevent *S. enteritidis* in experiment chicks.

Evaluation of the therapeutical effect of a BC to prevent *S. enteritidis* in native broiler chicks

One hundred and sixty of two-day-old native broilers were randomly divided into 4 treatments and 4 replicates of 10 chicks according to a completely randomized design. Dietary treatment groups were as follows: i) CD, ii) CD + 1 mL BC at a concentration of 10^9 PFU/mL (oral administration at days 2, 3, 6, 8, 10, 13, 15) + 1 mL *S. enteritidis* at a concentration of LD50 (oral administration at day 2) (BC+S), iii) CD + 1 mL *S. enteritidis* at a concentration of LD50 (oral administration at day 2) (S), iv) CD + 1 mL *S. enteritidis* at a concentration of LD50 (oral administration at day 2) + gentamicin at a concentration of 1g/4L water (oral administration at days 2, 3, 6, 8, 10, 13, 15) (S+A). The body weight of chicks was recorded weekly for each replicate until the end of the experiment (days 21). Furthermore, a total of 4 chicks (1 chick/replicate*4 replicates) through 4 treatments were necropsied, collected, and weighed the internal organs (heart, liver, spleen, cecum) on days 5, 11, and 21 of the experiment.

Bacteria enumeration, re-isolation, and identification of *S. enteritidis*

In the present study, *S. enteritidis* was re-isolated from the collected organs of chicks through two experiments. A total of 384 samples were cultured in XLD agar (Merck, Germany). The enumeration of bacteria was based on the guideline of the Vietnamese national standards (8400-12:2011). On the other hand, 4 samples from two experiments were carefully selected from chicks with clear lesions, and then extracted DNA, amplification, and sequencing to confirm *S. enteritidis*. The primer pairs and temperature cycling followed the previous study (Chiu and Ou, 1996). The nucleotide sequences after sequencing by the Sanger method were aligned and constructed the overlapping sequences, using BioEdit version 7.2.5. The sequences were then compared to the previous references of *S. enteritidis* from GenBank (CP085816, CP098831, CP098829, CP077424, CP092329, CP092258, CP082523, CP085806, CP085817, CP077760) via MEGA software version 7.0.26.

Statistical analysis

The collected data of the current study were calculated by Microsoft Excel 2016 and statistically analyzed by using the ANOVA test in Minitab version 16.2.1 software (State College, PA, USA). A p-value lower than 0.05 was statistically significant among treatments.

RESULTS

Bacteriophage isolation

Overall, 39 samples including 25 chicken intestines, 6 soil, 5 water, and 3 chicken feces were selected from local broiler households in Tra Vinh province. The isolated results indicated that bacteriophage was prevalent in chicken intestines (48.0%), following chicken feces (33.3%), soil (33.3%), and lower in water (20.0%) (Fig. 1). The isolated bacteriophage was then screened against *S. enteritidis*, the results indicated that 16 bacteriophages were chosen from water, soil, and chicken intestines samples. On double-layer agar, the selected bacteriophage produced a homogeneous

Table 2: Evaluation of pH tolerance of bacteriophages against *S. enteritidis*.

No.	Sample	pH level					
		2	3	4	5	6	7
1	G3D03	+	+	+	+	+	+
2	G3P01	-	-	+	+	+	+
3	G3P03	-	-	+	+	+	+
4	L1N01	+	+	+	+	+	+
5	L1R01	-	-	-	-	-	+
6	L1R04	-	-	+	+	+	+
7	L1R06	+	+	+	+	+	+
8	L1R13	-	-	+	+	+	+
9	L2N01	-	-	+	+	+	+
10	L2N02	+	+	+	+	+	+
11	L2N04	-	-	-	-	-	+
12	L2R01	-	-	-	+	+	+
13	L2R02	-	-	-	-	-	+
14	L2R03	-	-	+	+	+	+
15	L2D02	-	-	-	-	-	+
16	L2D03	+	+	+	+	+	+
Bacteriophage (+)		5	5	11	12	12	16
Percentage (%)		31.3	31.3	68.8	75.0	75.0	100

Table 3: The mortality rate of chicks through the LD50 experiment.

Treatment	Number of chicks	Concentration (CFU/mL)	Dose (mL)	Death (chick)	Percentage (%)
S.5	18	10^5	1.0	0/18	0
S.6	18	10^6	1.0	0/18	0
S.7	18	10^7	1.0	0/18	0
S.8	18	10^8	1.0	8/18	44.4
S.9	18	10^9	1.0	14/18	77.7

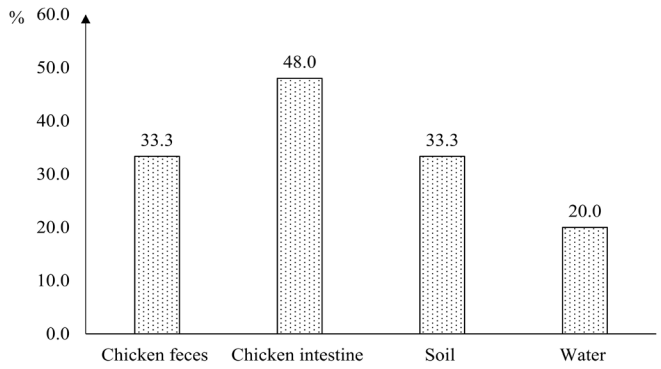

Figure 1: The isolated results of bacteriophages from broiler households in Tra Vinh province, Vietnam.

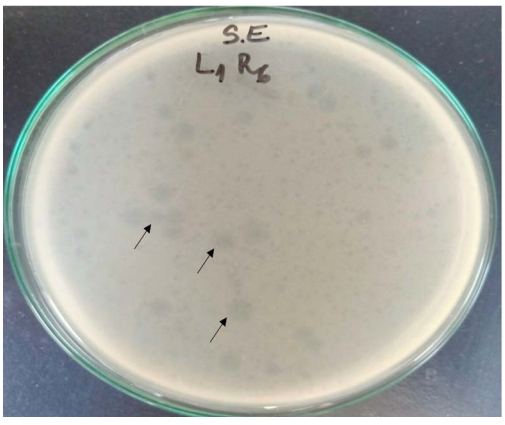

Figure 2: Plaques of bacteriophage cultured on *S. enteritidis* lawn. Clear transparent plaques of approximately 1-1.5 mm diameter were observed on a double-layer agar plate (black arrow).

transparent plaque over *S. enteritidis* lawn with a diameter of 1-1.5 mm (Fig. 2).

The isolated bacteriophages were evaluated for pH tolerance to select appropriate lineages against *S. enteritidis*. The results showed that 5/16 (31.3%) viability bacteriophages were found at pH = 2, 5/16 (31.3%) viability bacteriophag-

Table 4: The percentage of internal organ weights of chicks during 21 days in the protective experiment.

Day	CD	BC+S	S	S+A	SEM	p
Heart (%)						
5	0.69	0.52	0.70	0.51	0.05	0.07
11	0.91^{a}	0.59^{b}	1.06^{a}	0.54^{b}	0.05	0.01
21	0.53^{a}	0.50^{a}	0.40^{b}	0.57^{a}	0.01	0.01
Liver (%)						
5	4.19^{a}	3.54^{b}	3.16^{b}	3.55^{b}	0.14	0.01
11	2.45^{b}	2.98^{ab}	3.16^{ab}	3.75^{a}	0.20	0.01
21	2.25^{ab}	2.45^{a}	2.48^{a}	1.99^{b}	0.08	0.01
Spleen (%)						
5	0.10^{b}	0.18^{a}	0.10^{b}	0.16^{ab}	0.01	0.01
11	0.22^{a}	0.10^{b}	0.12^{b}	0.11^{b}	0.01	0.01
21	0.13^{ab}	0.07^{b}	0.13^{ab}	0.17^{a}	0.01	0.02
Cecum (%)						
5	0.67^{b}	0.84^{ab}	1.52^{a}	1.12^{ab}	0.19	0.04
11	0.71^{b}	0.87^{ab}	0.89^{ab}	1.37^{a}	0.13	0.02
21	0.60^{b}	0.64^{b}	1.32^{a}	0.56^{b}	0.05	0.01

Table 5: The percentage of internal organ weights of chicks during 21 days in the therapeutic experiment.

Day	CD	BC+S	S	S+A	SEM	p
Heart (%)						
5	0.68	0.50	0.75	0.59	0.06	0.08
11	0.57^{ab}	0.55^{ab}	0.52^{b}	0.62^{a}	0.01	0.01
21	0.53^{a}	0.50^{a}	0.40^{b}	0.57^{a}	0.01	0.01
Liver (%)						
5	3.44	3.04	2.66	3.30	0.29	0.31
11	2.95	2.73	3.16	3.75	0.30	0.15
21	2.67^{ab}	2.48^{b}	2.44^{b}	3.18^{a}	0.16	0.03
Spleen (%)						
5	0.13	0.17	0.13	0.17	0.02	0.41
11	0.13^{ab}	0.06^{b}	0.12^{ab}	0.18^{a}	0.02	0.04
21	0.16	0.10	0.15	0.17	0.02	0.35
Cecum (%)						
5	0.63^{b}	0.79^{ab}	1.52^{a}	1.12^{ab}	0.19	0.03
11	0.63^{b}	0.87^{ab}	0.89^{ab}	1.37^{a}	0.12	0.01
21	1.42^{ab}	1.47^{a}	0.88^{b}	0.85^{b}	0.13	0.01

Table 6: The similarity level of nucleotides (under the diagonal) and amino acid (above the diagonal) among *S. enteritidis* samples and the reference on GenBank.

Reference	1	2	3	4	5	6	7	8	9	10	11
1		99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8
2	99.3		100	100	100	100	100	100	100	100	100
3	99.3	100		100	100	100	100	100	100	100	100
4	99.3	100	100		100	100	100	100	100	100	100
5	99.3	100	100	100		100	100	100	100	100	100
6	99.3	100	100	100	100		100	100	100	100	100

7	99.3	100	100	100	100	100		100	100	100	100
8	99.3	100	100	100	100	100	100		100	100	100
9	99.3	100	100	100	100	100	100	100		100	100
10	99.3	100	100	100	100	100	100	100	100		100
11	99.3	100	100	100	100	100	100	100	100	100	

(1) *Salmonella*_020/CTU.TV; (2) CP085816; (3) CP098831; (4) CP098829; (5) CP077424; (6) CP092329; (7) CP092258; (8) CP085805; (9) CP082523; (10) CP085817; (11) CP077760

-es were observed at pH = 3, 11/16 (68.8%) viability bacteriophages were good at pH = 4, 12/16 (75.0%) bacteriophages were viability at pH = 5, 12/16 (75.0%) viability bacteriophages were recorded at pH = 6, and 16/16 (100%) viability bacteriophages were seen at pH = 7 (Table 2). Generally, three bacteriophages (G3D03, L1R06, and L1N01) were chosen via the pH tolerance results.

Identification of LD50 dose

All dead chicks were collected, and a necropsy was fully performed including the gross examination. Grossly, some white foci with pinhead size were found in the liver, the cecum was swollen (Fig. 3). After 5 days of the experiment, the LD50 dose was calculated as follows according to the results of Table 3.

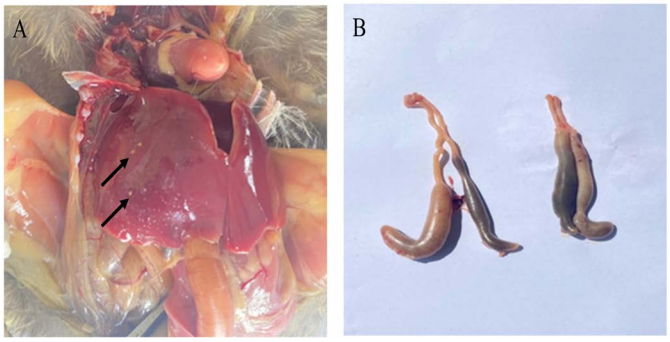

Figure 3: Macroscopic findings of liver and cecum of experimental chicks. The liver was white foci necrotic (black arrow) (**A**), and the cecum was swollen (**B**).

$LD50 = 10^{(9-0.8)} = 10^{8.2}$ (in which, PD = = 0.8)

Therefore, the LD50 dose of this experiment was $10^{8.2}$ CFU/mL. Furthermore, all samples from the internal organs of chicks (heart, liver, spleen, cecum) were harvested and cultured *S. enteritidis*. The cultured results showed that *S. enteritidis* was recorded from all collected samples.

The protective effect of BC on native broiler chicks against *S. enteritidis*

The mortality rate of chicks was documented during the time of the experiment. The results showed that the highest rate was in the S group (50.0%) and a significant difference ($p<0.05$) with other groups (BC+S, S+A). Meanwhile, there was no death chick in the CD group (Fig. 4A). The results demonstrated that no *S. enteritidis* was observed in

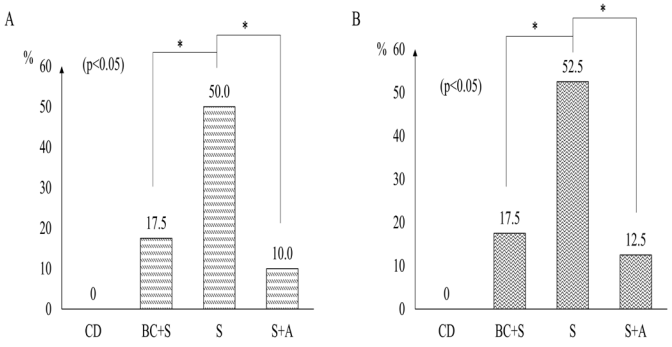

Figure 4: The mortality rate of chicks in the protective experiment (**A**), and therapeutic experiment (**B**).

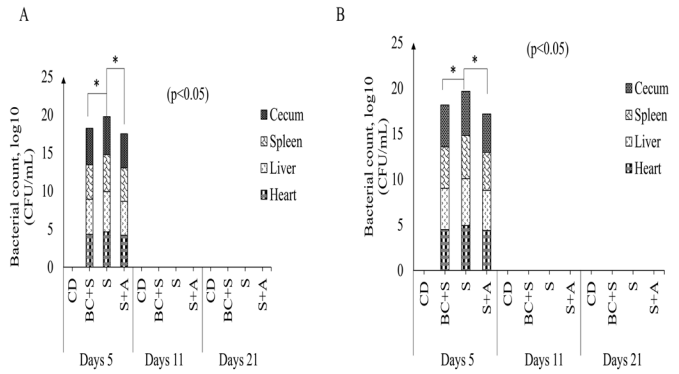

Figure 5: The number of *S. enteritidis* re-isolated from the internal organs of chicks on different days post-infection in the protective experiment (**A**), and therapeutic experiment (**B**).

the CD group while bacteria were found in all selected organs of other groups during day 5. There was a dramatic difference among the S group and BC+S, S+A groups ($p<0.05$). However, none were found between BC+S and S+A groups ($p>0.05$) (Fig. 5A). After the experimental period, the percentage of internal organ weights was recorded to be different among groups ($p<0.05$), which was highest in the S group, and lowest in the CD group. Meanwhile, none existed between BC+S and S+A groups ($p>0.05$) (Table 4). Furthermore, the weight gain of chicks (g/bird) was documented within 3 weeks of the experimental period. The results showed that there was a statistical difference between CD, and BC+S compared to the lowest group (S) ($p<0.01$) in the first week. It was the opposite in the next week ($p>0.05$). In the final week, differences emerged between the CD and S groups ($p<0.05$) (Fig. 6A).

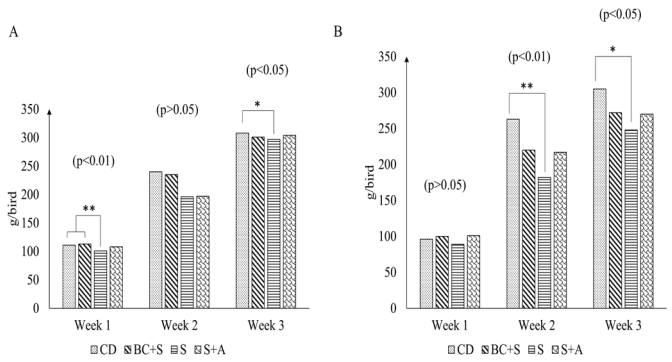

Figure 6: The weight gain of chicks in the protective experiment (**A**), and therapeutic experiment (**B**).

The therapeutic effect of BC on native broiler chicks against *S. enteritidis*

In this experiment, the mortality rate of chicks was not recorded in the CD group yet, it was dramatically increased in the S group (52.5%) in comparison with BC+S and S+A groups ($p<0.05$) (Fig. 4B). The number of *S. enteritidis* post-infection in the S group was of significant difference compared to the BC+S and S+A groups ($p<0.05$). No statistical differences were present among BC+S and S+A groups ($p>0.05$) (Fig. 5B), nor were there any between the CD, BC+S, and S+A groups in the percentage of internal organ weights ($p>0.05$). However, differences between the treatment groups (CD, BC+S, and S+A) and S group ($p<0.05$) were reported (Table 5). Meanwhile, the reverse was true for the weight gain results of chicks (g/bird) ($p>0.05$) in the first week of the experiment. However, chicks in the CD group significantly increased weight gain compared to those in the S group ($p<0.01$, $p<0.05$). Nevertheless, none prevailed among other groups ($p>0.05$) in weeks 2 and 3 (respectively) (Fig. 6B).

Identification of *S. enteritidis*

After sequencing, the harvested sequences were compared with the references of *S. enteritidis* on GenBank. The results demonstrated the high similarity level of nucleotides and amino acids between field bacteria samples and the references on GenBank. The similarity level of nucleotides among field bacteria samples and the strains from GenBank was 99.3-100%. The similarity level of amino acid of the field *S. enteritidis* samples was very high (99.8-100%) compared to the references on GenBank (Table 6).

DISCUSSION

Bacteriophage has been widely utilized as an alternative therapy to antibiotics in preventing *Salmonella* spp. in poultry worldwide (Atterbury et al., 2007; Lim et al., 2012; Duc et al., 2018; Upadhaya et al., 2021; Evran et al., 2022; Sarrami et al., 2022). However, the studies on the effect of bacteriophage against *Salmonella* spp. in the native chicken of Vietnam have been limited. Within the current study, three bacteriophages from local households were isolated and applied to prevent *S. enteritidis* in native broiler chicks (1-21 days old).

A general principle to screen bacteriophages is finding them in the host living environment. However, screening a bacteriophage to prevent a particular host could be a challenge due to a very host-specific characteristic of bacteriophage (Winfield and Groisman, 2003; Hyman, 2019). In the present study, three bacteriophages were isolated from various sources of both the host and the nearby environment of the host (mostly in the chicken intestines, chicken feces, soil, and water). In fact, possible bacteriophages against *Salmonella* spp. have easily been found in environmental reservoirs (Mattila et al., 2015). The bacteriophages were enriched, with the host tested, and pH tolerance evaluated (Watanabe et al., 2007) to determine appropriate lineages against *S. enteritidis*. Our results showed that the isolated bacteriophages were suitable for preventing *S. enteritidis* (Jurczak-Kurek et al., 2016). It is the initial basis for applying bacteriophages in further experiments of this study.

The mortality in the untreated chicks was the highest, whereas the treatment of bacteriophage administration reduced the death of chicks in the treated group. These findings were consistent with those previously reported, which indicated that the bacteriophage therapy significantly reduced the severity of signs, lesions, and mortalities of birds affected with *Escherichia coli* (*E. coli*) and *Salmonella* infections (Lau et al., 2010). The findings of this study demonstrated that bacteriophage treatment was efficacious in reducing *S. enteritidis* colonization in broiler chickens and could be used as an alternative to antibiotics (Nabil et al. 2018; Duc et al., 2018; Upadhaya et al., 2021; Evran et al., 2022).

In this study, BC was orally administered to chicks before oral bacterial infections, followed by 4 successive phage treatments after the bacterial challenge. Although *S. enteritidis* was still able to colonize the chicks, bacterial loads decreased after four successive phage treatments. After the 5th dose, no bacteria were detected, indicating that the chicks treated with bacteriophages were cured of *S. enteritidis*. This effect is most likely due to the lytic effect of the administered bacteriophages against *S. enteritidis*. Moreover, bacteriophages have previously been used to control intracellular pathogens (Ahn et al., 2015). Although the exact mechanism is understudied, it is reasonable to believe that bacteriophages could change the population of gut intestinal bacteria, which are necessary for the development of the gut and liver immune systems (Wang et al., 2013).

On the other hand, the percentage of liver weight was higher in the non-treated (S) group in the current study. Our results were similar to the publication of Kim et al. (2013) in that supplementing bacteriophage in the diet of chickens did not affect the relative weight of internal organs after 35 days. The size of the heart, liver, and spleen in *E. coli*-infected birds treated with bacteriophages was not different from that of the control groups. Also suggested in the study by Bao et al. (2011) was that phage therapy would not significantly affect organ mass relative to body mass.

Body weight is an important characteristic in broiler production due to the inverse correlation between body weight and broiler meat production costs. A clear tendency of improving the growth rate of the infected chicks treated with BC was identified in our study. The present findings corroborated the research outputs of a previous study (Toro et al., 2005) that *S. Typhimurium* phage treatment resulted in a favorable effect on weight gain. These authors also pointed out that chicks gained less weight following the challenge and maintained lower weights throughout the study, which was aligned with our results. In another study, Noor et al. (2020) concluded that antibiotics and bacteriophages did not body weight gain, feed intake, or FCR during the 15-32 days of commercial broilers. The phage-host interaction in the production traits of chickens could have been dependent on phage types and genotypes, which can be found in poultry and environmental isolates.

CONCLUSIONS

The present study illustrated the potential role of BC in native broiler chickens during the period of 1-21 days old, which decreased the mortality and the number of bacteria in the internal organs of chicks infected with *S. enteritidis*. Additionally, the weight gain of chicks tended to be facilitated, meanwhile, the percentage of internal organ weights was unaffected when administered bacteriophage cocktail. More elaborate studies should be carried out concerning other growth phases of chicken to highlight the anti-bacterial of bacteriophages in native broiler chickens, contributing to improving the quality of poultry production in Vietnam.

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CONFLICT OF INTEREST

The authors in this study declare no competing interests.

NOVELTY STATEMENT

Bacteriophage is suggested as a promising alternative to antibiotics in the poultry industry. Prior studies have shown that bacteriophage cocktails (BC) could prevent *Salmonella* spp. in chicken. The current study first illustrated the protective and therapeutic effects of BC isolated from the local broiler households in Tra Vinh province of Vietnam. The results revealed that BC showed a potential role in preventing *S. enteritidis* in native broiler chickens at 1-21 days old. The findings of this study may contribute to reducing the effect of *Salmonella* in the poultry industry of Vietnam.

AUTHORS CONTRIBUTIONS

NTAT, LTTL, NTL, and NTN designed the experiment. NTAT, CCD, NHP, and TDT managed the experiment, and collected and analyzed data. NTAT and NTN interpreted data and drafted the manuscript. NTAT, LTTL, NTL, and NTN edited the manuscript. All authors carefully read and approved the manuscript for publication.

REFERENCES

Ahn J., Lee H.Y., Biswas D (2015). Assessment of bacteriophage-induced inflammatory mediators in Salmonella-infected chicken macrophage HD11 cells. Poult. Sci. J., 52(3): 238-243. https://doi.org/10.2141/jpsa.0150025

Atterbury R.J., Van Bergen M.A.P., Ortiz F., Lovell M.A., Harris J.A., De Boer A., Wagenaar J.A., Allen V.M., Barrow P.A. (2007). Bacteriophage therapy to reduce Salmonella colonization of broiler chickens. Appl. Environ. Microbiol., 73(14): 4543-4549. https://doi.org/10.1128/AEM.00049-07

Bao H., Zhang H., Wang R. (2011). Isolation and characterization of bacteriophages of Salmonella enterica serovar Pullorum. Poult. Sci., 90(10): 2370-2377. https://doi.org/10.3382/ps.2011-01496.

Berchieri Jr, A., Murphy C.K., Marston K., Barrow P.A. (2001). Observations on the persistence and vertical transmission of Salmonella enterica serovars Pullorum and Gallinarum in chickens: effect of bacterial and host genetic background. Avian Pathol., 30(3): 221-231. https://doi.org/10.1080/03079450120054631

Chiu C.H., Ou J.T. (1996). Rapid identification of Salmonella serovars in feces by specific detection of virulence genes, invA and spvC, by an enrichment broth culture-multiplex PCR combination assay. J. Clin. Microbiol., 34(10): 2619-2622. https://doi.org/10.1128/jcm.34.10.2619-2622.1996

Chotinan S., Tadee P. (2015). Epidemiological survey of S. Enteritidis Pulsotypes from Salmonellosis outbreak in Chiang Mai and Samut Songkhram provinces, Thailand. Vet. Integr. Sci., 13(2): 73-80.

Desvaux S., Ton V.D., Phan Dang T., Hoa P.T.T. (2008). A general review and description of the poultry production in Vietnam. Agricultural Publishing House, Vietnam.

Duc H.M., Son H.M., Honjoh K.I., Miyamoto T. (2018).

Isolation and application of bacteriophages to reduce Salmonella contamination in raw chicken meat. Lwt., 91: 353-360. https://doi.org/10.1016/j.lwt.2018.01.072

Evran S., Tayyarcan E.K., Acar-Soykut E., Boyaci I.H. (2022). Applications of Bacteriophage Cocktails to Reduce Salmonella Contamination in Poultry Farms. Food. Environ. Virol., 1-9. https://doi.org/10.1007/s12560-021-09501-0

Ferrari R.G., Rosario D.K., Cunha-Neto A., Mano S.B., Figueiredo E.E., Conte-Junior C.A. (2019). Worldwide epidemiology of Salmonella serovars in animal-based foods: a meta-analysis. Appl. Environ. Microbiol., 85(14): e00591-19. https://doi.org/10.1128/AEM.00591-19

Hien N.D., Nguyen L.T., Hoang L.T., Bich N.N., Quyen T.M., Isoda N., Sakoda Y. (2022). First Report of a Complete Genome Sequence of a Variant African Swine Fever Virus in the Mekong Delta, Vietnam. Pathogens., 11(7): 797. https://doi.org/10.3390/pathogens11070797.

Hien N.D., Nguyen L.T., Isoda N., Sakoda Y., Stevenson M.A. (2022). Descriptive epidemiology and spatial analysis of African swine fever epidemics in Can Tho, Vietnam, 2019. Prev. Vet. Med., 2023; 211: 105819. https://doi.org/10.1016/j.prevetmed.2022.105819.

Hyman P. (2019). Phages for phage therapy: isolation, characterization, and host range breadth. Pharmaceuticals., 12(1): 35. https://doi.org/10.3390/ph12010035.

Jurczak-Kurek A., Gąsior T., Nejman-Faleńczyk B., Bloch S., Dydecka A., Topka G., Necel A., Jakubowska-Deredas M., Narajczyk M., Richert M., Mieszkowska A., Wróbel B., Węgrzyn G., Węgrzyn A. (2016). Biodiversity of bacteriophages: morphological and biological properties of a large group of phages isolated from urban sewage. Sci. Rep., 6(1): 1-17. https://doi.org/10.1038/srep34338

Kim K.H., Lee G.Y., Jang J.C., Kim J.E., Kim Y.Y. (2013). Evaluation of anti-SE bacteriophage as feed additives to prevent Salmonella enteritidis (SE) in broiler. Asian-Australas. J. Anim. Sci., 26(3): 386-393. https://doi.org/10.5713/ajas.2012.12138

Lau G.L., Sieo C.C., Tan W.S., Hair-Bejo M., Jalila A., Ho Y.W. (2010). Efficacy of a bacteriophage isolated from chickens as a therapeutic agent for colibacillosis in broiler chickens. Poult. Sci., 89(12): 2589-2596. https://doi.org/10.3382/ps.2010-00904

Lim T.H., Kim M.S., Lee D.H., Lee Y.N., Park J.K., Youn H.N., Lee J.H., Yang S.Y., Cho Y.W., Lee J.B., Park S.Y., Choi I.S., Song C.S. (2012). Use of bacteriophage for biological control of Salmonella Enteritidis infection in chicken. Res. Vet. Sci., 93(3): 1173-1178. https://doi.org/10.1016/j.rvsc.2012.06.004

Loc-Carrillo, C., Abedon, S.T. Pros and cons of phage therapy. Bacteriophage, 2011; 1(2): 111-114. https://doi.org/10.4161/bact.1.2.14590

Mattila S., Ruotsalainen P., Jalasvuori M. (2015). On-demand isolation of bacteriophages against drug-resistant bacteria for personalized phage therapy. Front. Microbiol., 6: 1271. https://doi.org/10.3389/fmicb.2015.01271.

Nabil N.M., Tawakol M.M., Hassan H.M. (2018). Assessing the impact of bacteriophages in the treatment of Salmonella in broiler chickens. Infect. Ecol. Epidemiol., 8(1): 1539056. https://doi.org/10.1080/20008686.2018.1539056.

Nam T.V.B., Anh L.H., Loc H.T., Trang C.T.H., Thiet N., Lan L.T.T., Diep T.H., Xuan N.H., Ngu N.T. (2022). Effects of probiotic (Lactobacillus plantarum and Bacillus subtilis) supplementation on mortality, growth performance, and carcass characteristics of native Vietnamese broilers challenged with Salmonella Typhimurium. Vet. World., 15 (9): 2302–2308. https://doi.org/10.14202/vetworld.2022.2302-2308

Nga V.T., Ngoc T.U., Minh L.B., Ngoc V.T.N., Pham V.H., Nghia L.L., Son N.L.H., Pham T.H.V., Bac N.D., Tien T.V., Tuan N.N.M., Tao Y., Show P.L., Chu D.T. (2019). Zoonotic diseases from birds to humans in Vietnam: possible diseases and their associated risk factors. Eur. J. Clin. Microbiol. Infect. Dis., 38: 1047-1058. https://doi.org/10.1007/s10096-019-03505-2

Ngu N.T., Phuong L.N.N., Anh L.H., Loc H.T., Tam N.T., Huan P.K.N., Diep T.H., Kamei K. (2022). The Efficiency of Bacteriophages Against Salmonella Typhimurium Infection in Native Noi Broilers. Braz. J. Poult. Sci., 24. https://doi.org/10.1590/1806-9061-2021-1561.

Nguyen-Thi T., Pham-Thi-Ngoc L., Nguyen-Ngoc Q., Dang-Xuan S., Lee H.S., Nguyen-Viet H., Padungtod P., Nguyen-Thu T., Nguyen-Thi T., Tran-Cong T., Rich K.M. (2021). An assessment of the economic impacts of the 2019 African swine fever outbreaks in Vietnam. Front. Vet. Sci., 8: 686038. https://doi.org/10.3389/fvets.2021.686038.

Noor M., Runa N., Husna A., Rahman M., Rajib D.M.M., Mahbub-e-Elahi A.T.M., Rahman M.M. (2020). Evaluation of the effect of dietary supplementation of bacteriophage on production performance and excreta microflora of commercial broiler and layer chickens in Bangladesh. MOJ proteom. Bioinform., 9(2): 27-31.

Phuong L.N.N., Anh L.H., Huan P.K.N., Loc H.T., Trang C.T.H., Mo T.T.H., Xuan N.H., Ngu N.T. (2022). The effect of different media and temperature conditions for *Salmonella* bacteriophage preservation. Vet. Integr. Sci., 20(2): 489-496.

Poxleitner M., Pope W., Jacobs-Sera D., Sivanathan V., Hatfull G. (2017). Phage Discovery Guide. Howard Hughes Medical Institute.

Reed L.J., Muench H. A (1938). simple method of estimating fifty per cent endpoints. Am. J. Epidemiol., 27(3): 493-497 https://doi.org/10.1093/oxfordjournals.aje.a118408.

Ruvalcaba-Gómez J.M., Villagrán Z., Valdez-Alarcón J.J., Martínez-Núñez M., Gomez-Godínez L.J., Ruesga-Gutiérrez E., Anaya-Esparza L.M., Arteaga-Garibay R.I., Villarruel-López A. (2022). Non-antibiotics strategies to control Salmonella infection in poultry. Animals,. 12(1): 102. https://doi.org/10.3390/ani12010102.

Sarrami Z., Sedghi M., Mohammadi I., Kim W.K., Mahdavi A.H. (2022). Effects of bacteriophage supplement on the growth performance, microbial population, and PGC-1α and TLR4 gene expressions of broiler chickens. Sci. Rep., 12(1): 14391. https://doi.org/10.1038/s41598-022-18663-1.

Toro H., Price S.B., McKee S., Hoerr F.J., Krehling J., Perdue M. (2005). Bauermeister, L. Use of bacteriophages in combination with competitive exclusion to reduce Salmonella from infected chickens. Avian Dis., 49(1): 118-124. https://doi.org/10.1637/7286-100404R

Torres-Acosta M.A., Clavijo V., Vaglio C., González-Barrios A.F., Vives-Flórez M.J., Rito-Palomares M. (2019). Economic evaluation of the development of a phage therapy product for the control of Salmonella in poultry. Biotechnol. Prog., 35(5): e2852. https://doi.org/10.1002/btpr.2852.

Tran H.T.T., Truong A.D., Dang A.K., Ly D.V., Nguyen C.T., Chu N.T., Hoang T.V., Nguyen H.T., Nguyen V.T., Dang H.V. (2021). Lumpy skin disease outbreaks in vietnam,

2020. Transbound. Emerg. Dis., 68(3): 977-980. https://doi.org/10.1111/tbed.14022.

Trinh T.B.N., Nguyen V.T., Nguyen T.T.H., Mai N.T.A., Le P.N., Lai T.N.H., Phan T.H., Tran D.H., Pham N.T., Dam P.V., Nguyen T.L., Ambagala A., Babiuk S., Le V.P. (2022). Molecular and histopathological characterization of lumpy skin disease in cattle in northern Vietnam during the 2020–2021 outbreaks. Arch. Virol., 167(11): 2143-2149. https://doi.org/10.1007/s00705-022-05533-4

Upadhaya S.D., Ahn J.M., Cho J.H., Kim J.Y., Kang D.K., Kim S.W., Kim H.B., Kim I.H. (2021). Bacteriophage cocktail supplementation improves growth performance, gut microbiome and production traits in broiler chickens. J. Anim. Sci. Biotechnol., 12(1): 1-12. https://doi.org/10.1186/s40104-021-00570-6

Verma V., Harjai K., Chhibber S. (2009). Characterization of a T7-like lytic bacteriophage of Klebsiella pneumoniae B5055: a potential therapeutic agent. Curr. Microbiol., 59(3): 274-281. https://doi.org/10.1007/s00284-009-9430-y.

Vidayanti I.N., Sukon P., Khaengair S., Pulsrikarn C., Angkittitrakul S. (2021). Prevalence and antimicrobial resistance of Salmonella spp. isolated from chicken meat in upper northeastern Thailand. Vet. Integr. Sci., 19(2): 121-131. https://doi.org/10.12982/VIS.2021.011

Wang J.P., Yan L., Lee J.H., Kim I.H. (2013). Evaluation of bacteriophage supplementation on growth performance, blood characteristics, relative organ weight, breast muscle characteristics and excreta microbial shedding in broilers. Asian-Australas. J. Anim. Sci., 26(4): 573-578. https://doi.org/10.5713/ajas.2012.12544

Watanabe R., Matsumoto T., Sano G., Ishii Y., Tateda K., Sumiyama Y., Uchiyama J., Sakurai S., Matsuzaki S., Imai S., Yamaguchi K. (2007). Efficacy of bacteriophage therapy against gut-derived sepsis caused by Pseudomonas aeruginosa in mice. Antimicrob. Agents Chemother., 51(2): 446-452. https://doi.org/10.1128/AAC.00635-06

Winfield M.D., Groisman E.A. (2003). Role of nonhost environments in the lifestyles of Salmonella and Escherichia coli. Appl. Environ. Microbiol., 69(7): 3687-3694. https://doi.org/10.1128/AEM.69.7.3687-3694.2003