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Virulent Genes and Genetic Relationship of *Salmonella* spp. Isolated from Chickens and Husbandry Environments in Small-Scale Farms in the Mekong Delta, Vietnam

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ABSTRACT

Salmonella is one of the most severe pathogens causing diseases in poultry and humans, and several factors could become transmission vectors in the husbandry environment. This study was conducted from April to July 2024 to clarify the prevalence of common *Salmonella* serovars in chickens and the husbandry environment and their pathogenicity and genetic relationship in small-scale farms in the Mekong Delta, Vietnam. A total of 279 samples were randomly collected from fresh chickens' feces (n = 54), husbandry environment (n=81), and pests (n=144), including rats, geckos, and ants, in four small-scale farms to examine the prevalence of *Salmonella* spp. By the conventional isolation method, 75 samples were positive for *Salmonella*, accounting for 26.88%. The prevalence of *Salmonella* in chicken feces, the environment, and pests were 27.78%, 12.35%, and 34.72%, respectively. Of 75 positive *Salmonella* isolates, two common serovars were identified, including *S. Gallinarum* (13.33%) and *S. Enteritidis* (10.67%); however, *S. Pullorum* and *S. Typhimurium* were not detected using PCR. These *Salmonella* isolates were detected virulent genes by using PCR, and found that these isolates harbored several virulent genes, including *InvA* (100%), *fimA* (100%), *stn* (93.33%), *sopB* (89.33%), and *sodC1* (54.57%). The ERIC-PCR method was used to determine the genetic relationship among *Salmonella* strains carrying virulent genes present in chickens, environment, and pests in these small-scale farms. The results showed diversity in phenotype and similarity in the genetic relationship (more than 75% similarity) among *Salmonella* strains isolated from chicken feces and the livestock environment. In conclusion, the study indicated that pathogenic *Salmonella* serovars could survive and be transmitted among sources, including chickens, the husbandry environment, and pests in small-scale poultry farms in the Mekong Delta.

Keywords: Chicken, Environment, Genetic relationship, Pest, *Salmonella*, Virulent gene

INTRODUCTION

Nowadays, the demand for meat and poultry products has promoted the development of the poultry industry (Henchion et al., 2021). *Salmonella* is one of the severe infectious pathogens causing diseases that directly affect the health of livestock and result in heavy economic losses to farmers (Cortés et al., 2022). *Salmonella* is present in the environment, including soil, water, food, barns, and livestock equipment, and can cross-contaminate from one source to another intermediate species (Tabo et al., 2013). The complicated epidemiology of *Salmonella* is due to horizontal and vertical transmission routes, and animals with weak resistance or immunodeficiency will be susceptible to salmonellosis (Gast and Porter, 2020). Examining pests (shrews, mice, rats, flies, ants, cockroaches, and birds) living around the broiler chicken farms on Reunion Island validated that they were resources of *Salmonella* spp. and infected chickens in these farms (Etheves et al., 2021). Nguyen et al. (2021) previously reported that chickens, the environment, and pests were reservoirs of *Salmonella* in poultry farms and households in the Mekong Delta.

Salmonella has more than 3,500 different serovars recorded in animals and the environment. Of which, the common pathogenic *Salmonella* strains in chickens can be divided into two groups, non-motile strains, including *S. Pullorum*, causing dysentery in chicks; *S. Gallinarum*, causing chicken typhoid; and motile strains, mainly including *S. Enteritidis* and *S. Typhimurium* causing paratyphoid in animals and humans (Al-baqir et al., 2019; Wales and Lawes, 2023). The pathogenicity of *Salmonella* strains depends on the presence of several virulent genes encoded in *Salmonella* pathogenicity islands (SPIs), plasmids, and other gene cassettes (Pavon et al., 2022). Several virulence genes have

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essential roles in adhesion, invasion, intracellular survival, systemic infection, and toxin production of *Salmonella* in hosts, including *InvA*, *fimA*, *stn*, *sopB*, and *sodC1* (Kim and Lee, 2017; Tarabees et al., 2017). In research of Siddiky et al. (2021), all *Salmonella* isolated from chickens in wet markets of Bangladesh underwent polymerase chain reaction (PCR) screening for eight virulence genes—*invA*, *agfA*, *IpfA*, *hilA*, *sivH*, *sefA*, *sopE*, and *spvC*; the results revealed that *S. Enteritidis* carried all the genes while *S. Typhimurium* contained six genes, lacking *sefA* and *spvC*.

In the Mekong Delta, chickens are mainly raised in small-scale farms or households; thus, hygiene is not taken seriously because the low income of farmers affects the investment for these farms. Besides, managing risk factors, such as pests and the husbandry environment, was also limited. The transmission route of *Salmonella* in these farms was not determined. Therefore, this study elucidates the prevalence of common *Salmonella* serovars in chickens, environments, and pests and examines their genetic relationships. The findings contribute to a better understanding of *Salmonella* epidemiology in small-scale poultry farms, thereby aiding in the prevention of salmonellosis in chickens and humans in the Mekong Delta, Vietnam.

MATERIALS AND METHODS

Ethical approval

This study was conducted following the guidelines outlined in the Helsinki Declaration and the animal welfare and safety procedures of Can Tho University, Vietnam.

Isolation of *Salmonella*

From April to July 2024, 279 samples were collected from hybrid broilers (Noi chicken, a local chicken breed). These cocks were one-month-old with an average weight of 700 g, and raised in four small-scale farms in the center of the Mekong Delta, Vietnam (two farms in Vinh Long province, one farm in Can Tho City, and one farm in Hau Giang province). The Mekong Delta is located in the southern region of Vietnam, covering latitudes from 8°34' to 11°10' N and longitudes from 104°25' to 106°48' E. This region encompasses a total land area of approximately 40,547 square kilometers, with around 1.7 million hectares designated for agricultural use. The area's air temperature typically ranges between 26.5 and 29.3°C, while the average annual precipitation fluctuates between 1,287.6 and 2,832.8 mm, with 80% of this rainfall occurring during the wet season (Vu-Thanh et al., 2014; Lee and Dang, 2019; Dinh and Dang, 2022).

These farms raised about 1,000 broilers/farms. The samples were collected, including fresh chicken feces (n = 54), bedding (n = 36), feed (n = 27), drinking water (n = 18), rats (n = 18), geckos (n = 54), and ants (n = 72) in these farms. In this study, the number of samples collected was based on the number of chickens, the farm design, and the captured pests in each farm when collecting samples. The average number of samples collected on each farm includes 13 chicken feces, 9 bedding samples, 6 drinking water samples, and 36 pests.

Chicken feces (about 1 g) were collected via cloacal swabbing and put into Cary-Blair medium (Merck, Germany), while environmental samples comprising 250 g of feed, 1,000 mL of drinking water, and 250 g of bedding were obtained directly from the farms, placed in sterilized bags, and stored at 2-8°C. Pest animals, including geckos, ants, and rats, were captured in traps and housed separately in sterilized plastic boxes with ventilation. All pest' samples were transported to the laboratory on the same day as collection. Geckos and rats were dissected to collect feces in the rectum, while whole bodies of ants were used in this study. The procedures for animal dissection and feces collection followed the laboratory biosafety guidelines of Can Tho University and the guidelines of Nguyen et al. (2021).

Salmonella was isolated on Brilliant-green Phenol-red Lactose Sucrose agar (BPLS, Merck, Germany) and examined for biochemical characteristics, such as triple-sugar iron fermentation, VP test, urea test, H_2S, lysine, and idol mobility test following previously described by Tran et al. (2004) and Nguyen et al. (2021).

Identification of *Salmonella* serovars

This study used the PCR method to identify four *Salmonella* serovars, including *S. Gallinarum*, *S. Pullorum*, *S. Enteritidis*, and *S. Typhimurium*, which could commonly cause disease in chickens and humans. Firstly, the DNA of *Salmonella*-positive strains was extracted using the TopPURE Genomic DNA extraction kit (ABT, Vietnam), following the manufacturer's guidelines. Then, it was stored at -20 °C for further use. The primer sequences and PCR conditions for the detection of *Salmonella* serovars were carried out following the guidelines of Paião et al. (2013) for *S. Enteritidis* and *S. Typhimurium* and Xiong et al. (2018) for *S. Gallinarum* and *S. Pullorum*. The kit of Mastermix 2X (Bioline, Canada) was used in these PCR reactions. A total volume of 25 µl PCR reaction included 12.5 µl of Mastermix, 0.5 µl of forward primer, 0.5 µl of reverse primer, 9.5 µl of distilled water, and 2.0 µl of DNA of *Salmonella* strains. The purified water served as the negative control. *Salmonella* serovars isolated previously from chickens in the Mekong Delta served as a positive control and were maintained at the Veterinary Food Hygiene Lab, Faculty of Veterinary Medicine, College of Agriculture, Can Tho University.

Detection of virulent genes

The PCR procedure was conducted to detect virulent genes of *Salmonella* isolates, similar to the method used to identify *Salmonella* serovars in the previous experiment. This study detected five virulent genes, including *InvA, fimA, stn, sopB,* and *sodC1*. The primers and thermocycling were carried out as described by Li et al. (2021).

Genetic relationship of *Salmonella* isolated from chicken and environment

The Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) method was used to determine the genetic relationship between *Salmonella* strains isolated from chicken feces, the environment, and pests in small-scale farms. The mixture composition of each ERIC-PCR reaction was similar to that of the PCR reaction used to identify serovars and recommendations by Tawfik et al. (2022).

The ERIC-PCR primers (Forward: 5'-ATGTAAGCTCCTGGGGATTCAC-3', Reverse: 5'-AAGTAAGTGACTGGGGTG AGCG-3') and thermocycling were carried out as described by Tawfik et al. (2022). The electrophoresis figure was inserted and analyzed by GelJ software (GNU General Public License version 3.0) following the guidelines of Heras et al. (2015).

Statistical analysis

The Chi-square test was used to determine the difference in the prevalence of *Salmonella* and its virulent genes detected from chickens and the environment. The Pearson chi-square statistic was used at the significance level of 95% with $p < 0.05$ in the Minitab 17.0 software (Minitab Pty Ltd, Australia).

RESULTS

In this study, *Salmonella* was detected at a high rate (26.88%) in the collected samples of small-scale farms in the Mekong Delta (Table 1). The presence of *Salmonella* in chickens' feces (27.78%) and pests (34.72%) was higher than that in the husbandry environment (12.35%, $p > 0.05$). In environmental samples, *Salmonella* was detected from bedding (16.67%) and feed (14.81%); however, *Salmonella* was not found in drinking water. In pests, *Salmonella* was detected at the highest rate in gecko feces (70.37%).

Of 75 positive *Salmonella* samples, 75 *Salmonella* isolates were selected (one isolate/sample) to identify *Salmonella* serovars. The results indicated that *S. Typhimurium* and *S. Pullorum* were not detected in this study. In contrast, *S. Enteritidis* and *S. Gallinarum* were detected at a relatively high rate of 10.67% and 13.33%, respectively (Table 2). Among them, *S. Gallinarum* was mainly found in chickens' feces (46.67%), while *S. Enteritidis* was detected in both chicken feces (13.33%), environment (10.00%), and pests (10.00%).

Of 75 *Salmonella* isolates examined, the genes *InvA* (100%) and *fimA* (100%), followed by *stn* (93.33%), *sopB* (89.33%), and *sodC1* (54.57%), were harbored. There was no significant difference ($p > 0.05$) in the presence of each virulent gene among identified *Salmonella* serovars, and all pathogenic genes could be found in *S. Enteritidis* and *S. Gallinarum* (Table 3).

Using ERIC-PCR, the results indicated that *Salmonella* isolated from chickens, the environment, and pests showed diverse genetic relationships, with eighteen patterns obtained, which were noted from P1 to P18 in Figure 1. The results especially revealed the close genetic similarity between *S. Enteritidis* isolated from feces and bedding, geckos, and rats (Pattern 9), and *S. Gallinarum* isolated from feces, bedding, and geckos (Pattern 11), with more than 80% similarity. On the other hand, other *Salmonella* isolates showed homologous patterns (from 50% to 75%) among isolates from chickens, the environment, and pests. Moreover, most of the *Salmonella* isolates shared the same virulent gene patterns among *Salmonella* strains from chicken feces, the environment, and pests in these farms.

Table 1. Prevalence of *Salmonella* in chickens, environment, and pests in small-scale farms in the Mekong Delta, Vietnam, from April to July 2024

Samples	No. of examined samples	No. of positive samples	Percentage (%)
Feces	54	15	27.78[a]
Environment			
Bedding	36	6	16.67
Drinking water	18	0	0.00
Feed	27	4	14.81
Subtotal	81	10	12.35[b]
Pests			
Rat	18	8	44.44
Gecko	54	38	70.37
Ant	72	4	5.56
Subtotal	144	50	34.72[a]
Total	279	75	26.88

[a,b]: These letters indicate the significant statistical difference at 95% confidence; No: The number of; Subtotals is for each factor: chickens, environment, and pests; Total is for all samples collected.

Table 2. Distribution of identified *Salmonella* serovars by PCR in chickens, environment, and pests in small-scale farms in the Mekong Delta, Vietnam, from April to July 2024

Samples	No. of examined isolates	*S. Enteritidis*		*S. Gallinarum*	
		No. of positive isolates	Percentage (%)	No. of positive isolates	Percentage (%)
Feces	15	2	13.33[a]	7	46.67[a]
Environment					
Bedding	6	1	16.67	2	33.33
Feed	4	0	0.00	0	0.00
Subtotal	10	1	10.00[a]	2	20.00[a]
Pests					
Rat	8	2	25.00	0	0.00
Gecko	38	2	5.26	1	2.63
Ant	4	1	25.00	0	0.00
Subtotal	50	5	10.00[a]	1	2.00[b]
Total	75	8	10.67	10	13.33

[a,b] The letters indicate the significant statistical difference at 95% confidence in each column; No: The number of; *S.: Salmonella;* Subtotal is for each factor: chickens, environment, pests; Total is for all samples collected.

Table 3. Presence of virulent genes in *Salmonella* isolated from chickens, environment, and pests in small-scale farms in the Mekong Delta, Vietnam, from April to July 2024

Genes	*S. Enteritidis* (%) (n=8)	*S. Gallinarum* (%) (n=10)	Other serovars (%) (n=57)	Total (%) (n=75)
InvA	8 (100.00)	10 (100.00)	57 (100.00)	75 (100.00)
fimA	8 (100.00)	10 (100.00)	57 (100.00)	75 (100.00)
stn	7 (87.50)	8 (80.00)	55 (96.49)	70 (93.33)
sopB	8 (100.00)	9 (90.00)	50 (87.72)	67 (89.33)
sodC1	6 (75.00)	4 (40.00)	31 (54.39)	41 (54.57)

S.: Salmonella

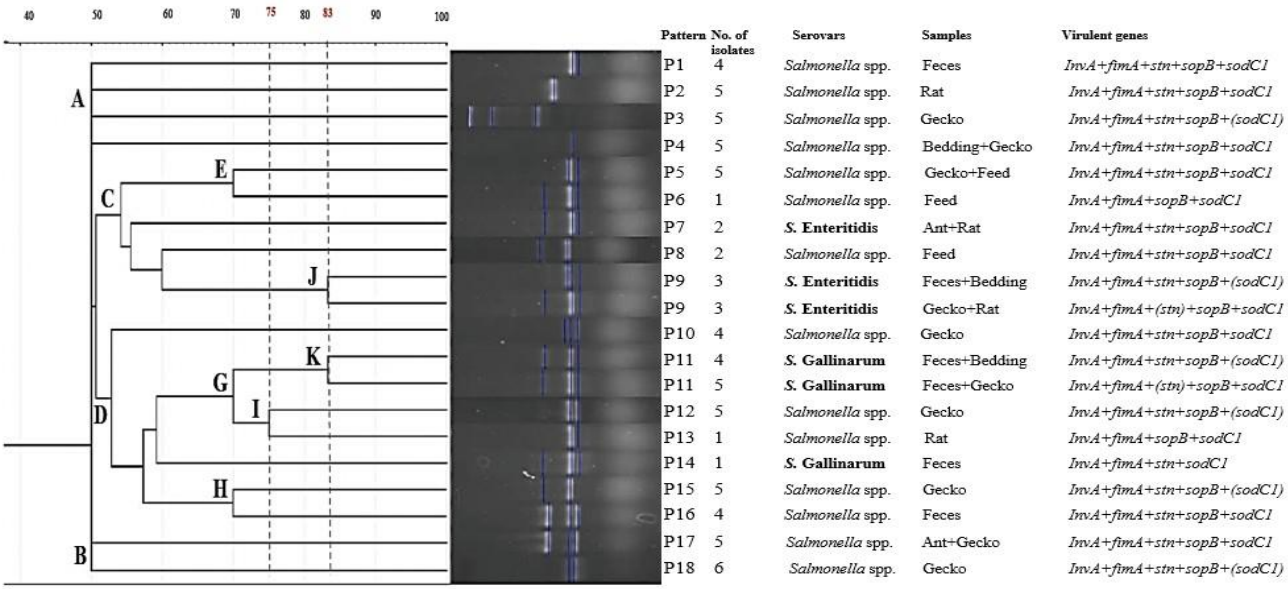

Figure 1. The dendrogram of the genetic relationship of *Salmonella* isolated from chickens, the environment, and pests in small-scale farms in the Mekong Delta, Vietnam, from April to July 2024. The dendrogram revealed diverse patterns of *Salmonella* isolated in small-scale farms (18 gene patterns); Moreover, there was a homogenous genetic characteristic among *S. Enteritidis* (P9, 83.00%) and *S. Gallinarum* (P11, 83.00%) isolated from chicken feces, environment, and pests in these farms. The gene, which was put in the bracket, is present or absent in some isolates in one group; P: Pattern

DISCUSSION

Chicken manure can contain *Salmonella* after being excreted; it can contaminate the environment and surrounding pests. In addition, farmers have raised several domestic animals, such as cattle, ducks, and dogs, on these small-scale farms, which could increase the risk of *Salmonella* contamination among animals and the environment (Lowenstein et al., 2016). There is a close relationship and interaction between livestock, the environment, and wildlife regarding hygiene; animals in the area are sources of pathogens and vectors for *Salmonella* infection (Tessier et al., 2016; Ame et al., 2022). In the study of Nguyen et al. (2021), *Salmonella* was isolated at a low rate in farms and households in the Mekong Delta, including 7.67% in chickens' feces, 4.33% in the environment, and 5.98% in wild animals. In commercial chicken farms in Nigeria, a farm-level prevalence of 47.9% and a sample-level prevalence of 15.9% for *Salmonella* were recorded (Jibril et al., 2020). Compared to the findings of this study in the Mekong Delta, Vietnam, these differences in the prevalence of *Salmonella* in chickens and the husbandry environment could be affected by the sampling location, hygiene conditions, and farm scales. Moreover, poorly preserved leftover food is a potential food source for pests, which can be contaminated with *Salmonella* through their feces and contact with their bodies (Gwenzi et al., 2021). According to research by Gosling et al. (2022), the prevalence of *Salmonella* in water, feed, and litter (husbandry waste) caused *Salmonella* contamination in chicken farms, which was harmful to human health. The bedding, where waste materials from chicken farming activities are stored, and chickens come into direct contact with feces (Dunn et al., 2022). Chickens walking and digging increase contact and diffusion of feces, increasing the risk of infection by harmful microorganisms in general and *Salmonella* in particular (Chinivasagam et al., 2010). Furthermore, this study showed that *Salmonella* was detected at a high rate in geckos in small-scale farms in the Mekong Delta, Vietnam. It was the same statement of Nguyen et al. (2021) regarding geckos and ants being a source of *Salmonella* on poultry farms. In other reports, ants living in the residential areas sporadically contain *Salmonella* at rates of 8% in Mauritius (Simothy et al., 2018), and Etheves et al. (2021) indicated that pests (shrews, mice, rats, flies, ants, cockroaches, and birds) were resources of *Salmonella* infection to chickens in the broiler farms on Reunion Island. Therefore, the prevalence of *Salmonella* in chickens and the environmental agents could become a source of *Salmonella* outbreaks in chickens and humans in these small-scale farms in the Mekong Delta.

The prevalence of the same *Salmonella* serovars in most of the samples (feces, environment, and pests) showed contamination among chickens, the environment, and pests in these small-scale farms in the Mekong Delta. Wales and Lawes (2023) stated that *S. Gallinarum* and *S. Pullorum* are limited to poultry and can be transmitted vertically and horizontally to cause fowl dysentery or typhoid. Haque et al. (2021) reported that *S. Gallinarum* was detected in 25.75% of samples in small-scale layer flocks in Bangladesh and highlighted the urgent need for effective control measures to reduce the prevalence of antibiotic-resistant *S. Gallinarum* in these farms to promote improved egg production and bolster food security and safety in resource-limited environments. Shalaby et al. (2021) reported a higher infection rate in younger broiler chicks in Egypt and identified isolates primarily as *S. Enteritidis*, *S. Shangani*, and others. In Iran, Bahramianfard et al. (2021) clarified that 2.3% of examined poultry samples and 1.3% of eggs were contaminated precisely with *S. Enteritidis*. In Singapore, Aung et al. (2020) conducted the epidemiological distribution of *Salmonella* serovars in humans, food, animals, and the environment. Their findings demonstrated that *S. Enteritidis* was the most prevalent serovar among isolates from chicken (28.5%) and egg products (61.5%). In contrast, over 80% of isolates from farms and wildlife were identified as serovars distinct from *S. Enteritidis* or *S. Typhimurium*. Thus, it underscored the importance of a coordinated one-health approach for enhanced surveillance of *Salmonella* epidemiology.

Virulent genes are essential for the survival and pathogenicity of *Salmonella* in hosts. *Salmonella* has been observed to gain virulence from other species via horizontal gene transfer, which is believed to be a primary factor in the evolution and emergence of highly pathogenic strains (Van Asten and Van Dijk, 2015; Zakaria et al., 2021). The high presence of virulence genes in *Salmonella* isolates identified in this study exhibited significant virulence, potentially leading to severe disease outcomes in susceptible humans and animals. El-Saadony et al. (2022) reported that various *Salmonella* species possess numerous virulent genes that enhance their pathogenic potential, with the *invA* gene being the most prevalent among the examined isolates. Zakaria et al. (2021) observed that the virulence genes present in *Salmonella*, particularly *S. Enteritidis*, were obtained from chickens in Malaysia. These genes predominantly included *PefD, SpvC, Spv, Rck, SseK1, T3SS, InvA*, and *Spa*. Shittu et al. (2022) identified the genes *InvA* and *sopB* (100%) in *Salmonella* strains isolated from the feces of layer chickens in Nigeria. In other research, all *Salmonella* spp. strains isolated from broiler chickens in Colombia contained virulence genes *(lpfA, csgA, sitC, sipB, sopB, sopE*, and *sivH*), which were also detected in humans within the same region. Identifying virulent genes in *Salmonella* from broilers and humans raises concerns regarding potential public health risks in Colombia (Lozano-Villegas et al., 2023). In addition, Shu et al. (2022) stated that *Salmonella* isolates from chicken in China frequently carried extended-spectrum beta-lactamases (ESBLs),

such as *blaTEM*, *blaOXA*, and *blaCTX-M*, and various virulence genes, including *invA*, *stn* (100%), *sopE* (94.87%), *spvR* (87.18%), *ssaQ* (85.47%), *avrA* (77.78%), *spvB* (71.79%), *bcfC* (69.23%), *spvC* (54.70%), *sopB* (51.28%), and *mgtC* (29.06%). These genes were horizontally transferred, significantly contributing to the spread of antimicrobial resistance and pathogenesis, thereby enhancing the pathogenic potential of *Salmonella* through the interplay of resistance and virulence factors. Kanaan et al. (2022) reported that the distribution of various virulence factors was observed, such as *phoP/Q* (40.0%), *traT* (30.0%), *stn* (22.0%), *slyA* (11.0%), and *sopB* (9.0%) in *Salmonella* isolated from chicken meat and egg samples in Iraq, especially in carbapenem-resistant *S. Enteritidis* isolates. Carbapenem-resistant *S. Enteritidis* contains various virulent and antibiotic resistance genes in chicken meat and egg samples; it poses the issue that hygienic practices are essential to prevent *Salmonella* transmission from animals to humans. In the study in the Mekong Delta, virulent genes, which were selected to clarify, were not specific for each *Salmonella* serovar and were still limited. Therefore, studies on the prevalence of virulent genes in *Salmonella* isolated from chickens and the environment should be conducted more to evaluate the pathogenicity of *Salmonella* circulating in the Mekong Delta region, Vietnam.

Gast and Porter (2020) indicated that *Salmonella* can be transmitted from chickens to the environment and vice versa. Consequently, it is crucial to comprehend the epidemiology of *Salmonella* in small-scale farms to avert disease outbreaks or further transmission. In this study, *Salmonella* isolated from chickens, the environment, and pests showed diverse genetic relationships and close genetic similarity between *S. Enteritidis* isolated from feces and bedding, geckos, and rats (Pattern 9), or *S. Gallinarum* isolated from feces, bedding, and geckos (Pattern 11). These *Salmonella* isolates also shared the same virulent patterns. These serovars were mainly detected in chickens' feces (Table 2); this demonstrated that *Salmonella* isolates could be transmitted from chickens to the environment and pests. In contrast, the environment and pests might become a source of *Salmonella* and contaminate chickens. The other *Salmonella* homologous patterns (from 50% to 75%) proved the transmission ability of *Salmonella* isolates among chickens, the environment, and pests. Zhao et al. (2016) used ERIC-PCR to analyze the genetic characteristics of *Salmonella* isolated from free-range chickens in China and found diverse gene patterns belonging to three genotypes. These genotypes were also found in humans previously; thus, free-ranging chickens could act as potential reservoirs for pathogenic *Salmonella*, representing a risk to public health. In South Africa, Ramtahal et al. (2022) reported that distinct ERIC-PCR patterns were identified across various *Salmonella* subtypes isolated from poultry, and they concluded that poultry and their environments were reservoirs for resistant and pathogenic *Salmonella* strains. Elsayed et al. (2024) reported that ERIC-PCR was effectively utilized to create biologically significant clusters of *Salmonella* strains, revealing various genetic patterns and relationships among *Salmonella* isolated from chickens and their husbandry environment in Egypt. Therefore, understanding the genetic diversity of *Salmonella* in chicken farms was essential for protecting chicken health and humans.

CONCLUSION

There was a high prevalence of *Salmonella* detected from chickens, environments, and pests in small-scale farms in the Mekong Delta, Vietnam. In addition, *S. Enteritidis* and *S. Gallinarum* were commonly detected, and these *Salmonella* isolates harbored virulent genes in the function of adhesion, invasion, intracellular survival, systemic infection, and toxin production, including *InvA, fimA, stn, sopB,* and *sodC1*. Moreover, there was a close genetic relationship between *Salmonella* isolated from chickens, the environment, and pests in these farms. It indicated that there was a transmission of *Salmonella* among these factors, especially the environment and pests, which could become a source of *Salmonella* in chickens. Therefore, working on hygiene status in small-scale farms is essential to prevent chicken salmonellosis outbreaks.

DECLARATIONS

Competing interests

The authors declare that they have no conflicts of interest.

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Authors' contributions

Thuan Khanh Nguyen, Luan Minh Huynh, Tan Van Duy Vo, and Duy Duc Tran conceptualized, designed, and supervised the research. Thuan Khanh Nguyen and Luan Minh Huynh critically reviewed the study. Tan Van Duy Vo, Duy Duc Tran, Thu Thanh Kha, and Chi Thi Hanh Nguyen collected samples and processed the data. Thu Thanh Kha, Khai Thi Lien Ly, and Chi Thi Hanh Nguyen analyzed and interpreted the data. All authors revised and approved the final manuscript.

Availability of data and materials

The authors of this article confirm that all data supporting the findings of this research are available upon reasonable request.

Ethical considerations

The authors checked the validity of the data before writing the manuscript. This article was written originally without any copy from data from published articles and books.

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