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Salmonella spp. Infection in Chicken at Markets in Hanoi City: a Cross-Sectional Study for the First Quarter of 2023

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***Abstract* – Foodborne disease is a significant health concern for scientists and populations in Vietnam. While Salmonella is considered the most cause of foodborne illnesses, chicken meat is a main source of this disease. We conducted this study to investigate the presence of Salmonella in distributed chicken meat in Hanoi city, Vietnam. In the first quarter of 2023, we collected 100 samples of chicken parts (head and neck, breast, drumstick, and wing) from street markets around 8 districts in Hanoi city to test the presence of Salmonella. TCVN 10780-1:2017/ISO 6579-1:2017 Microbiology of the food chain - Horizontal method for the detection, enumeration, and serotyping of Salmonella-Part 1: Detection of Salmonella spp. was used to detect Salmonella in the sample. In parallel with that, polymerase chain reactions to detect Salmonella with DNA taken from typical Salmonella colonies on selected agar were also performed. The results show that 70 chicken samples (70%) were contaminated with Salmonella spp.. None of them was Salmonella enterica (Salmonella typhimurium and Salmonella enteritidis). Results show a high prevalence of Salmonella in chicken meat. Therefore, the improvement of food conditions in street markets is highly recommended.**

***Keywords* – Salmonella, Poultry Products, Foodborne Diseases, Methods for Detection of Salmonella, Serotyping of Salmonella.**

I. INTRODUCTION

Foodborne disease is a big concern and a serious health problem worldwide. Every year, many people are affected by foodborne diseases in the world, especially people with immune system deficiency and malnutrition [1]. Pathogens can survive in food products, especially in meat, until distribution in the markets [2]. Different types of organisms cause foodborne infections. One of the most important causes of foodborne disease is *Salmonella* [3], which affects human health by using contaminated and raw meat [4]. *Salmonella* is usually found in animal-derived foods including chicken, beef and pork meat, egg, and milk. It also spreads through zoonotic transmission [5]. Poultry meat is one of the most popular food products worldwide. Because, several nutritional factors such as high levels of protein, low fat content, and favorable unsaturated fatty acid content contribute to the popularity of poultry meat, of which sensory, dietary, and economic factors are of considerable importance [6].

Salmonella is a gram-negative bacillus belonging to the Enterobacteriaceae family. In common, the genus *Salmonella* consists of only two species: *S. enterica* and *S. bongori*. *Salmonella enterica* is divided into the following six subspecies: *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae* and *S. enterica* subsp. *indica*. *S. enterica* subsp. *enterica* comprises more than 1500 serotypes and is responsible for more than 99% of human salmonellosis cases, such

as those caused by *Salmonella typhi*, *Salmonella paratyphi*, *Salmonella typhimurium*, and *Salmonella enteritidis* [7]. The White-Kauffmann-Le Minor scheme is used to serotype *Salmonella* isolates based on the identification of surface O (somatic) and H (flagella) antigenic epitopes. Over 2600 serotypes have been reported [7]. The prevalence of *Salmonella* in chicken has been reported as 6.79% to 97.6% [8]. Despite significant improvements in public health, *Salmonella* remains the most important cause of foodborne diseases worldwide. In Vietnam, the prevalence of street markets with unpackaged raw meat products may be a factor in the increased prevalence of *Salmonella* in chicken meat [9]. The periodic evaluation of *Salmonella* presence in raw meat is necessary to control and reduce salmonellosis in humans.

A. *Objectives*

- To evaluate the presence of *Salmonella* spp. in chicken meat sold at the street market in Hanoi city, Vietnam for the first quarter of 2023.
- Initial identification of some strains of *Salmonella* is that *Salmonella enterica (Salmonella typhimurium* and *Salmonella enteritidis)* in chicken meat sold at street markets in Hanoi city, Vietnam for the first quarter of 2023.

II. MATERIALS AND METHODS

A. *Sample Collection and Preparation*

In this study, we collected 100 samples of chicken meat (drumstick, breast, head and neck, and wing) from street markets/ traditional markets in Hanoi city. Samples were collected in the morning around 6-9 am, transported, and implemented for the experiment in the morning of the same day at the Laboratory Center of the School of Preventive Medicine and Public Health, Hanoi Medical University, Vietnam.

B. *Isolation and Identification of Salmonella*

TCVN 10780-1:2017/ISO 6579-1:2017 Microbiology of the food chain - Horizontal method for the detection, enumeration, and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp. was used to detect *Salmonella* in the sample. In detail, twenty-five grams of tissue samples were added to 225 mL of Buffered peptone water (Merck) and incubated at 37°C for 18 ± 2 hours. Then, 0.1 mL of this medium was added to 10 mL of Rappaport Vassiliadis (Oxoid), and incubated at 41, 5 ± 1^0C for 24 ± 3 hours. One loop of the current medium was transferred to Hektoen Enteric agar (Himedia) and Xylose Lysine Deoxycholate (XLD) agar (Merck), separately. On XLD agar: typical *Salmonella* colonies have a black center and a transparent pale red halo due to the color change of the indicator. On Hektoen Enteric agar: typical *Salmonella* colonies are usually green, with or without a black center. These colonies were cultured in Triple Sugar Iron agar (Merck), Lysine decarboxylase broth (Himedia), DL-Tryptophan (Merck), and Urea agar base (Merck). Finally, Isolates with typical *Salmonella* colony characteristics were confirmed by agglutination using Salmonella poly (O) and poly (H) antisera (Institute Pasteur Ho Chi Minh, Vietnam). *Salmonella* spp. VTCC 12271 and *Salmonella enterica* VTCC 12270 strains obtained from the Institute of Microbiology and Biotechnology, Vietnam National University were used as known positive control.

C. *Polymerase Chain Reaction*

Taking typical *Salmonella* colonies on selective agar and transferring to nutrient agar. Culture bacteria on nut-

-rient agar at 37^{o}C for 16-24 hours. From the nutrient agar plate, take a loop of bacteria and put it into a 1.5 ml centrifuge tube with 500 µl distilled deionized water and process at 100^{o}C for 10 minutes. Then, centrifuge the tube at 10000 rpm x 5 min. The supernatant was used as a template for the polymerase chain reaction (PCR). PCR was performed using primer pairs to detect *Salmonella* spp. and *Salmonella enterica* [10] including Sal InvA F: 5' TGG CAT TAT CGA TCA GTA CCA G 3', Sal invA R: 5' AAC AGC TGC GTC ATG ATA TTC C 3', En spvC F: 5' TCC CTC CTT TGA ATA TTG TAG CTG 3' En spvC R: 5' GGG CTT GTT GAA CGA CCT TC 3'. Samples were prepared by using Taq 2X master mix (NEB, USA) according to the manufacturer's instruction and reacted in the thermal cycler C1000 Bio-Rad for PCR. The thermal cycle for 25 µl PCR (12.5 µl of Taq 2X master mix, 2.5 µl of primer each, 2.5 µl of ADN template) included three steps: the first step with initial denature at 94^{o}C for 2 mins; the second step with 35 cycles: denaturation at 94^{o}C for 45 secs, annealing at 60^{o}C for 1 min, extension at 72^{o}C for 90 secs; the third step with a final extension at 72^{o}C for 7 mins. After the reaction, PCR products were electrophoresis to check the ADN band of the *Salmonella* spp. and *Salmonella enterica* on 2% agarose in 1X TAE (Invitrogen) for 45 mins at 100 V. Read DNA electrophoresis results based on Gene Ruler 50bp DNA Ladder with the DNA bands included 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000 bp (Thermo Scientific): conclusion the analyzed sample is *Salmonella* spp. in the presence of a 600 bp DNA band (detected by Sal InvA primers) and the analyzed sample is *Salmonella enterica (Salmonella typhimurium* and *Salmonella enteritidis)* in the presence of 600 bp and 400 bp DNA bands (detected by Sal InvA primers and En spvC primers).

III. Results and Discussions

Table 1. Prevalence of Salmonella spp. infection in chicken samples.

No.	District	Market	Sample (N)	Positive PCR (%)	Positive PCR (%)
1.	Hai Ba Trung	Goc De, Nguyen Cao	13	11	84, 6
2.	Hoan Kiem	Thanh Ha	10	4	40, 0
3.	Cau Giay	Nghia Do	13	11	84, 6
4.	Hoang Mai	Giap Bat	13	8	61, 5
5.	Ba Dinh	Ngoc Ha, Chau Long	13	9	69, 2
6.	Thanh Xuan	Hoang Van Thai, Vuong Thua Vu	13	8	61, 5
7.	Dong Da	Truong Chinh, Ton That Tung, Phuong Mai, Nam Dong	13	9	69, 2
8.	Tay Ho	Buoi, Nhat Tan	12	10	83, 3
	Total		100	70	70

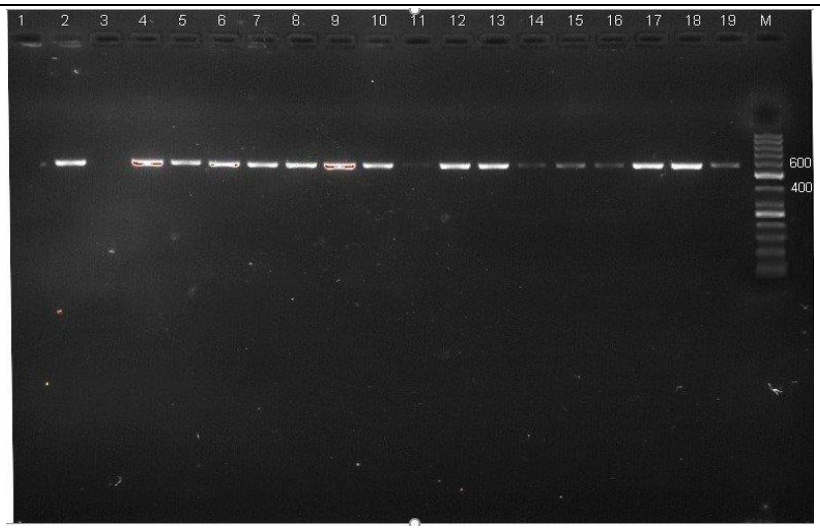

Fig. 1. PCR results from sample number 1 to sample number 19. Negative wells 1, 3, 11; *Salmonella* spp. wells: 2, 4-10, 12-19; M well is 50 bp DNA ladder.

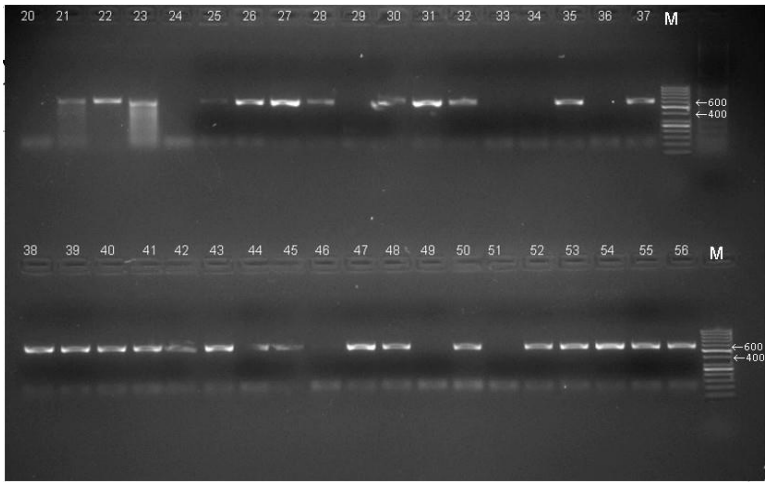

Fig. 2. PCR results from sample number 20 to sample number 56. Negative wells: 20,24,25,29,33,34,36,46,49,51; *Salmonella* spp. wells: 21-23,26-28,30-32, 35,37,38-45,47,48,50,52-56; M well is 50 bp DNA ladder.

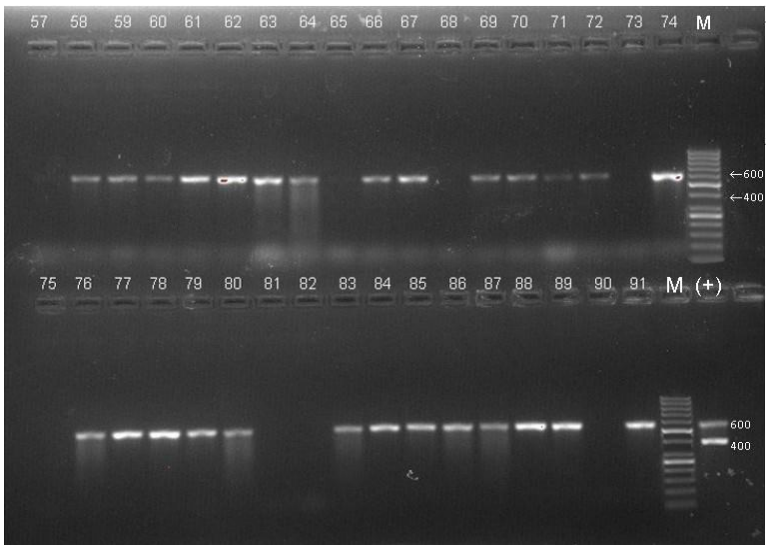

Fig. 3. PCR results from sample number 57 to sample number 91. Negative wells: 57, 65, 68, 73, 75, 81, 82, 90; *Salmonella* spp. wells: 58-64, 66, 67, 69-72, 74, 76-80, 83-89, 91; M well is 50 bp DNA ladder. Rightmost well: *Salmonella enterica* VTCC 1227.

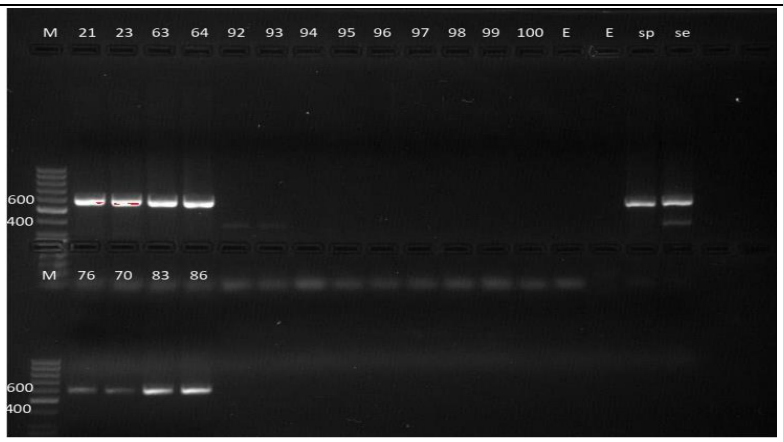

Fig. 4. PCR results from sample number 92 to sample number 100. M well is 50 bp DNA ladder; Negative wells: 92-100; *E. coli* (next 2 wells); *Salmonella* spp. VTCC 12271 (next 1 well); *Salmonella enterica* VTCC 12270 (next 1 well).

The rate of *Salmonella* contamination detected in the poultry collected between October 2012 and March 2015 from slaughterhouses, wholesale fish market, and retail markets in Ho Chi Minh City-Vietnam is 65.3% [11]. According to research in 2012 by Yen T.T. *et al*., the prevalence of *Salmonella* spp. on chicken carcasses from street markets in Vietnam was 45.9%, in detail: Hanoi 51.1%; Hai Phong 45.6%; Da Nang and Can Tho 45.5%; Bac Ninh and Ho Chi Minh City 44.7%; Dong Nai 44.6%; Ha Tinh 44.4%; Phu Tho 43.8%; Lao Cai 43.5%; Kien Giang 41.9%; and Lam Dong 40.9% [12]. In our study, 30 chicken samples out of 100 samples (70%) were contaminated with *Salmonella* spp. The prevalence of *Salmonella* in my study was higher than in Yen *et al.* possibly due to problems with sample size, analytical methods, test time, and chicken population.

Looking out to the world, in 2012, the prevalence of 88.2% of *Salmonella* isolated from poultry carcasses sold in street markets in Phnom Penh, Cambodia had been reported [13]. The study by Vannith Hay in 2018 in Phnom Penh indicated that raw animal meats and fresh vegetables sold in Kilo No. 4 and Derm Kor market might be highly contaminated with *Salmonella* with a prevalence of 100 % in animal meats and 97% in vegetables [14]. The high rate of their study can be attributed to the original food sources, the transport of food products to the market, and the improper storage of the food [14].

So which areas have a lower rate of *Salmonella* contamination in food? The study by Dhaher *et al*. showed that 24.76% of chicken samples were contaminated with *Salmonella* in Iraq [15]. Alali *et al* also showed that 31.5% of retailed chicken meat samples were contaminated with *Salmonella* in Russia [16]. In another study, Todd reported that the prevalence of *Salmonella* in chicken was 13.3% in Ethiopia [17]. A higher prevalence of *Salmonella* was reported by Tibaijuka, as 42% of chicken samples were contaminated with *Salmonella* [18]. In this study, the authors also concluded that the presence of *Salmonella* might be indicative of poor hygiene and a potential danger to consumers. According to research in 2012 by Ramya *et al*., the rates of positive for *Salmonella* spp. were that chicken meat 64%, feces 92%, and cloacal swabs 92% by PCR methods, whereas samples were positive by the cultural method including chicken meat 56%, poultry feces 88%, and cloacal swabs 84%. Also by PCR methods, the rate of positive for *Salmonella enteritidis* including chicken meat was 48%, poultry feces was 68%, and cloacal swabs were 60%. The high incidence of *Salmonella enteritidis* in these research samples is indicative of unhygienic conditions in poultry farms [8]. Thus, it is possible to explain the high prevalence of *Salmonella* spp. in our study samples thought from all stages, from farm to market: the slaughter stage that may be transmitted from feces to raw meat like Ramya *et al*., storage and distribution that t-

-hese process can cause cross-contamination of the product in street markets.

According to a study in 2019-2020 by Truong Huynh Anh Vu, the prevalence of *Salmonella* spp. infection on fresh meat samples (pork, chicken, and beef) randomly collected at traditional markets in Ho Chi Minh City was 42.37%. Which, the infection rate is 49.62% for chickens [19]. In several studies, the most frequently isolated serotype was *S. Enteritidis.* For example, Jalili *et al.* reported that *S. Enteritidis* was the most frequently isolated serotype (29%) from chicken samples in Iran [20]. Besides, Molla and Mesfin's research showed that *S. Braenderup* and *S. Typhimurium* were the dominant serotypes in Ethiopia [21]. Moreover, Abdellah *et al*. reported that *S. Typhimurium* (40.35%) was the dominant serotype among 4 different serotypes isolated from chicken and giblets [22]. The rate of *Salmonella* spp. infection on fresh meat after washing was 19.4% in slaughterhouses and 40% in markets in the research by Le The Bien, in Binh Thuan. The prevalence of *Salmonella* spp. from pork, beef, and chicken was 29.2%, 23.0%, and 36.7%, respectively. Which, *S. Typhimurium* and *S. Braenderup* together accounted for a low proportion with one sample out of 60 samples of chicken meat (1.67%) [23]. According to research in 2014-2015 by Pham Thi Ngoc, in some districts of Hanoi city, there is a common prevalence of *Salmonella* contamination according to the chicken production chains from parent breed chicken farms, hatcheries, household chicken farms, slaughterhouses, and street markets are 32.8%, 11%, 32.08%, 43.3%, 36.9%, respectively [24]. Which, the prevalence of *Salmonella* in samples collected at the point of consumption for carcass-washed samples was 37%, for meat pieces were 35.8%, and serotype *S. Typhimurium* and *S. Enteritidis* in chicken samples at the point of consumption market accounts for 26% [24]. According to research in 2011 by T.T.X. Mai *et al*., the prevalence of *Salmonella* spp. in chicken samples collected at the morning market accounted for 26.67%, of which none were contaminated with *Salmonella typhimurium* and *Salmonella enteritidis*. The prevalence of *Salmonella* spp. in chicken samples collected at the afternoon market, the rate was 63.33%, of which one sample was infected with *Salmonella typhimurium* / *Salmonella enteritidis* [10]. In our study, the prevalence of *Salmonella* infection here is *Salmonella* spp.. *Salmonela enterica* (*Salmonella typhimurium* and *Salmonella enteritidis*) was not detected so the risk of Salmonellosis would be reduced in line with the findings in this study's data. The research in 2016 by Nguyen Thanh Viet at Hanoi street markets indicated that the total rate of Salmonella infection from pork, beef, and chicken samples was 27.8%. For chicken samples, the prevalence of *S. Typhimurium* infection was 4 samples out of 30 chicken samples (13.33%) [25]. According to research in 2001 by Tran Thi Phan *et al*. from six provinces of the Mekong Delta, the infection rate of *Salmonella* spp. was isolated from 69.9% of the pork samples, 48.6% of the beef samples, 21.0% of the chicken samples. Which, *S. Typhimurium* accounted for 4 samples out of 202 chicken samples (1.98%) [26]. In our study, the most frequently isolated serotype was *Salmonella* spp. not *Salmonella typhimurium* and *Salmonella enteritidis*. This is likely because the sample size has not yet expanded for the identification of bacteria, possibly because the biological pattern of *Salmonella* has changed according to the sample collection area and over the years.

Salmonella is composed of two species, *S. enterica* and *S. bongori*. Although *S. bongori* have been reported to infect humans [27, 28], the species is predominantly associated with cold-blooded animals whereas serovars causing disease in humans and other warm-blooded animals mostly belong to *S. enterica* subsp. *enterica*. It is well known that, *S. enterica* subsp. *enterica* is composed of more than 1500 serotypes with some of great importance, such as *S. Typhimurium* and *S. Enteritidis* [29]. In our study, the serotyping results by PCR showed that *Salmonella enterica (Salmonella typhimurium and Salmonella enteritidis*) did not be detected in chicken

samples collected from the street market in Hanoi city. Since this study did not detect *S. Typhimurium* and *S. Enteritidis*, the production and consumption of these chicken samples resulted in a low risk of human salmonellosis. However, the original food sources, slaughter, the transport of food products to the market, and the storage of the food need to be carefully controlled because the prevalence of *Salmonella* spp. in this study range was 70% as an indicator of microbiological failure for the *Salmonella* criterion and suggest a high probability of failure for other microbiological agents as well. All chicken carcasses were collected without labels. Therefore, it is difficult to trace the origin of the collected samples. Meanwhile, supermarkets are large chain stores, mainly selling chilled Vietnamese chicken, frozen meats, and cool meats of clear origin. However, these supermarket items are priced higher than in the street markets/ traditional markets, so people still mainly consume chicken at traditional markets, although buying meat in supermarkets still gives customers a more secure feeling.

IV. Conclusion and Recommendations

Our study shows that 70% of chicken samples were contaminated with *Salmonella*. In the current period, especially after the Covid-19 pandemic, it seems the practice of food safety and hygiene in processing, transporting, storing, preserving, and distributing chicken meat in markets is not guaranteed according to regulations. Thus, strengthening the inspection and supervision of the production, circulation, and distributi--on of fresh meat, especially chicken, contribute to proactive monitoring and detection of hazards, and potential foodborne diseases from the community.

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