Full Length Research Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 24/02/2023;
Date Revised: 06/05/2023;
Date Published Online: 30/06/2023;

Authors' Affiliation:
1. Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universitas Wijaya Kusuma Surabaya - Indonesia
2. Veterinary Microbiology Laboratory, Faculty of Veterinary Medicine, Universitas Wijaya Kusuma Surabaya - Indonesia
3. Student of Faculty of Veterinary Medicine, Universitas Wijaya Kusuma Surabaya - Indonesia
4. Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Mulyorejo, Kampus C FKH UNAIR, Surabaya - Indonesia
5. Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki - Nigeria

***Corresponding Author:**
Mustofa Helmi Effendi
Email: mustofa-h-e@fkh.unair.ac.id

How to Cite:
Wibisono FJ, Rahmaniar RP, Syaputra DE, Zuriya Z, Aziz KM, et al (2023). Risk Factors for Non-typhoidal Salmonella Contamination in chicken meat: A cross-sectional study on Traditional Markets in Surabaya. Adv. Life Sci. 10(2): 282-288.

Keywords:
Chicken Meat, *Non-typhoidal Salmonella*, Public health, Risk Factors, *Salmonella enterica;* Traditional Markets, Surabaya

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Risk Factors for *Non-typhoidal Salmonella* Contamination in chicken meat: A cross-sectional study on Traditional Markets in Surabaya

Freshinta Jellia Wibisono[1], Reina Puspita Rahmaniar[2], Dandi Ernando Syaputra[3], Zuriya Zuriya[3], Khoirul Muhroni Aziz[3], Lusitania Darmayanti Ikeng[3], Mustofa Helmi Effendi[4]*, Agumah Nnabuife Bernard[5]

Abstract

Background: Food of animal origin with good quality is needed by the community. Poor environmental conditions especially bad sanitary conditions are a factor in the entry of microbes. This study aims to identify the presence and risk factors of non-typhoidal Salmonella contamination of chicken meat sold in traditional markets of Surabaya City.

Methods: Chicken meat samples and Questionnaires from the traders (respondents) were collected for this study. A total of 117 samples were used. Isolation and identification of non-typhoidal Salmonella-causing contamination were carried out using standard microbiological techniques. The risk factors for contamination considered in this study included: the cleanliness of chicken meat, means of transporting the meat, market sanitation, and tool sanitation.

Results: The identification test results showed that 42.74% (50/117) were positive for Salmonella enterica contamination. The significance test results indicated a positive relationship between risk factors and the presence of non-typhoidal Salmonella contamination of chicken meat in the traditional markets in Surabaya.

Conclusion: The fulfillment of standards of hygiene and sanitation in traditional markets can prevent contamination by non-typhoidal Salmonella bacteria in chicken meat. Proper handling of chicken meat can increase the fulfillment of the need for safe and healthy protocols for public health requirements.

Introduction

Food is one of the primary needs of humans. Consequently, many standard criteria of food should be met from the variety, the amount, and the fulfillment of nutrition to the quality of food ingredients. The quality of food ingredients is indicated by the absence of physical damage, the addition of chemicals, or biological contamination originating from microbes. Chicken meat is the most widely consumed animal protein source among Indonesian people due to its high protein content, simple processing, relatively large stock in the market, and affordable prices[1–3]. Chicken meat is a good source of animal protein for public consumption because it contains complete essential amino acids as well as vitamins and minerals. Due to its high nutrient content, chicken meat becomes a vulnerable place for bacterial growth[4,5]. Chicken meat in the environment is closely related to the presence of bacterial contamination that can cause foodborne diseases, such as *Escherichia coli*, *Salmonella* sp., *Klebsiella* sp, *Citrobacter* sp., and others [6–8]. According to the Indonesian National Standard, *Salmonella* sp. should not be found in animal-source foods because it can cause zoonotic diseases that can be transmitted from animals to humans or vice versa[9–12].

Salmonellosis is an infection caused by *Salmonella sp*. that has been identified with over 2500 serotypes [13,14]. *Non-typhoidal Salmonellosis* (NTS) is caused by all serotypes of *Salmonella sp* except *Salmonella Typhi, Salmonella Paratyphi A, Salmonella Paratyphi B,* and *Salmonella Paratyphi c*. While *typhoid/paratyphoid Salmonella* causes typhus/paratyphoid disease in Indonesia, NTS causes foodborne disease in humans (self-limiting gastroenteritis in humans). NTS often spreads through the consumption of foods that have been contaminated with animal feces. Poultry can be infected with host-specific *Salmonella* such as *Salmonella Pullorum* and *Salmonella Gallinarum* which cause disease with systemic symptoms similar to *typhoid* or *non-typhoidal salmonellosis*. The etiology of NTS includes *Salmonella enterica* serovar *Enteritidis* and *Typhimurium*, along with other serovars such as *Salmonella Newport*, *Salmonella Heidelberg*, and *Salmonella Javiana*. *Salmonella enterica* serotypes enteritidis and *Typhimurium* are the two most common serotypes and are often transmitted from animals to humans [14–17].

Non-typhoidal Salmonella is one of the most common causes of foodborne disease and is responsible for high rates of morbidity and mortality compared to *Typhoid/paratyphoid Salmonella* [18]. *Non-typhoidal Salmonella* is widespread in domestic and wild animals such as poultry [19–21], pigs [22–24], cattle [25] and domesticated dogs and cats [18,26–29]. These bacteria can persist in the natural environment and are difficult to be eradicated using a disinfectant. *Non-typhoid Salmonella* resides in the animal's gastrointestinal tract, skin, and fur, with clinical symptoms that include mild and self-limiting gastroenteritis in case of the absence of other risk factors such as malnutrition, malaria, anemia, and HIV [14,18,30]. Gastroenteritis symptoms caused by *Salmonella* appear within 8 to 48 jam after the pathogenic bacteria infect the host [31,32].

The incidence of *non-typhoidal Salmonella* infections in Indonesia is still very high and quite alarming. Indonesia is one of the countries with the highest incidence of foodborne salmonellosis endemic in Asia after China and India, followed by Pakistan and Vietnam [18]. With the high risk of *non-typhoidal Salmonella* infection, it is necessary to conduct a field survey to find out whether *non-typhoidal Salmonella* contamination is present in chicken meat distributed in the Traditional Markets in the City of Surabaya. This study also aims to determine the incidence and risk factors of *non-typhoidal Salmonella* contamination in chicken meat sold at the Traditional Markets in Surabaya.

Methods

Ethical approval

Ethical approval was not collected. The samples were not live animals. Chicken meat was collected randomly from traditional markets.

Study period and location

This research was conducted from April until August 2022. Samples were collected from chicken meat traders in traditional markets from 5 regions of Surabaya City (Central Surabaya, East Surabaya, South Surabaya, West Surabaya, North Surabaya).Sample processed at the Laboratory of the Department of Veterinary Public Health, Faculty of Veterinary Medicine, University Wijaya Kusuma Surabaya.

Sample collection

A cross-sectional study design was employed for this study. A total of 117 samples of chicken meat which consist of 18 samples from Central Surabaya, 15 samples from East Surabaya, 15 samples from South Surabaya, 19 samples from West Surabaya, and 50 samples from North Surabaya were collected. The Chicken meat samples collected weighed 10 grams and were covered with plastic and placed in cold boxes to avoid microbial contamination and meat spoilage during transportation to the laboratory. Questionnaires were also distributed among the traders (respondents) to get information on the processing and transportation of the Chicken meat. Information on the sanitary conditions of the slaughterhouses and

butchers tools were also collected from the respondents.

Isolation and Identification of *non-typhoidal Salmonella*

The samples were cultured in Tetrathionate Broth enrichment media, incubated at 45°C for 24 hours, and were observed for color changes and cloudy appearance [20,33].Identification and isolation was conducted on the selective medium of Salmonella-Shigella Agar (SSA) with an incubation period at 37°C for 24 hours, colorless colonies on the edges and black spots in the middle of the colonies were noted. The resultant colonies were subsequently examined with the Triple Sugar Iron Agar (TSIA) and other Biochemical tests which include Urease, Simmon's Citrate Agar (SCA) Test, and Sulfide Indole Motility Test (SIM) [20,34,35].

Statistical analysis

The risk factors for contamination considered in this study include several variables, namely cleanliness (water used to wash slaughtered chicken and chicken meat), the means by which the meat was transported, market sanitation (puddles and waste disposal), and tool sanitation. Information on these risk factors were obtained from Questionnaire distributed to the traders (respondents). Data processing included editing by checking the completeness, clarity of the responses, consistency, and errors among responses in the questionnaire, data coding for data processing, and data entry activities by recording data to be processed using a computer. Furthermore, the data that were inputted were also subject to re-checking to avoid errors in data entry, ensure completeness and corrections, and were tabulated according to the study variables to facilitate data analysis. Data analysis was performed using a qualitative descriptive method by presenting the results of identification of *non-typhoidal Salmonella* as well as risk factor analysis with bivariate correlation analysis and the strength of correlation of causal factors on the incidence of *Non-typhoidal Salmonella* contamination in chicken meat in traditional markets in the City of Surabaya.

Results

Incidence of *Non-typhoidal Salmonella* Contamination

In terms of the incidence of *non-typhoidal Salmonella* contamination in chicken meat in the traditional markets in Surabaya, it was found that 42.74% (50/117) of the samples were positive for *Salmonella enterica* bacteria contamination (Table-1). The result of identification showed a higher contamination incidence compared to the incidence in previous studies in Lampung (3.3%)[36], Makassar (12,5%) [33], Yogyakarta (5%) [37].

Isolation and Identification of *Non-typhoidal Salmonella*.

From the examination of *non-typhoid Salmonella* bacteria (Figure-1) on Salmonella-Shigella Agar (SSA) media, it was shown that black colonies were formed in the middle and colorless at the edges. Black colonies are formed as *Salmonella* produces H_2S. Microscopic examination using Gram stain shows the bacteria were rod-shaped and red. In TSIA media, the red color of the slant (alkaline) indicated that the bacteria cannot ferment lactose, while the black butt indicates that *Salmonella* sp. can form H_2S. The results of the Simmons Citrate Agar (SCA) test showed positive for *Salmonella sp.* with a color change on the SCA media from green to blue because *Salmonella* sp. uses citrate as the sole carbon source. From the Sulfide Indole Motility (SIM) test results, bacteria were motile, did not form indole (red ring), and formed H_2S. Furthermore, the biochemical urease test of *Salmonella* sp. showed negative results because they cannot convert urea to ammonia. This urease test can be used to distinguish the genus *Salmonella* from *Proteus*. Proteus bacteria showed a positive urease test result (the urease medium may change to pink).

Analysis of Risk Factors for Chicken Meat Quality

From the descriptive analysis, risk factors for *non-typhoidal Salmonella* contamination in chicken meat in the traditional markets in Surabaya (Table-2) were the chicken meat hygiene variables. In terms of water source, more than half of the sellers, 54.7% (64/117), used well water, while 45.3% (53/117) used municipal water (PDAM). From the analysis of the incidence of *non-typhoidal Salmonella* contamination, it was shown that 46 (71.8%) of 64 Chicken meat samples washed with well water were identified positive for *non-typhoidal Salmonella* contamination. While only 4 (7.5%) of 53 chicken meat washed with municipal water were identified positive for *non-typhoidal Salmonella* contamination [38, 39]. The use of well water sources to clean chicken meat becomes one of the factors associated with *non-typhoidal Salmonella* contamination. Some of the wells often do not meet construction standards and their wall often is not waterproof. The walls are usually cracked and in some cases the wells are not properly closed and this which can allow biological contamination to occur. As reported in other studies, other factors involve damaged floors and lack of elevated well head (minimum of 70 cm). Wells that do not have a cement width of 1.5 m are also at high risk of pollution from water seepage of pollutant sources [40–42].

In terms of slaughtering criteria for the process of chicken meat, all 117/117 (100%) of the sellers slaughtered chickens in the slaughterhouses. As for the

Traditional Market	Total Sample	*S. enterica*		*S. typhi*	
		Positive	(%)	Positive	(%)
Central Surabaya	18	6	33.33	2	11.11
East Surabaya	15	2	13.33	4	26.67
South Surabaya	15	11	73.33	-	-
West Surabaya	19	13	68.42	-	-
North Surabaya	50	18	36.00	1	2.00
Total	117	50	42.74	7	5.98

Table 1: *Salmonella* sp. contamination in chicken mean sold at some traditional markets in Surabaya.

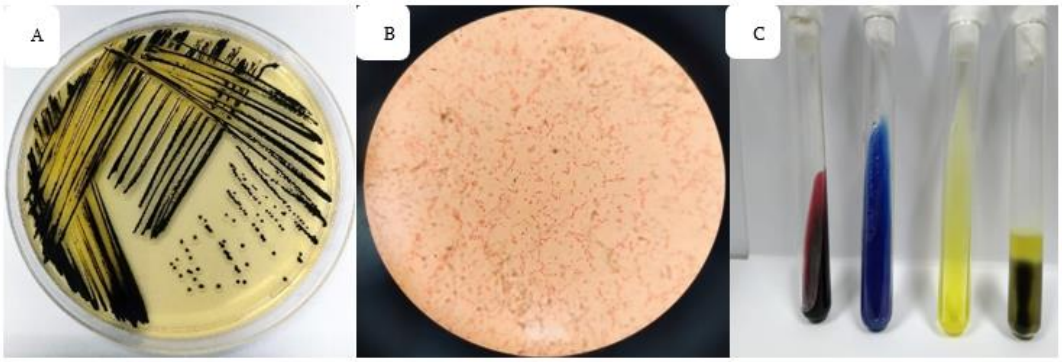

Figure 1: Isolation and Identification of *Non-typhoidal Salmonella*.
Note; Colonies of *Salmonella* sp. On SSA (A), Macroscopic examination (B), TSIA, SCA, Urease, and SIM tests (C).

No.	Risk Factors	Notes	Variable	Value (F+)	Positive NTS (D+/F+)
1	Hygiene of Chicken Meat	Water Source	Well	64/117 (54.7%)	46/64 (71.8%)
			Municipal	53/117 (45,3%)	4/53 (7.5%)
		Slaughter Place	Private Slaughter	0/117 (0%)	0/0 (0%)
			Slaughterhouse	117/117 (100%)	50/117 (42.7%)
2	Transport	Transport from slaughterhouse to market	Motor	48/117 (41%)	36/48 (75%)
			Mobil Box	69/117 (59%)	14/69 (20.3%)
3	Market sanitation	Puddle	Present	61/117 (52.1%)	46/61 (75.4%)
			Absent	56/117 (47.9%)	4/56 (7.1%)
		Waste Disposal	Absent	61/117 (52.1%)	29/61 (47.5%)
			Present	56/117 (47.9%)	21/56 (37.5%)
4	Tool sanitation	Meat butcher tools (Seller tools)	Unwashed	48/117 (41%)	33/48 (68.8%)
			Washed	69/117 (59%)	17/69 (24.6%)

Table 2: Description of *non-typhoid Salmonella* contamination on each variable of risk factors of the incidence.

No.	Risk Factors	Notes	Variable	+	-	χ^2	p-value	OR	RR
1	Chicken meat hygiene	Water source	Well	46	18	46.4	.0001	31.1	9.52
			Municipal	4	49				
2	Transportation	Transportation from slaughterhouses to the markets	Motorbike	36	12	34.4	.0001	11.8	3.7
			Box Pickup cars	14	55				
3	Market sanitation	Water puddle	Present	46	15	52.8	.0001	39.8	10.6
			Absent	4	52				
		Waste Disposal	Absent	29	32	1.2	.27	-	-
			Present	21	35				
4	Tool sanitation	Meat butcher tools (Seller tool)	Unwashed	33	15	22.5	.0001	6.7	2.8
			Washed	17	52				

Note: χ^2= *Chi-Square Test*, OR= *Odds Ratio* and RR=*Relative Risk*
Table 3: Associative risk factors of *Non-typhoid Salmonella* contamination.

incidence of *non-typhoidal Salmonella* contamination, 42.7% (50/117) of 117 chicken samples from slaughterhouses were identified positive for *non-typhoidal Salmonella* contamination. Slaughter activities in slaughterhouses do not rule out the possibility of bacterial contamination by the hands of the butcher, sanitation of equipment, and sanitation of the slaughterhouse. The already contaminated meat in the slaughterhouse can deteriorate during the distribution to each traditional market [22,25,43,44,45,46]. For the transport variable, 59% (69/117) of the sellers transport the chicken using a closed box pickup car from the slaughterhouses to the market, while 41% (48/117) of the sellers rode motorcycles. The activity of chicken slaughtering in the slaughterhouses is associated with the transport variables from the chicken slaughterhouses to the traditional markets. A total of 75% (36/48) samples that were transported using motorcycles were identified positive for *non-typhoidal Salmonella* compared to only 20.3% (14/69) samples with positive *non-typhoidal*

Salmonella contamination that were transported using a box pickup car.

The results of significance testing (Table-3) showed that there was a significant relationship between the use of well water, transportation using a motorbike, the presence of puddles, and washing equipment with a significance value of $p< 0.05$, so the initial hypothesis (H_0) was rejected, and the final hypothesis (H_1) accepted. This means these conditions are associated with the presence of *non-typhoidal Salmonella* contamination in the traditional markets in Surabaya. The highest risk factors for contamination were the presence of water puddles surrounding the markets (OR=39.8), the use of well water (OR=31.1), the use of motorcycles to transport the meat from slaughterhouses to the traditional markets (OR=11.8), and sanitation of meat butcher tools (OR=6.7). In terms of the market sanitation variable, the presence of water puddle in the traditional markets showed the RR coefficient of 10.6, indicating that the risk of *non-typhoidal Salmonella* contamination is 10 times greater in the traditional markets where the water puddle exists around there than in those where there was no water puddle. Water puddles are one of the factors causing bacterial contamination because this place can be a breeding ground for bacteria. Following the Decree of the Minister of Health of the Republic of Indonesia No. 519/MENKES/SK/VI/2008, market areas should be free from water puddles[47,48].The variable for meat hygiene criteria for using well water shows the RR coefficient of 9.52, indicating that the risk of *non-typhoidal Salmonella* contamination in traditional markets in Surabaya is 9 times greater among the sellers who use well water to wash the chicken than among the sellers who use municipal water. Municipal water contains lime or chlorine which is the most common disinfectant used to kill bacteria that contaminate water [49,50].

Discussion

This finding should draw greater public attention to improving hygiene and sanitation of poultry products as the Indonesian National Standard (SNI; 01-7388-2009) concerning the Maximum Limit of Microbial Contamination in Food has stipulated that fresh chicken meat should not contain *Salmonella* [12].

This study also found that 2 *Salmonella* sp. serovars isolated from chicken meat sold at the traditional markets in Surabaya, namely *Salmonella enterica* and *Salmonella typhi* do not cause food borne zoonotic disease. *Salmonella typhi* can only be transmitted from humans to humans and animals are not carrier agents for this serovar. Meanwhile, *non-typhoidal Salmonella* is the leading cause of food borne disease. The common symptoms of *non-typhoidal Salmonella* infection are primarily related to the digestive organs. These bacteria live in the digestive tract of animals and humans and can be spread through food, especially meat, eggs, and milk. People who suffer from *salmonellosis* have diarrhea, nausea and vomiting, stomach cramps, and fever within 8-72 hours after consuming food contaminated by *Salmonella* sp. [13,32,38,39].

In terms of the market sanitation variable, this study indicated that water puddle was absent in 47.9% (56/117) of the sample cases, while it existed in 52.1% (61/117). As for waste disposal criteria, 47.9% (56/117) of the sellers have waste disposal bins/sites, while 52.1% (61/117) did not have a waste disposal site. Concerning market sanitation, it was shown that 75.4% (46/61) of samples collected from those having water puddle surrounding the market tested positive for *non-typhoidal Salmonella* contamination, while only 7.1% (4/56) of those having no puddle water surrounding the market were identified positive for *non-typhoidal Salmonella*. The criteria for water puddles in traditional markets were related to the waste disposal from sellers and sanitation of the tools. A total of 47.5% (29/61) of samples from sellers who did not have a waste disposal site were identified positive for *non-typhoidal Salmonella* contamination, while those collected from sellers who had a waste disposal site, 37.5% (21/56) were identified positive for *non-typhoidal Salmonella* contamination. The other variable was sanitation of equipment at the time of selling, a total of 59% (69/117) of the sellers reported they washed the equipment either before or after use, while 41% (48/117) of the sellers said they did not wash the equipment. Poor sanitation of equipment also became a risk factor. A total of 68.8% (33/48) of samples collected from those who did not wash or clean their tools were positive for *non-typhoidal Salmonella* contamination, while 24.6% (17/69) of samples from those who clean their tools were positive for *non-typhoid Salmonella* contamination. Water puddles, waste disposal, and the meat butcher tools are sources of contamination at the time of sale. Based on previous studies, cutting tools the sellers used became the major source of *Salmonella* contamination. The other sources of *Salmonella* contamination were hair removal tools, washing tubs, knives, and meat tables [11,45,46].

Transportation variable from slaughterhouses to traditional markets also plays a role in the emergence of *non-typhoidal Salmonella* bacterial contamination in traditional markets in Surabaya, with a RR coefficient of 3.7, which means the risk of non-typhoid *Salmonella* contamination in traditional markets in Surabaya is 4 times greater in chicken meat transported using a motorcycle than using a box pickup car. The use of poor transportation can easily lead to bacterial

contamination. Microbial contamination of food is the result of direct or indirect contamination. To minimize bacterial contamination, the meat should be transported using a refrigerated van to prevent bacterial growth [51,52]. In terms of tool sanitation, criteria for washing meat butcher tools carried out by traders have a RR coefficient of 2.8, indicating that the risk of non-typhoid *Salmonella* contamination in traditional markets in Surabaya City is 3 times greater for sellers who did not wash their tools than those who often washed their tools at the time of sale. Tools used in or related to food handling should fulfill hygiene requirements to prevent *Salmonella* sp. and other bacterial contamination. Tool sanitation is associated with the presence of *Salmonella* sp. in food and is related to the amount of germ contamination in food [53]. In conclusion, from the result of this study investigating *Non-typhoidal Salmonella* contamination in chicken meat sold at the traditional markets in Surabaya, it can be concluded that 42.74% (50/117) of the samples were contaminated with *Salmonella enterica.* The highest risk factors for the contamination were the presence of water puddles in the market, the use of well water, the use of motorcycles to transport the meat from the slaughterhouses to the traditional markets and poor sanitation of meat butcher tools.

Competing Interest

The authors declare that there is no conflict of interest.

Author Contributions

FJW and RPR: Conceptualization and supervision of the study and drafted the manuscript. DES, and ZZ: Data curation. KMA and LDI: Formal analysis and Investigation. MHE and ANB: Methodology. MHE, FJW, and RPR: Validation. MHE and ANB: Review and editing. All authors have read and approved the final manuscript.

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