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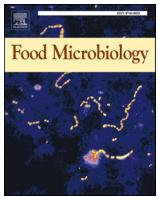

# Prevalence of *Salmonella* spp. in meat, seafood, and leafy green vegetables from local markets and vegetable farms in Phnom Penh, Cambodia

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ABSTRACT

*Salmonella* is a major bacterial concern for public health globally. Although there are limited documentation on the prevalence of *Salmonella* species in Cambodia's food chain, some reports indicate that salmonellosis is a severe gastrointestinal infection in its population and especially in children. To investigate the presence of *Salmonella* spp., 285 food samples (75 meat, 50 seafood, and 160 leafy green vegetable samples) were randomly collected from various local markets in Phnom Penh capital and nearby farms in Cambodia. Concurrently, field observations were conducted to collect data on food hygiene and practices among the relevant actors. All food samples were analyzed using bacterial culture and plate counts, and the findings were confirmed serially with biochemical, serological, and PCR tests. The observational data on food hygiene and practices from farm to market revealed that the spread of *Salmonella* in the food-value chain from farm to market could pose health risks to consumers. The overall prevalence of *Salmonella* spp. was 48.4% (138/285), while the prevalence in meat, seafood, and vegetables was 71% (53/75), 64% (32/50), and 33% (53/160), respectively. Mean *Salmonella* plate count ranged from 1.2 to 7.40 log10 CFU/g, and there was no significant difference in bacterial counts between meat, seafood, and vegetable samples ($p > 0.05$). The most common serogroups among the isolated *Salmonella* spp. were B and C. These results suggest that a large proportion of meat, seafood, and vegetable products sold at local markets in Phnom Penh are contaminated with *Salmonella* spp. This is likely linked to inadequate hygiene and sanitation practices, including handling, storage, and preservation conditions. Observations on farms suggested that the prevalence of *Salmonella* in vegetables sold at the market could be linked to contamination relating to agricultural practices. Thus, controlling the spread of foodborne salmonellosis through the food-value chain from farms and retailers to consumers is warranted to enhance food safety in Cambodia.

## 1. Introduction

Foodborne illnesses are a significant public health concern and may result from improper food handling, inadequate cooking or storage, and use of water contaminated with pathogens such as bacteria, viruses, and parasites (St Amand et al., 2017). It has been estimated that foodborne diarrheal illnesses cause at least 230,000 deaths worldwide, with non-typhoidal *Salmonella enterica* being reported to be one of the four leading foodborne diarrheal agents, accounting for approximately 59,000 global deaths (Havelaar et al., 2015; WHO, 2018).

*Salmonella* is a group of Gram-negative rod-shaped bacteria belonging to the *Enterobacteriaceae* family and consists of two species known as *Salmonella bongori* and *S. enterica* (Issenhuth-Jeanjean et al., 2014; Reeves et al., 1989). Based on three distinct surface structures, i.e., lipopolysaccharides (LPS), flagella, and capsular polysaccharides, approximately 2600 serovars of *S. enterica* have been distinguished (Ferrari et al., 2019; Popoff et al., 2004). *Salmonella* species can also be categorized based on their capacity to induce particular medical conditions in humans, distinguishing between typhoidal strains that affect only humans and non-typhoidal (NTS) strains that affect both humans

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and animals (Diaz et al., 2022; Ferrari et al., 2019).

*Salmonella* can spread to humans through contaminated foods such as fresh meat, vegetables, and dairy products (Huoy et al., 2014; Abatcha et al., 2018; Rönnqvist et al., 2018), through environmental sources, including water, (St Amand et al., 2017; Gu et al., 2019), and through contact with human or animal feces (Penakalapati et al., 2017). Several studies have shown that *Salmonella* contamination can occur at several points in the food production chain (Arnold et al., 2010; Lassnig et al., 2012; Bonardi, 2017; Ehuwa et al., 2021). *Salmonella* is of particular importance in Southeast Asian countries, such as Cambodia. According to recent estimates by the Global Burden of Disease, more than 24,000 typhoid cases (123 cases per 100,000) resulting in around 300 deaths occurred in Cambodia during 2019 (Havelaar et al., 2015). Studies in other Southeast Asian countries have found a high prevalence of NTS *Salmonella* contamination in fresh meat and vegetables at farm and market level (Lettini et al., 2016; Meunsene et al., 2021; Patra et al., 2021; Vidayanti et al., 2021).

Fresh meats and vegetables are popular in the Cambodian diet and can be important sources of *Salmonella* spp. infection. Cambodia's food production chains and markets are informal, with opportunities for bacterial cross-contamination during transportation, distribution, and sale (Chhean et al., 2004; Mosimann et al., 2023). Currently, there are three forms of markets in Cambodia: i) wholesale markets (meat and vegetables are primarily sold in large quantities, while fresh products are distributed to other vendors or small open markets); ii) retail markets (selling foodstuffs or other items directly to end users); and iii) superstore/close markets (supermarkets and private stores selling self-claimed organic produce). There are few published reports on the occurrence of *Salmonella* spp. along the food chain in Cambodia, but previous reports indicate that salmonellosis is a severe gastrointestinal illness in humans, particularly children (Emary et al., 2012; Wijedoru et al., 2012; Chheng et al., 2013; Kheng et al., 2020). A recent study found that the prevalence of *Salmonella* spp. in chicken meat and pork collected at Cambodian markets was 42% (Rortana et al., 2021). It has been found that the prevalence of *Salmonella* spp. in fresh lettuce is higher during the dry season (56.5 %) compared with the rainy season (15.4%) (Desiree et al., 2021).

More science-based data are needed to understand microbial contamination mechanisms for different food types at markets, farms, and slaughterhouses and to enable mitigation and preventive methods to be implemented. This study aimed to investigate the prevalence of *Salmonella* spp. in meat, seafood, and leafy green vegetables collected from various local markets in Phnom Penh and from farms located close to Phnom Penh, Cambodia, and to investigate food safety procedures at markets that might pose risks of *Salmonella* cross-contamination.

## 2. Materials and methods

### 2.1. Study area

Cambodia is located in Southeastern Asia, bordering Laos, Vietnam, Thailand, and the Gulf of Thailand. The total land area is 181,035 square kilometers, with a population of around 14.6 million people. Cambodia's climate is dominated by the tropical monsoon, with temperatures ranging from 21 to 35 °C and with peaks of up to 40 °C during April. There are two main seasons: the rainy season (May to November) and the dry season (December to April) (NAP-GSP, 2022). Cambodia has 24 provinces, and the central capital is Phnom Penh (NIS, 2019). Phnom Penh acts as a hub for receiving and distributing fresh food from different locations in Cambodia, e.g., 98% of vegetables (choysum, lettuce, and yard long been) from Kandal province were supplied to local markets in Phnom Penh in 2002 and 2003 (Chhean et al., 2004). Most Cambodians buy groceries, fresh meats, and vegetables at local markets, which can be either formal or informal. The informal markets are unregulated and consist of a decentralized community of producers, distributors, and sellers (Desiree et al., 2021). Phnom Penh City and Kandal Province were selected as study areas for our analysis, enabling easy access to the laboratory.

### 2.2. Study design and data collection

The design of the present study was a cross-sectional study which was conducted between November 2020 and November 2021. A previous study in Cambodia showed *Salmonella* prevalence in poultry samples of 88 % (Lay et al., 2011), a value which we used for calculating the number of samples required in the present study. The aim was to include 250 samples from different food categories collected from the selected markets (see below) to give an estimated a prevalence of 80%, with a confidence level of 95% and precision of 5%.

Food samples were collected from one wholesale market (Market 1), three retail markets (Markets 2, 3 and 4), and superstore/closed markets (Market 5, including organic shops and supermarkets) in Phnom Penh Capital (Fig. 1). The number of vendors from each market depended on their availabilities of the sample types included in the study. Selected vendors could provide more than one sample type, but each sample type was collected from each individual vendor separately. The sampling teams decided the vendors to include to ensure equal distribution of the vendors in the markets. Twenty-eight vendors selected from Market 1, 26 from Market 2, 24 from Market 3, 19 from Market 4 were included in our sampling. To extend the understanding of the *Salmonella* contamination for common market forms across Cambodia, samples were collected from 11 organic shop and supermarkets to represent Market 5.

We planned to include 50 samples per market (25 leafy vegetable samples, 15 meat samples (pork, beef and chicken), and 10 from seafood samples), and 15 vegetable samples per farm (Table 1). Two vegetable farms located in Kandal province, known for supplying vegetables to the markets in the capital Phnom Penh, were also sampled (Fig. 1).

All samples were randomly collected. Each sample consisted of 500g and were collected aseptically using sterile gloves. Each sample package was cleaned with 70% ethanol to prevent cross-contamination. All samples were stored in a cool box with ice packs for a maximum of 5 h before analysis. Samples from vendors were collected within the shelf life of up to seven days, counted from the harvest date for vegetables and seafood and one day for meat from the slaughterhouse. All samples were given unique identification numbers and were placed in secure sterile re-sealable plastic bags in a cool box containing ice packs for immediate transport to the laboratory. Samples that had been improperly packaged or visibly damaged were discarded before analysis. All samples were brought to the Microbiology Laboratory at the Department of Food Chemistry, Faculty of Science and Technology, International University (IU), Phnom Penh, Cambodia.

Epidemiological data related to food hygiene and practices at the selected farms and markets were collected through observations and interviews during sampling, using pre-made checklists at the included markets and farms. Training on sample collection and on conducting observations and interviews was provided to the research team (third- and fourth-year BSc students from the Royal University of Phnom Penh (RUPP) and IU) before the start of the study. The observational part of the study focused on measuring ambient temperature, noting the storage containers used, checking whether the vendors or farmers used personal protection equipment (PPE), and recording other potential risk factors for *Salmonella* transmission related to food-contact surface areas. The interviews with vendors and farmers focused on how the food was processed and handled at markets and on farms, as well as on the transportation of the food. All data were recorded using the pre-made checklists. The research team informed the vendors and farmers about the study and said that participation would be voluntary and anonymous. They also asked for oral consent to participate in the study. At the end of each day, data were transferred to Microsoft Excel for further analysis and checked for accuracy. The study was approved by the Royal University of Phnom Penh under the grant agreement between Sweden and RUPP (19000439) on January 22, 2019.

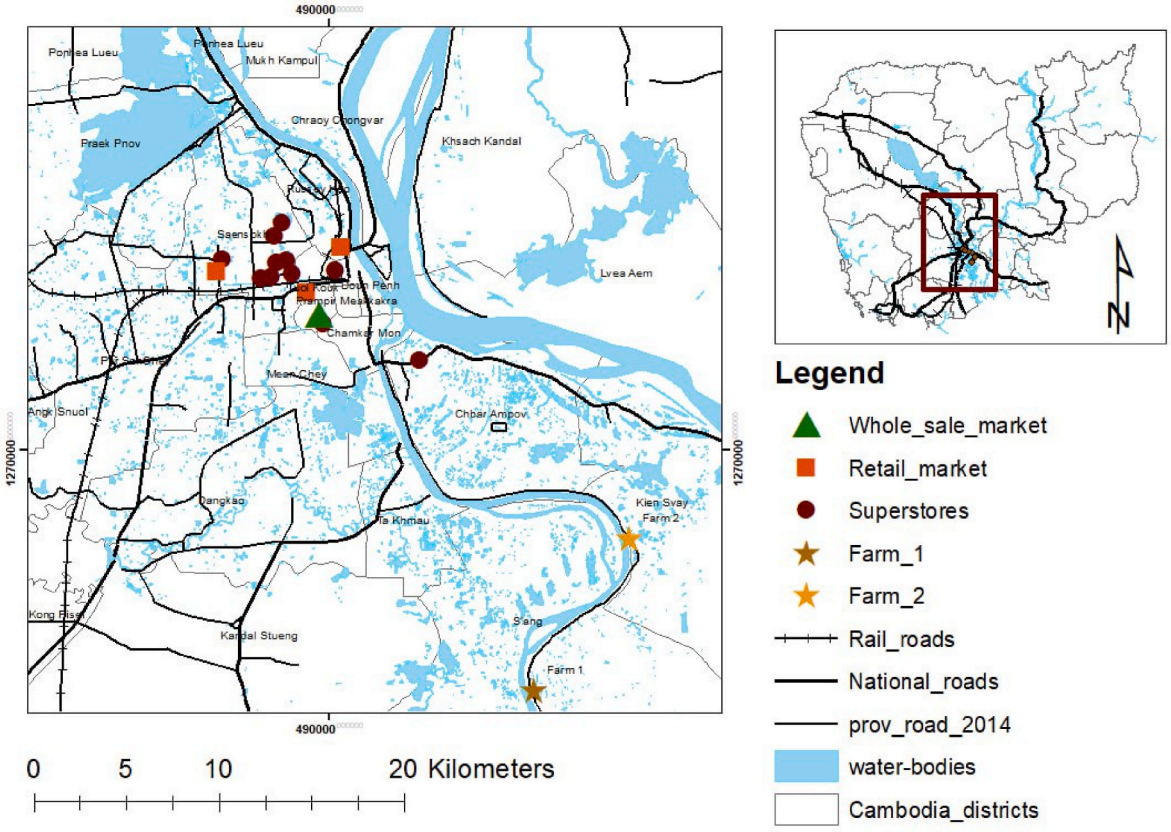


**Fig. 1.** Map of sampling sites.

**Table 1**
Food samples collected from markets and vegetable farms in Cambodia and analyzed to determine the prevalence of *Salmonella* spp.

| Sample types | | Scientific name | No. of Samples Collected | | | | | | | Total no. of samples |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Market 1 | Market 2 | Market 3 | Market 4 | Market 5 | Farm 1 | Farm 2 | |
| **Meat** | Beef | *Bos indicus* | 5 | 5 | 5 | 5 | 5 | – | – | 25 |
| | Chicken | *Gallus gallus domesticus* | 5 | 5 | 5 | 5 | 5 | – | – | 25 |
| | Pork | *Sus scrofa domesticus* | 5 | 5 | 5 | 5 | 5 | – | – | 25 |
| **Seafood** | Fish | *Channidae* (Snake Head Fish) | 5 | 5 | 5 | 5 | 5 | – | – | 25 |
| | Seafood | *Caridean* shrimp | 5 | 5 | 5 | 5 | 5 | – | – | 25 |
| **Vegetable** | Bok Choy | *Brassica rapa* subsp. *chinensis* | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 35 |
| | Salad | *Lactuca sativa* var. *crispa* | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 35 |
| | White cabbage | *Brassica oleracea* var. *capitata f. alba* | 5 | 5 | 5 | 5 | 5 | 5 | – | 30 |
| | Water morning glory | *Ipomoea aquatica* | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 35 |
| | Curly cabbage | *Brassica oleracea* var. *capitata f. sabauda* | 5 | 5 | 5 | 5 | 5 | – | – | 25 |
| **Total no. of samples** | | | **50** | **50** | **50** | **50** | **50** | **20** | **15** | **285** |

### 2.3. Microbiological analysis

In all microbiological analysis, a negative control (*E. coli* ATCC 25922) and a positive control (*Salmonella enterica* subspecies *enterica* serotype Typhimurium ATCC 14028) were used.

#### 2.3.1. Bacterial isolation

*Salmonella* was isolated according to ISO method 6579–1:2017. In brief, meat, seafood, and vegetable samples were cut into small pieces using a sterile knife, and 25 g of sample were mixed thoroughly with 225 mL buffer peptone water (BPW, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). Then, 1 mL was transferred to a 1.5 mL centrifuge tube for serial dilution and plating on Salmonella-Shigella agar (SS, HiMedia, Maharashtra, India) for *Salmonella* quantification (Malorny et al., 2008). The plates were incubated at 37 °C for 18–24 h. Colorless colonies with a black center were presumed to be *Salmonella*. Colonies typical of *Salmonella* were counted all over the entire plates, if less than 300 colonies. The total *Salmonella* concentration (CFU/g) was calculated using actual colony counts multiplied by the dilution factor, then divided by the volume of the sample used.

The remaining sample mixture was incubated in a shaker (KS 4000 i control, IKA-Werke GmbH & Co.KG, Staufen, Germany) at 37 °C for 18 h. The pre-enrichment culture was then incubated for 18–24 h in two selective enrichment media, Muller-Kauffmann Tetrathionate Novobiocin broth (MKTTn, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and Rappaport Vassiliadis Soya Peptone broth (RVS, Sigma-

Aldrich Chemie GmbH, Taufkirchen, Germany), at 37 °C and 42 °C, respectively. Each sub-culture was cultured on xylose-lysine-desoxycholate agar (XLD, HiMedia, Maharashtra, India), and Brilliant Green Agar (BGA, Sigma- Aldrich Chemie GmbH, Taufkirchen, Germany) at 37 °C for 24 h and then checked for typical *Salmonella* colonies. Each sample was analyzed in triplicate.

#### 2.3.2. Bacterial confirmation

*2.3.2.1. Morphological and serological confirmation.* Three to five typical colonies were chosen from each XLD and BGA plate. Selected colonies were confirmed by Gram staining with microscopic morphology identification. Colonies with red/pink color containing rod-shaped bacteria were considered suspected *Salmonella* spp. Presumptive *Salmonella* isolates were confirmed using the Latex slide agglutination commercial LK02-HiSalmonella™ Latex test kit (HiMedia, Maharashtra, India). First, autoagglutination was tested, and any colony material from isolates that did not show autoagglutination was picked from XLD or BGA agar, mixed with 20 μL Latex reagents and observed for agglutination in accordance with the kit instruction. The *Salmonella* Sero-Quick Group kit (SSI Diagnostica A/S, Hillerød, Denmark) was used to identify the most common *Salmonella* serogroups.

*2.3.2.2. Biochemical confirmation.* Once a pure culture was obtained, each isolate was tested with the KBM002 Himotility™ Biochemical kits for Salmonella (HiMedia, Maharashtra, India). A single isolated colony was inoculated in 5 mL of Brain Heart Infusion broth (BHI, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and incubated at 37 °C for 4 h until turbidity of more than 0.1 OD at 620 nm was measured with a UV Spectrophotometer (UV-3000 spectrophotometer, LC-Instrument). A loopful of mixed culture was then inoculated into each well, except the second well, for detecting the motility of the strain. After incubation at 37 °C for 24–48h, the color change was recorded by comparing the result with the standard data sheet provided with the kit.

*2.3.2.3. PCR-based confirmation.* Genomic DNA from each isolate was prepared using a QIAamp blood Mini kit (Hilden, Germany) following the manufacturer's instructions. To confirm presence of the *Salmonella invA* gene, the commercial Primer set SIN-1, SIN-2 (Takara Bio Europe SF, Göteborg, Sweden) was selected with amplified fragment 378bp, with *S.* Typhimurium ATCC14028 as the positive control. PCR amplification was performed in a final volume of 25 μL in a thermocycler with conditions described in the manufacturer's manual. PCR Master Mix reagent (Thermo Fisher Scientific, Vilnius, Lithuania) was used for PCR amplification. A 12.5 μL aliquot of PCR Green Master Mix was dispensed into each PCR tube with 9.5 μL deionized distilled water and 10 pmol/μL of each forward and reverse primer (0.5 μL of each primer per reaction). Template DNA (2 μL) was added before loading into the SimpliAmp™ Thermal Cycler. The PCR amplification steps were performed as following the instructions in the primer set: 1 min initial denaturation at 94 °C, followed by 35 cycles (94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min), and final extension at 72 °C for 10 min. The PCR products were run through 1.5 % agarose gel in 1X TAE buffer at a constant voltage of 85 V for 80 min. As size marker, 1 μL of 1 kb ladder plus was used, and 5 μL gel red staining solution was added per 50 mL agarose gel. The PCR product was visualized as a single fluorescent band using Chemidoc Touch images (Bio-Rad, Image Lab Gel Doc™ EZ system).

### 2.4. Statistical analysis

IBM SPSS statistic version 27 was used to perform statistical analysis. All bacterial plate counts were converted into $\log_{10}$ CFU/g. A one-way ANOVA test was performed to check for significant differences in mean *Salmonella* spp. plate count on meat, seafood, and vegetable samples. The prevalence of *Salmonella* spp. in different sample types and different markets was compared using the Chi-square ($\chi^2$) test, with $P \leq 0.05$ considered to represent a statistically significant difference.

## 3. Results

In total, 285 fresh vegetables and meat samples were collected at local markets in Phnom Penh city and from farms located in Kandal Province (Fig. 1). Of these, 125 were meat (n = 75) and seafood (n = 50) samples from local markets, and 160 were leafy green vegetables from local markets and farms (Table 1).

### 3.1. Prevalence of Salmonella spp. in food samples

Based on serial testing of each sample, from conventional culture followed by biochemical and serological tests and finally with PCR confirmation with *invA* gene detection, 138 (48.4%) of the 285 samples tested positive for *Salmonella* spp. These comprised 53 (71%) meat and 32 (64%) seafood samples collected from local markets and 53 (33%) vegetable samples from local markets and farms (Table 2). At the market level, combined *Salmonella* spp. prevalence in meat, seafood, and vegetables from the wholesale market (78%) was significantly higher than that recorded at retail markets (Markets 2, 3, and 4, 43%) and the superstores/closed market (Market 5,46%), ($P < 0.001$). There was no difference in the prevalence of *Salmonella* spp. between vegetables collected at the different markets and farms (Fig. 2).

The *Salmonella* spp. plate count results for meat, seafood, and vegetable samples from the selected markets are presented in Fig. 2. The mean total count of *Salmonella* spp. on meat samples ranged from 1.8 to 3.4 $\log_{10}$ CFU/g, while that on seafood samples ranged from 1.8 to 7.4 $\log_{10}$ CFU/g and that on fresh vegetables ranged from 1.2 to 4.1 $\log_{10}$ CFU/g. There was no significant difference in total *Salmonella* spp. counts between meat, seafood, and vegetable samples. On the other hand, there were significant differences in total *Salmonella* spp. count between vegetable samples taken from Market 1, Market 2, Market 3, Market 4, and Market 5 ($p < 0.05$) (Fig. 2).

According to the serogroup results, serogroups B and C were the most common serogroups among the positive samples accounting for 20% (28 out of 138 isolates) and 33% (45 out of 138 isolates), respectively (Fig. 3). Serogroup C was present in all food commodities, while serogroup B was not found in white cabbage samples.

### 3.2. Observed food safety risk

Data on food hygiene and practices were collected from 27/28

**Table 2**
Proportion of meat and vegetable samples from selected markets and farms in Cambodia testing positive for *Salmonella* spp.

| Sampling region | | Meat | | *Vegetable* (pos/ total) | No. of positives | Prevalence (%) |
|---|---|---|---|---|---|---|
| | | *Meat* (pos/ total) | *Seafood* (pos/ total) | | | |
| **Market** | ***Market 1*** | 15/ 15 | 10/10 | 14/25 | 39/50 | 78.0 |
| | ***Market 2*** | 10/ 15 | 2/10 | 6/25 | 18/50 | 36.0 |
| | ***Market 3*** | 8/15 | 1/10 | 4/25 | 13/50 | 26.0 |
| | ***Market 4*** | 13/ 15 | 9/10 | 12/25 | 34/50 | 68.0 |
| | ***Market 5*** | 7/15 | 10/10 | 6/25 | 23/50 | 46.0 |
| **Farm** | ***Farm 1*** | – | – | 4/20 | 4/20 | 20.0 |
| | ***Farm 2*** | – | – | 7/15 | 7/15 | 46.7 |
| **Total** | | 53/ 75 | 32/50 | 53/160 | 138/285 | 48.4 |

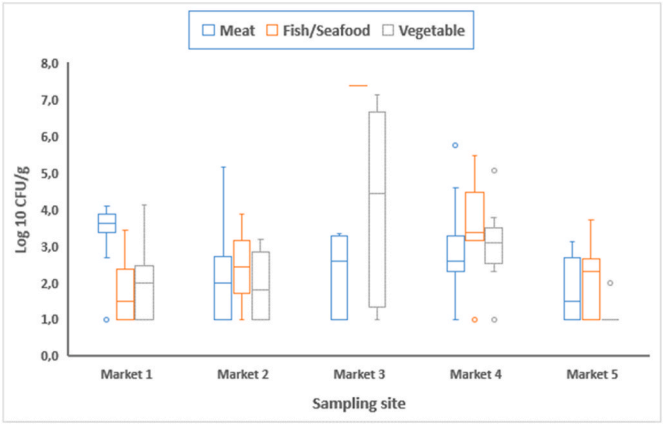


**Fig. 2.** Boxplot of Mean total plate count of *Salmonella* spp. different meat, seafood, and vegetable samples collected from selected markets in Cambodia.

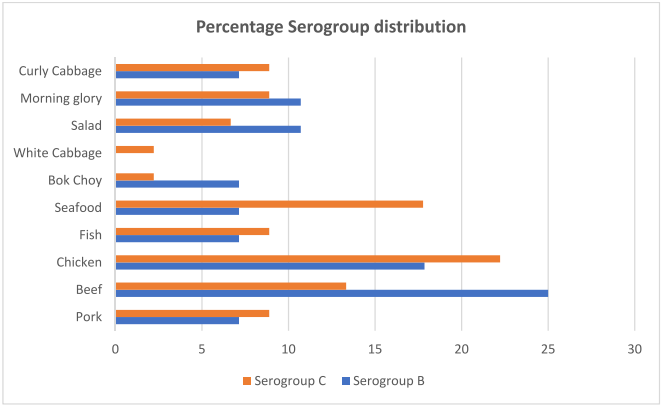


**Fig. 3.** Percentage of serogroup distribution among food commodities.

vendors from Market 1, 25/26 from Market 2, 18/24 from Market 3, 19/19 from Market 4, and 11/11 from Market 5. Farm observation data were collected from both included farms. The observation of vegetable farms showed that the actual temperature ranged between 30 °C and 31 °C. Fresh vegetables were kept directly on the ground after harvest before being packed in plastic bags for transport to market. There was no PPE used during vegetable harvesting, with only 20% (1 out of 5 farms) of farm 1 and 33% (1 out of 3 farms) of farm 2 rinsed the vegetables using mainly pond or stream water before transport to the markets. Other observed risks for bacterial cross-contamination during vegetable growing and harvest were, e.g., wet ground with some animal feces-contaminated soil near the growing areas and visibly dirty irrigation water due to dirty pipes or unclean water sources (Supplement Table 1).

Risks relevant to food hygiene were also observed in the selected markets. Significant temperature variations between the selected markets ranged from 19 to 36° C. Table 3 describes the details of the potential risk for the spread of *Salmonella* among those markets. Different storage containers were used, including wooden, metal, and plastic. Most vendors used steel plates (21%), plastic bags (21%), directly placed on wooden tables (17%), and plastic containers (15%). Still, proper shelves were limited in wholesale and retail markets, except in super-stores/closed markets, where they were stored on a proper shelf with cold storage conditions. The PPE investigation showed limited use among markets, with 27 % (27 out of 100 response records) using mask, gloves, and apron, while 21 % (21 out of 100 response records) using masks only. Moreover, the observed risk arising during food handling shows that 48% (48 out of 99 responses) rinsed vegetable samples using recirculated tap water, while only 5% (5 out of 99 responses) used

**Table 3**
Observed/reported potential food safety risks among local markets.

| Risk categories | Risky practice | Number of Responses | Percentage (%) |
|---|---|---|---|
| **Storage** | Plastic bag | 21/100 | 21 |
| | Steel plate | 21/100 | 21 |
| | Directly on the table | 17/100 | 17 |
| | Plastic basket | 15/100 | 15 |
| | Wooden board | 12/100 | 12 |
| | Cold storage (fridge) | 6/100 | 6 |
| | Tent mat | 4/100 | 4 |
| | Proper shelf | 2/100 | 2 |
| | Cardboard | 2/100 | 2 |
| **Rinsing** | Rinsing with tap water | 48/99 | 48 |
| | No rinsing | 43/99 | 43 |
| | Soaking | 5/99 | 5 |
| | Rinsing with pond water | 3/99 | 3 |
| **Personal Protection Equipment (PPE)** | No PPE | 49/100 | 49 |
| | Mask, gloves, and apron | 27/100 | 27 |
| | Mask | 21/100 | 21 |
| | Mask and gloves | 3/100 | 3 |
| **Transportation** | Motorcycle | 37/97 | 38 |
| | Car | 28/97 | 29 |
| | Trailer | 22/97 | 23 |
| | Tuk Tuk | 8/97 | 8 |
| | Bicycle | 2/97 | 2 |
| **Flies** | Around food vendors | 24/100 | 24 |
| **Dirty ground** | Next to the food vendors | 32/100 | 32 |
| **Food display** | Meat kept close to vegetables | 23/100 | 23 |
| | Different food types in the same containers | 16/100 | 16 |

*Note: PPE Including mask, gloves, apron, or other protection material.

soaking for dirt removal and keeping the vegetables looking fresh. Other cross-contamination factors were also observed at the markets, including 16% (16 out of 100 responses) mixing food types in the same storage container, 32% (32 out of 100 responses) showed food vendors close with dirty ground, and 24% (24 out of 100 response) revealed abundant flies around the food areas.

In addition, the food supply sources were recorded for local supplies from Kandal, Kampong Cham, Kampot, Mondolkiri, and Svay Reang Provinces. The farms near Phnom Penh supplied most of the leafy green vegetables sold at the markets. Various means of transportation were used to transport fresh products from farms to the market, such as motorcycles (37%), cars (28%), and trailers (22%). The length of the pre-processing period showed that transport of vegetables, fish, and seafood could take one to two days, depending on the distance to the market, while transport of meat samples took less than 5 h. Therefore, the processing and storage period for all food commodities ranged from 2 h up to two days (Supplement Table 2).

## 4. Discussion

Our analysis revealed a high prevalence of *Salmonella* spp. on fresh meat, seafood, and vegetable samples at both local market and farm level. In addition, observations and interview responses revealed food safety practices that could contribute to the spread of *Salmonella* from farm to market and further to consumer. These practices included, e.g., reuse of storage containers without cleaning, mixing of meat and vegetable products in the same storage container, inadequate rinsing, a long period from harvest to market, and poor hygiene in food court areas. This confirms findings in previous studies, that lack of sanitation and improper practices by both farmers and vendors increase the risk of microbial cross-contamination at the market level (Trongjit et al., 2017; Schwan et al., 2021). Schwan et al. (2021) also observed a high

prevalence of *S.* enterica on food contact surfaces, indicating that microbial cross-contamination can occur when the same food containers are used interchangeably for different food types. A study on risk factors associated with *Salmonella* in pork shops in Vietnam revealed several risk factors, including the presence of flies or insects and the use of cloth material (Dang-Xuan et al., 2019). Additionally, another study indicated that livestock sourced meat, such as pork, can become contaminated with *Salmonella* within 30 min to 6 h of exposure to low concentrations of *Salmonella* in a slaughterhouse environment (Hurd et al., 2001). Thus, various practice enhancements, such as improved hygiene measures in slaughterhouses and at the market level, could potentially reduce the risk of salmonellosis from meat consumption.

Our analysis showed that fresh meat sold at local markets in Phnom Penh was frequently contaminated (71%) with *Salmonella* spp., which is consistent with findings from previous studies in Cambodia. For instance, one study found 88.2% *Salmonella* spp. prevalence in poultry from markets in Phnom Penh (Lay et al., 2011), while another detected 42.1% *Salmonella* spp. prevalence in chicken and pork samples collected from local markets across 25 Cambodian provinces (Rortana et al., 2021). Our study also revealed higher prevalence of *Salmonella* contamination in meat samples than reported in neighboring countries. For example, a study in Malaysia detected *Salmonella* spp. in 21% of raw chicken meat samples at retail markets (Thung et al., 2016), while a study in Thailand found that 23% of raw food samples at retail were contaminated with *Salmonella* (Kong-Ngoen et al., 2022). We found that fresh seafood sold in local markets in Phnom Penh was also highly contaminated with *Salmonella* spp. (64%). In contrast, in a study in Bangkok around 36% of samples collected from seafood markets tested positive for *Salmonella* (Atwill and Jeamsripong, 2021). A previous study on *S. enterica* persistence revealed a survival time of up to 15 days in fish and shrimp stored in ice (Don et al., 2020). *Salmonella* contamination of seafood can occur in the natural aquatic environment, in aquaculture, or during processing (Amagliani et al., 2012). Our results indicated significant occurrence of *Salmonella* in both meat and seafood sold at local markets in Phnom Penh.

Moreover, analysis of fresh vegetables revealed *Salmonella* spp. prevalence of 33%. A previous study in Cambodia found that 57% and 15% of lettuce samples tested positive for *Salmonella* during the dry and rainy season, respectively (Desiree et al., 2021). In another study, *Salmonella* spp. was detected in 25% (78/310) of samples collected from vegetable food contact surfaces and non-food contact surfaces in markets in Cambodia (Schwan et al., 2021). We observed slightly higher occurrence of *Salmonella* on vegetables than reported in some neighboring countries, where *Salmonella* was detected in e.g., 23% (28/120) of samples of green leaf lettuce collected from open markets and supermarkets in Cambodia and Thailand (Chhay et al., 2018), in 13% (74/572) of retail fresh vegetables sampled in Vietnam (Nguyen et al., 2021), and in 33% (26/80) of various vegetables collected from retail markets in Laos PDR (Meunsene et al., 2022). While the occurrence of *Salmonella* in vegetable samples was lower than in meat and seafood samples, our results still indicated high levels of contamination with *Salmonella* among vegetables sold in the selected local markets and farms.

The *Salmonella* plate count data revealed no significant difference between meat, seafood, and vegetable samples, with the maximum count being just above 7.0 $\log_{10}$ CFU/g (for seafood). The average counts were higher than those reported in a previous study in Cambodia, which detected 3–4 $\log_{10}$ CFU/g in poultry samples (Lay et al., 2011), but similar to levels reported for on fresh lettuce collected from local farms and markets in Phnom Penh, Kampong Speu and Kandal province, Cambodia, with mean values ranging from 4.24 to 7.62 $\log_{10}$ CFU/g (Chhim et al., 2022). This high contamination of fresh vegetables, meat, and seafood with *Salmonella* spp. indicates a considerable risk of infection on eating fresh food products. Therefore, an effective pretreatment process should be applied before consumption, and cross-contamination should be avoided.

Furthermore, the study also showed that serogroup C was the most prevalent *Salmonella* serogroup in fresh food samples collected from local markets and farms around Phnom Penh. A previous review showed that serogroup C is the most common serogroup in the United States, and its serovars are found increasingly in Europe and the United States, accounting for the majority of human infections (Fuche et al., 2016). Strains in serogroup C include Infantis, Thompson, Rissen, Newport, and Virchow, among others (Grimont and Weill, 2007; Herrera-León et al., 2007). *Salmonella* enterica serovar Rissen is reported to be the most common serotype (29% prevalence) in meat products in Cambodian border provinces, while *S. enterica* serovar Virchow has been found in 9.1% of broiler chickens in Egypt (Trongjit et al., 2017; Moawad et al., 2022). The second most common serogroup in our study was serogroup B, with serovars such as Typhimurium, Agona, Paratyphi B, Indiana, Haifa, Derby, and Stanley (Grimont and Weill, 2007; Herrera-León et al., 2007). Many serovars in this group are commonly found in food samples, e.g., studies of chickens, pigs, and meat products in the border provinces of Thailand have found that the most common serovar is *S.* Typhimuirum (29%) (Trongjit et al., 2017; Gomes et al., 2022). These findings indicate that human salmonellosis can be linked with food sources at both farm and local market level.

The on-site observations described some general hygiene factors that could partly explain the high prevalence and counts of *Salmonella* in meat, seafood, and vegetables, such as varying temperature (ranging from 19 to 36 °C), improper storage and processing before transfer to the market, and long-distance transportation. At high temperatures, *Salmonella* will not only survive but also grow. It has been shown that *Salmonella* can not only survive, but actively grow at temperatures ranging from 2 to 54 °C depending on the serotype (Pui et al., 2011; Bintsis, 2017). Lack of an appropriate hygiene program is suggested to be the main factor behind the spread of *Salmonella* infection through freshwater fish (Bibi et al., 2015). Lack of sanitation and hygiene practices in vegetable production may also result in high levels of *Salmonella* survival and growth on vegetables sold in local markets (Desiree et al., 2020). In addition, Cambodian's poultry production largely relies on traditional or small-scale systems, which often lack effective practices and management. Despite the provision of food safety and hygiene training programs for producers and for government staff in the Cambodian livestock sector, many small-scale animal production systems show limited implementation of good food safety and hygiene practices (Birhanu et al., 2021). Moreover, a quantitative microbial risk assessment for salmonellosis among Cambodian consumers indicated an 11.1% probability of illness per person per year. This risk is potentially associated with cross-contamination during food preparation and raw material contamination at the market level (Rortana et al., 2022). These findings indicate that in combination, insufficient access to resources, inadequate training, limitations of the regulatory process, and the requirement for substantial shifts in behavior on both individual and community levels can lead to gaps in translating food safety knowledge into actual food safety practices. More attention to risk factors and a good understanding of *Salmonella* epidemiology in food products between farms and markets would minimize the negative impact on consumers. This study can provide essential data for future research design and influence plans emphasizing the necessity of a One-Health approach to safeguard public health from *Salmonella* in fresh food products from farms and markets.

## 5. Conclusions

Science-based data on the prevalence of foodborne pathogens and on existing food safety practices are needed as a basis for interventions to improve food safety and public health in low and middle-income countries. High prevalence and high levels of *Salmonella* in food products sold both at informal, and formal, markets can be the result of cross-contamination, as well as from the survival and growth of the bacteria at any point in the food-value chain. In the present study, a high prevalence

of *Salmonella* was confirmed and contributing risk factors were identified during production, transport, processing and sale of foods. To mitigate the adverse health impact of food borne bacteria, controlling or eliminating the spread of *Salmonella* spread in the food-value chain is essential. This can be achieved by enhancing the food safety practices of farmers, retailers, and consumers.

## CRediT authorship contribution statement

**Laingshun Huoy:** Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sireyvathanak Vuth:** Methodology, Investigation. **Sophanith Hoeng:** Methodology, Investigation. **Chilean Chheang:** Methodology, Investigation. **Phalla Yi:** Methodology, Investigation. **Chenda San:** Methodology, Investigation. **Panha Chhim:** Methodology, Investigation. **Sopacphear Thorn:** Methodology, Investigation. **Bunsopheana Ouch:** Methodology, Investigation. **Dengrachda Put:** Methodology, Investigation. **Lyna Aong:** Methodology, Investigation. **Kongkea Phan:** Writing – review & editing, Supervision, Investigation. **Leila Nasirzadeh:** Writing – review & editing, Supervision, Conceptualization. **Siteng Tieng:** Writing – review & editing, Supervision, Conceptualization. **Erik Bongcam-Rudloff:** Writing – review & editing, Supervision, Conceptualization. **Susanna Sternberg-Lewerin:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Formal analysis, Data curation, Conceptualization. **Sofia Boqvist:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

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