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Foodborne bacteria in the Cambodian meat value chain:

Emphasis on the risk of *Salmonella* in chicken and pork from traditional markets to household consumption

Rortana Chea

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Rortana Chea
Faculty of Veterinary Medicine and Animal Sciences
Department of Biomedical Sciences and Veterinary Public Health
Uppsala

SLU
SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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Cover: Activities during sample collection at traditional market, Phnom Penh, Cambodia (photo: Rortana Chea)

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Foodborne bacteria in the Cambodian meat value chain: Emphasis on the risk of *Salmonella* in chicken and pork from traditional markets to household consumption

Abstract

Foodborne pathogens, such as *Salmonella* and *Staphylococcus aureus*, pose a high risk to human health globally. Using quantitative microbial risk assessment (QMRA), this thesis estimated the risk of salmonellosis to Cambodian consumers eating contaminated chicken and pork salad. Chicken meat and pork samples (n=204 each) were collected from traditional markets in 25 provinces in Cambodia for analyses of *Salmonella* and also *S. aureus*. Practices used for preparing chicken and pork salads in 93 Cambodian households were surveyed and used to design an experiment assessing *Salmonella* cross-contamination from raw meat to ready-to-eat salad in four scenarios. *Salmonella* prevalence in chicken meat and pork was found at 42.6% (87/204) and 45.1% (92/204), respectively, with mean *Salmonella* concentration of 10.6 MPN/g in chicken meat and 11.1 MPN/g in pork. *Salmonella* contamination, salad consumption and dose-response were modelled using Monte Carlo simulations with 10,000 iterations. The QMRA model showed that the annual estimated risk of salmonellosis from consuming chicken salad, pork salad and both salads was 11.2% (90% CI 0.0-35.1), 4.0% (0.0-21.3) and 14.5% (0.0-33.5), respectively. Thus one in 10 chicken salad consumers in Cambodia was at risk of contracting salmonellosis annually. The factor with the most decisive influence on risk estimates was cross-contamination while preparing salad, followed by high prevalence of *Salmonella* on chicken meat/pork at the market. These results indicate a need to implement control measures, including monitoring the safety of retail chicken and pork at markets and improving hygiene in practices and equipment used during salad preparation in households.

Keywords: animal-source food, traditional market, food safety, *Salmonella*, *S. aureus*, cross-contamination, hygiene practices, microbial risk assessment.

Author's address: Rortana Chea, Department of Biomedical Science and Veterinary Public Health, Swedish University of Agricultural Sciences, 75007 Uppsala, Sweden.

Livsmedelsburna bakterier i Kambodja: fokus på *Salmonella* i kyckling och griskött i värdekedjan från marknad till konsumtion

Abstrakt

Salmonella är en viktig livsmedelsburen bakterie med stor global betydelse för hälsan. Syftet med studien var att uppskatta risken att insjukna i salmonellos efter att ha konsumerat salmonellakontaminerad kyckling- eller fläsksallad i Kambodja. Detta gjordes genom en kvantitativ mikrobiell riskbedömning (QMRA). Prov från kyckling och griskött (n=204 vardera) samlades in från lokala matmarknader i 25 provinser och analyserades för *Salmonella*, men även för *S. aureus*. Information om tillagningsmetoder för salladerna samlades in från 93 hushåll och användes för ett experiment för att bedöma korskontaminering av *Salmonella* från rått kött till ätfärdig sallad i fyra olika scenarier. Prevalensen av *Salmonella* i kyckling och griskött var 42,6 % (87/204) respektive 45,1 % (92/204). Den genomsnittliga koncentrationen av *Salmonella* i kycklingkött var 10,6 MPN/g och i griskött 11,1 MPN/g. Variablerna *Salmonella* kontaminering, salladskonsumtion och dos-respons modellerades med Monte Carlo-simuleringar (10 000 iterationer) för att få fram risken för salmonellos. QMRA-modellen visade att den årliga risken för salmonellos efter att ha konsumerat kyckling respektive fläsksallad, eller båda, var 11 % (90 %CI 0,0 – 35,1), 4,0 % (90 %CI 0,0 – 21,3) och 14,5 % (90 %CI 0,0 – 33,5). Uppskattningsvis en av tio personer som äter kycklingsallad riskerar att insjukna i salmonellos varje år. Variablerna med störst inverkan för att insjukna var korskontaminering vid tillagning och förekomst av *Salmonella* i kyckling- och griskött. Studien visar att det finns behov av ökade kontrollåtgärder, till exempel för att förbättra hygien och säkerhet kring försäljning av kyckling och griskött på marknader, samt att det finns behov av att förbättra livsmedelshygien inom hushållen.

Nyckelord: kyckling, griskött, lokal marknad, livsmedelssäkerhet, *Salmonella, S. aureus*; korskontaminering, hygien, mikrobiell riskbedömning.

Författarens adress: Rortana Chea, Sveriges lantbruksuniversitet, Institutionen för biomedicin och veterinär folkhälsovetenskap, Uppsala, Sverige.

Dedication

To my parents, my wife, my children.

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Rortana Chea*, Hung Nguyen-Viet, Sothyra Tum, Fred Unger, Sofia Boqvist, Sinh Dang-Xuan, Sok Koam Grace Delia, Osbjer Kristina, Heng Theng, Sarim Seng, Phirum Or, Sophia Roeurn and Johanna Lindahl. Prevalence of *Salmonella* spp. and *Staphylococcus aureus* in chicken meat and pork from Cambodian Markets. *Pathogens* 10, no. 5 (2021): 556.

II. Rortana Chea*, Hung Nguyen-Viet, Sothyra Tum, Fred Unger, Johanna Lindahl, Delia Grace, Chhay Ty, Sok Koam, Vor Sina, Huy Sokchea, Son Pov, Theng Heng, Or Phirum and Sinh Dang-Xuan. Experimental cross-contamination of chicken salad with *Salmonella enterica* serovars Typhimurium and London during food preparation in Cambodian households. *PloS One* 17, no. 8 (2022): e0270425.

III. Rortana Chea*, Sinh Dang-Xuan, Hung Nguyen-Viet, Johanna F. Lindahl, Sothyra Tum, Chhay Ty, Delia Grace, Fred Unger, Kristina Osbjer and Sofia Boqvist. Quantitative microbial risk assessment of salmonellosis through chicken and pork salad consumption at Cambodian households. *Frontiers in Sustainable Food Systems* (accepted for publication).

Papers I-III are reproduced with the permission of the publishers.

The contribution of Rortana Chea to the papers included in this thesis was as follows:

I. Led study design, developed data collection tool and led field sampling, performed the laboratory testing, applied for ethical approval, analysed the results in collaboration with the supervisors and researchers from ILRI, wrote the manuscript with the supervisors and all co-authors, submitted the manuscript, and corresponded with the journal.

II. Led the design of study scenarios with the research team from ILRI and supervisors, jointly developed the questionnaire with input from the co-authors, performed the experimental and laboratory work, conducted statistical analyses with input from the research team, supervisors and ILRI, wrote the manuscript with input from all co-authors, submitted the manuscript and corresponded with the journal.

III. Was involved in pathogen prioritisation for the study, jointly designed the study, jointly developed the questionnaire, analysed questionnaire results with input from the co-authors, searched the literature for the study design, analysed data with input from supervisors and ILRI research team, wrote the manuscript with input from all co-authors, submitted the manuscript and corresponded with the journal.

Other papers and policies brief not included in this thesis:

I. Rortana Chea*, Sinh Dang-Xuan, Hung Nguyen-Viet, Johanna F Lindahl, Fred Unger. Incentive intervention for safer pork in the traditional Cambodian market to reduce the burden of foodborne disease (manuscript).

II. Theng Heng, Rotana Chea*, Sothyra Tum, Fred Unger, Johanna F Lindahl, Delia Grace, Chhay Ty, Arsooth Sanguankiat, Sinh Dang-Xuan, Sok Koam, Vor Sina, Huy Sokchea, Or Phirum, and Hung Nguyen-Viet. Food safety interventions: changes of knowledge, attitude, and practices of pork retailers in traditional Cambodia market (manuscript).

III. Minh-Cam Duong, Shivani Patel, Hung Nguyen-Viet, Rortana Chea, Sinh Dang, Sothyra Tum, Usha Ramakrishnan and Melissa F. Young. 2023. Access to food markets, household wealth and child nutrition in rural Cambodia: findings from nationally representative data. *Food Policies* (under review).

IV. Hung Nguyen-Viet, Delia Grace, Fred Unger, Johanna F Lindahl, Silvia Alonso, Kristina Roesel, Tezira Lore, Chi Nguyen, Steven Lam, Sothyra Tum, Rortana Chea and Theng Heng, Chhay Ty, Teng Srey, Melissa Young, Candice Duong and Morgan Brown. 2021. Pork and poultry safety in traditional markets in Cambodia: Understanding complexities and scaling up good interventions. *ILRI Policy Brief*. Nairobi, Kenya: ILRI. https://hdl.handle.net/10568/114472.

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Abbreviations

ASF	Animal-source food (known as livestock derived food)
CAC	Codex Alimentarius Commission
CPS	Coagulase-positive staphylococci
DALYs	Disability-adjusted life years
DoM	Department of Meteorology
FAO	Food and Agriculture Organization of the United Nations
FBD	Foodborne disease
FGD	Focus group discussion
FORT	Foodborne Disease Outbreak Investigation and Response Team
GDAHP	General Directorate of Animal Health and Production
GMS	Greater Mekong Sub-region
ILRI	International Livestock Research Institute
LMIC	Low- and middle-income countries
MAFF	Ministry of Agriculture Forestry and Fisheries
MPN	Most probable number
NAHPRI	National Animal Health and Production Research Institute
OR	Odds ratio
PDAFF	Provincial Department of Agriculture Forestry and Fisheries

RTE	Ready-to-eat
Taskforce	Refer to the taskforce on Food Safety Risk Assessment in Cambodia consist of stakeholders involved during SFFF project implementation.
WHO	World Health Organization
WOAH	World Organization for Animal Health (formerly known as OIE)

1. Introduction

1.1 Global perspective on food safety

1.1.1 Foodborne pathogens and foodborne illnesses

Foodborne diseases (FBD) are illnesses caused by ingesting food containing biological, chemical or physical hazards. Bacteria, viruses and parasites are significant biological hazards causing FBD (Todd, 2014). Around 200 different types of FBD cause illness in 600 million people. The burden of such illness falls most heavily on the poor and on the young (CAC, 2022). Food may contain pathogenic microorganisms (such as bacteria), either because they were present at the time of harvest or slaughter or because they were introduced by contamination during processing and handling (Uyttendaele *et al*., 2016). Foodborne diseases can cause various short-term symptoms, including nausea, vomiting and diarrhoea (generally referred to as food poisoning), and also many longer-term illnesses, including cancer, kidney or liver failure, brain disease and neural disorders. Diarrhoeal diseases are the most common type of FBD (Devleesschauwer *et al*., 2018; Havelaar *et al*., 2013; Kothary & Babu, 2001; World Health Organization, 2016).

1.1.2 Impact of foodborne illnesses

Foodborne diseases are one of the leading causes of human mortality and morbidity, comparable in effect to major infectious diseases such as malaria, HIV/AIDS and tuberculosis (Gourama, 2020). According to a review by the World Health Organization (WHO), the global health burden of FBDs caused by 26 priority hazards and 31 common microbes results in an estimated 33 million disability-adjusted life years (DALYs), a time-based measure that combines years of life lost due to premature mortality, poor health or disability (Havelaar *et al*., 2015). The impact is highest in children under five years of age because they are more susceptible to FBD and more commonly affected by chronic diseases as a result of FBD (Havelaar *et al*., 2015; World Health Organization, 2016). In children under five years, the mortality caused by FBD can reach up to 8%. FBD constitutes a significant health threat and impediment to social and economic development worldwide, especially in low- and middle-income countries (LMIC) (Grace, 2015). This is due to high population density, limited general knowledge, and lack of good hygiene practices when handling fresh meat, vegetables and other foodstuffs and preparing food (Grace, 2015; Lund, 2015; Lund, 2019). Heavy metals are also an important health hazard, with a related study by the WHO estimating an additional burden of 9 million DALYs associated with four heavy metals in food (arsenic , cadmium , lead and mercury) (Gibb *et al.*, 2019). A recent study reported estimated annual losses in LMICs of more than US$110 billion in productivity and medical expenses from unsafe food (Jaffee *et al*., 2018). However, few LMICs monitor the presence of FBD and thus, data on the burden are limited, while more data are available for high-income countries (Grace, 2015; Hoffmann *et al*., 2019; Jaffee *et al*., 2018).

1.2 Foodborne pathogens

Foodborne pathogens are biological agents that contaminate food and cause various illnesses in humans on ingesting that food. The most common foodborne pathogens are viruses, bacteria, parasites, fungi and prions (Labbé & García, 2013; Todd, 2014). Foodborne pathogens are considered one of the most important hazards in food, and risk-based food safety approaches, such as hazard analysis and critical control points (HACCP), are commonly recommended to control FBD. Such food safety management systems should be designed to estimate the risks to human health from food consumption and to identify, select and implement mitigation strategies to control and reduce these risks (Motarjemi *et al*., 2013; Todd, 2020).

1.2.1 Foodborne bacteria

Foodborne bacteria are bacterial species that can be present on food and cause either food spoilage or sickness in people consuming the contaminated food. Foodborne bacteria are among the most important biological hazards in raw and undercooked meat (Havelaar *et al*., 2015). Common foodborne bacteria in animal-source foods (ASF) include *Staphylococcus aureus, Bacillus cereus* and *B. anthracis, Clostridium botulinum, Cl. perfringens, Cl. difficile, Listeria monocytogenes, Escherichia coli, Salmonella enterica, Campylobacter, Yersinia enterocolitica and Y. pestis, Vibrio cholerae, V. parahaemolyticus, V. vulnificus* and *Shigella* species (Bintsis, 2017; Gourama, 2020; Pérez-Rodríguez, 2020). Most of these bacteria are also zoonotic pathogens (Bhunia, 2018; Gourama, 2020; Kirk *et al*., 2015). The most common bacteria, particularly on raw meat, are *Salmonella, E. coli, Campylobacter* spp. and *Staphylococcus aureus* (Bhunia, 2018; Sugrue *et al*., 2019). The most frequent foodborne bacteria that cause diarrhoeal disease are *Campylobacter* and non-typhoidal *Salmonella enterica*, and these pathogens are also responsible for the majority of deaths due to FBD (Devleesschauwer *et al*., 2018; Havelaar *et al*., 2013; Kirk *et al*., 2017; Li *et al*., 2019).

1.2.2 *Salmonella* species

Salmonella species are a genus of Gram-negative rod-shaped bacilli of the family Enterobacteriaceae, which are facultative anaerobes (Andino & Hanning, 2015; OIE, 2016). The two most common species of *Salmonella* frequently contaminating ASF are *S. enterica* and *S. bongori,* with *S. enterica* being the species most frequently associated with human salmonellosis. *Salmonella* can be classified into typhoidal (TS) and non-typhoidal *Salmonella* (NTS). Non-typhoidal serotypes are zoonotic and can be transferred from animal to human and human to human. They are common foodborne pathogens that can cause gastroenteritis and other foodborne illnesses in people and are also among the most common foodborne bacteria along the ASF value chain (Yates, 2011). Typhoidal serotypes, including *Salmonella* Typhi Paratyphi A, B and C, can only be transferred from human to human. Typhoidal *Salmonella* causes disease by passing through the lymphatic system of the human intestine to the blood system, and then moving to infect other target organs (Dougan & Baker, 2014; Hu & Kopecko, 2003).

1.2.3 Foodborne disease caused by non-typhoidal *Salmonella*

Non-typhoidal serotypes of *Salmonella* are those that generally result in food poisoning. They are usually associated with animal hosts, with the most common vehicle for NTS transmission being animal-based products such as poultry, pork and raw eggs. Infection can occur when a person ingests foods with a high concentration of *Salmonella* and it results in a variety of symptoms (Crump & Wain, 2017; Jajere, 2019; Yates, 2011). In humans, the clinical signs include diarrhoea, fever, abdominal cramps and vomiting. Infected humans show clinical signs 6-48 hours after ingestion and for up to 4-7 days (Brenner *et al*., 2000; Sánchez-Vargas *et al*., 2011; Yates, 2011). *Salmonella* infection contributes to an estimated 230,000 deaths annually (Eng *et al*., 2015; Havelaar *et al*., 2013). Based on recommendations from WHO and US-CDC, *Salmonella* is categorised into 2,463 serovars (Table 1). In 2020, salmonellosis was the second most reported zoonotic disease in Europe, with 52,702 cases documented (ESFA, 2021).

Table 1. *Salmonella* species, subspecies, serotypes and their usual habitats, according to the Kauffmann-White scheme

Salmonella species and subspecies	No. of serotypes/ subspecies	Usual habitat
S. enterica subsp. *Enterica* (I)	1,454	Warm-blooded animals
S. enterica subsp. *Salamae* (II)	489	Cold-blooded animals and the environment
S. enterica subsp. *Arizonae* (IIIa)	94	Cold-blooded animals and the environment
S. enterica subsp. *Diarizonae* (IIIb)	324	Cold-blooded animals and the environment
S. enterica subsp. *Houtenae*(IV)	70	Cold-blooded animals and the environment
S. enterica subsp. *Indica* (VI)	12	Cold-blooded animals and the environment
S. bongori (V)	20	Cold-blooded animals and the environment
Total	2,463	

Note: Isolates of all species can contaminate animal products and/or the environment, and some subspecies have been found in humans (sources: Brenner *et al*., 2000; Popoff & Le Minor, 1997)

1.2.4 *Staphylococcus aureus*

S. aureus is a Gram-positive bacterium, regarded as a commensal, that can occasionally cause disease in both humans and animals, sometimes as secondary infections (Grace & Fetsch, 2018; Le Loir *et al*., 2003). It is an opportunistic pathogen in humans and can cause a broad spectrum of conditions, from superficial skin infections to severe and potentially fatal and invasive diseases (Götz *et al*., 2006; Kadariya *et al*., 2014). *S. aureus*, a coagulase-positive staphylococci (CPS) species, is more frequently pathogenic to humans, resulting in a wide range of clinical symptoms, including diarrhoea and vomiting, caused mainly by its wide range of enterotoxin production (Le Loir *et al*., 2003; Logue *et al*., 2017). The enterotoxins commonly cause human diseases through contaminated food, even if properly cooked (Le Loir *et al*., 2003; Varijakshapanicker *et al*., 2019). *S. aureus* is frequently isolated from meat and ready-to-eat (RTE) foods, in traditional markets or supermarkets (Kadariya *et al*., 2014; Rortana *et al*., 2021). In Cambodia, it has been found to cause foodborne outbreaks, with one large outbreak occurring when people ate contaminated food, including noodles and salad, at a wedding ceremony (Huong *et al*., 2010; Kimsean *et al*., 2017).

1.3 Animal-source food value chain and food safety in Cambodia

1.3.1 Cambodian animal-source food value chain

In Cambodia, ASF are an essential part of the diet, with pork, fish and poultry being widely consumed. Small and medium-sized farms produce most livestock products. Most markets sell fresh, unpackaged meat from the early morning and close at about 6 pm, which means that people must purchase meat for lunch and dinner in the morning (Borin *et al*., 2010). Meat is commonly sold in traditional markets where women are the main retailers (Borin *et al*., 2010). Most of these traditional markets are operated as open-air or household shops (Borin *et al*., 2010; Nguyen-Viet *et al*., 2019), often as so-called wet markets (Naguib *et al*., 2021). There is seldom access to clean water or cooling systems in these markets. As in other countries in Asia, growing concerns over food safety issues have been reported in Cambodia during recent decades. Moreover, as is common in LMICs, until recently there was very little scientific evidence on the health burden of foodborne disease in Cambodia or on how to manage food safety in traditional markets, which are known to be responsible for most foodborne illness in LMICs (Mulugeta, 2021; Roesel & Grace, 2014).

People in Cambodia may also buy meat from supermarkets. In contrast to traditional markets, clean water, cooling systems and appropriate processing are readily available in supermarkets, but these types of markets are still uncommon in Cambodia and mostly found in the major cities (Khieu *et al*., 2009; Pen *et al*., 2014). Recent surveys have shown that 480 traditional markets serve and sell fresh meat to Cambodian people countrywide, whereas only a few supermarkets and minimarts sell different types of meat (Khieu *et al*., 2009; PIN, 2015; Rortana *et al*., 2021).

1.3.2 Bacterial contamination at slaughterhouses

In livestock, such as pigs and chickens, *Salmonella* contamination of meat can occur during slaughter, and the numbers of bacteria may increase along the value chain to the market due to an ambient temperature allowing bacterial growth (Juneja *et al*., 2007). A likely pathway for *Salmonella* transmission in food to the consumption stage is via meat purchase at traditional markets and preparation at the household level using unhygienic practices (Botteldoorn *et al*., 2003; Carrasco *et al*., 2012). According to respondents in a study in Cambodia, an official veterinarian at each slaughterhouse inspects all pigs before slaughter (ante mortem) and the carcasses after slaughter (post mortem) (Palmer & Slauch, 2017). Post mortem inspection specifically targets indications of tuberculosis, liver fluke, cysticercosis and other infectious diseases that are revealed by clinical signs (NAHPRI, 2019). However, screening for bacterial contamination is only conducted as part of active surveillance projects in which samples are sent to the central veterinary laboratory at the National Animal Health and Production Research Institute (NAHPRI) to perform bacteria isolation (Siengsanan-Lamont *et al*., 2021; Tum, 2008). Many foodborne bacteria, including *Salmonella*, can contaminate fresh meats during slaughter, processing, handling and sale in markets (Botteldoorn *et al*., 2003; Rortana *et al*., 2021; Tum, 2008).

1.3.3 Background to food safety in Cambodia

Previously published studies on food safety in Cambodia have focused on non-foodborne hazards (*e.g*. non-pathogenic food contaminants), laboratory-based studies, with few studies on antimicrobial resistance or studies on animals only. There is limited evidence on the health and economic impacts of FBD in Cambodia (Nguyen-Viet, 2018). A study by Roesel *et al*. (2018) found only 25 published articles regarding food safety in Cambodia since 1990s. A regional WHO study in 2010, which included Cambodia, found a substantial foodborne disease burden caused by biological rather than chemical hazards (Havelaar *et al*., 2015).

1.3.4 The burden of foodborne bacterial infection in Cambodia

In terms of food safety, Cambodians are among the most exposed citizens in Greater Mekong Sub-region (GMS) countries. This is due to high poverty and lack of proper food handling management (Sante, 2016; World Health Organization, 2016). Most diarrhoea cases are reported to be caused by foodborne bacteria (Paul Ebner, 2020). It has also been reported that the number of FBD outbreaks is increasing (MOH, 2020), although it is not known whether this is a true increase or due to better reporting. According to a study by the Mekong Institute in collaboration with the Ministry of Health in Cambodia, 135 foodborne disease outbreaks were officially reported from 2014 to 2019, affecting 5,825 individuals and leading to 81 deaths (MOH, 2021). In January 2022, multiple FBD cases were reported by the media, with at least 52 individuals infected (Battambong-News, 2022). These FBDs were reported to involve different kinds of food and drinks, including methanol poisoning, pesticides, homemade rice wine and foodborne pathogens (*Salmonella*, *Bacillus*, *Staphylococcus*, *Vibiros*) (Kimsean *et al*., 2017; Kunthear, 2022; Thul, 2016; Vandy *et al*., 2012). Studies have found that water spinach, the most important vegetable in Phnom Penh, which is cultivated in wastewater, can be highly contaminated with parasites, especially protozoa (*Giardia* and *Cryptosporidium* spp.), while levels of potentially toxic elements are generally below maximum tolerable limits. Prevalence studies in humans have detected high prevalence of *Salmonella enterica* (more than 70%) due to consumption of chicken and pork, *Campylobacter jejuni* (~50%) due to chicken consumption and *Opisthorchis viverrini* due to fish consumption (Lay *et al*., 2011; Roesel, 2018; Schwan *et al*., 2021; Trongjit *et al*., 2017).

1.3.5 Chicken meat consumption in Cambodia

In Cambodia, chicken is one of the most popular ASF as it is low-cost, widely available (Figure 1, Figure 2A) and provides protein and micronutrients essential for growth and health (Darapheak *et al*., 2013; Mulugeta, 2021; Sary *et al*., 2019). According to the annual report of the General Directorate of Animal Health and Production (GDAHP) in 2020, poultry meat accounts for almost 20% (62,000 tons) of total Cambodian meat consumption (301,000 tons) per year (GDAHP, 2021). The consumption rate is expected to increase by 5.5% annually in future, due to increased demand driven by population growth, urbanisation and increasing incomes (MAFF, 2015). Native black chicken is preferred by the locals due to its taste and is often produced as backyard chickens, usually by smallholders but sometimes by medium-scale producers (GDAHP, 2021; Mulugeta, 2021; PIN, 2015).

1.3.6 Pork consumption in Cambodia

Pork is more widely consumed than other ASF in Cambodia (Borin *et al*., 2010). Pork is affordable for most Cambodians and there is year-round availability and broad accessibility (Figure 2B). The most frequently consumed pork products are fried, boiled and dried pork. According to a previous study, pork is purchased mainly from wet markets (45.9%), street vendors (28.6%) and mobile vendors (21.1%) (Duong *et al*., 2022). People tend to select meat from stalls based on cleanliness, personal relationships, accessibility and price. The most important concerns among consumers regarding food safety relate to imported food and food additives, followed by pesticides, cancer-causing chemicals and preservatives (Duong *et al*., 2022). Bacterial hazards are of less concern than chemical hazards (Borin *et al*., 2010; Duong *et al*., 2021).

Figure 1. Traditional Cambodian market where fresh meats are sold.

Figure 2. Chicken (A) and pork (B) at a retail stall in a traditional Cambodian market.

1.3.7 Bacteria contamination and other hazards in ready-to-eat food

Salmonella can persist in the intestinal tract of animals without causing clinical signs, and contamination of the meat cannot be detected by macroscopic meat inspection (Kumar *et al*., 2020). Unhygienic practices at slaughter and at markets can be the source of *Salmonella* contamination of carcasses and of meat to consumers. Inappropriate raw pork consumption or use of the same knife and cutting board while preparing raw meat and vegetables for cooking can increase foodborne disease risks, but unfortunately food hygiene often receives insufficient attention since Cambodian consumers are more concerned about chemicals than microbial contamination in ASF (Brown *et al*., 2022; Duong *et al*., 2022; Duong *et al*., 2021; Schwan, 2020).

1.4 Current food safety policies in Cambodia

In July 2022, the Cambodia Government endorsed the first Food Safety Law of Cambodia, which represents a commitment to respond to these challenges and provides a farm-to-table framework for ensuring that food is safe for all (CAC, 2022). There are six ministries involved in governing food safety from farm to table, namely the Ministry of Commerce (MOC), Ministry of Health (MoH); Ministry of Agriculture, Forestry and Fisheries (MAFF); Ministry of Industry, Mines and Energy (MIME); Ministry of Trade (MoT); and Ministry of Industry, Science, Technology and Innovation (MISTI). The technical departments within each ministry has a different mandate, *e.g.* in MAFF, NAHPRI works under GDAHP to monitor animal health through the slaughter process and until the meat is sold at the market. The current situation in Cambodia needs to improve towards a risk-based approach and interventions in the retail stage of meat (FAO, 2019; Nguyen-Viet, 2020; Unger *et al*., 2020). A recent review stressed the importance of improved surveillance and targeted intervention research and suggested strengthening the country's current food safety regulatory structures (Thompson *et al*., 2021).

1.5 Quantitative microbial risk assessment

The Codex Alimentarius Commission risk analysis framework aims to ensure public health protection (CAC/GL-30, 1999). The overall microbial risk analysis is a process to ensure public health protection that consists of three components: risk assessment, risk management and risk communication (CAC/GL-30, 1999; Possas *et al.*, 2020). Microbial risk assessment is a developing science for establishing standards, guidelines and recommendations for food safety to increase consumer protection and facilitate international trade. The microbial risk assessment procedure involves either qualitative or quantitative estimation of potential adverse health effects associated with exposure of individuals or populations to hazards (materials or situations, physical, chemical and/or microbial agents) (Haas *et al*., 2014; Pérez-Rodríguez, 2020). There are four steps in quantitative microbial risk assessment (QMRA) according to CAC/GL-30 (1999), namely hazard identification and prioritisation; hazard characterisation; exposure assessment; and risk characterisation. These are described in the following sections.

1.5.1 Hazard identification and prioritisation

Hazard identification is a way to identify biological, chemical and physical agents capable of causing adverse health effects that may be present in a particular food or group of foods. Potential microbial hazards include bacteria and their toxins, viruses and parasites. Hazard identification is an initial effort to evaluate the public health significance of food hazards (CAC/GL-30, 1999). It is an important part of the process used to evaluate some adverse effects that may have the potential to cause harm to health. It is the first part of QMRA and can be modelled quantitatively or qualitatively (Sperber, 2001). Typical components of hazard identification are the prevalence of pathogenic microorganisms, the sources of pathogens, transmission routes, the survival rate of pathogens in foods, the adverse health outcomes resulting from infection and the population burden of disease (Pérez-Rodríguez *et al.*, 2014; Possas *et al.*, 2020).

1.5.2 Hazard characterisation

Once a hazard/pathogen has been identified, information on the basic characteristics of the hazard is gathered (Crawley & Tyler, 2003). Hazard characterisation involves qualitative and/or quantitative evaluation of the nature of the adverse effects associated with biological agents that may be present in food (CAC/GL-30, 1999; Perez-Rodriguez, 2020). This aids in subsequent steps of the assessment, such as deciding on the method for identifying the pathogen. The characteristics of a hazard will vary depending on the type of microorganism and will comprise a combination of genotypic and phenotypic characteristics (Lammerding & Fazil, 2000; Possas *et al.*, 2020). For bacterial foodborne pathogens, the general pathogen characteristics of interest may include methods for detecting microorganisms that have a negative effect on food and human health (CAC/GL-30, 1999).

1.5.3 Exposure assessment

Exposure assessment aims to study the primary critical step/activities/ behaviour that could cause bacteria to cross-contaminate along the chain from farm to fork (CAC/GL-30, 1999). Use of models in exposure assessments for QMRA is sometimes complex, due to the aim to evaluate public health status regarding a specific biological hazard in a food product (Oscar, 2021b). Experiments are usually designed to assess response at relatively high doses (microbial detection limit and a limited number of experimental units), and a minor frequency of infection or illness cannot be precisely assessed. Some pathogens can infect humans exposed to only a single dose (Bernstein *et al*., 2020; Oscar, 2004, 2021b).

1.5.4 Risk characterisation

Risk characterisation, the final step of QMRA, is the biological foundation for dose-response models and covers major components of the disease process: exposure, infection, illness and consequences (recovery, sequelae or death) (CAC/GL-30, 1999). The risk outcome results from the interactions between the pathogen, the host and the food matrix, giving the daily incidence to annual incidence of disease due to the pathogen under study (Perez-Rodriguez, 2020). Mathematically, there is always a non-zero probability of infection or illness when a host is exposed to an infectious pathogenic organism (CAC/GL-30, 1999; Wagner & Hensel, 2011; WHO, 2009). Stochastic risk assessment involves a variety of calculations in modelling the probability of illness using different parameters, including the concentration of the hazard, frequency and amount of ingestion, probability of contamination, storage temperature and the time until consumption (Makita *et al*., 2019).

2. Aims of the thesis

The main aims of this thesis were to estimate the risk of salmonellosis along the ASF value chain, from market to household, through consuming chicken and pork salad; and to gather evidence on food-borne bacteria, hazardous hygiene in markets and household food preparation in order to guide food safety policies in Cambodia. The intention was to produce information that can help policymakers improve food safety standards and reduce the risk of foodborne diseases. Specific objectives were to:

1. Determine the prevalence of *Salmonella* and *Staphylococcus aureus* on chicken meat and pork in Cambodian markets.
2. Assess cross-contamination of *Salmonella* from raw chicken and pork during handling and preparation of RTE chicken and pork salad.
3. Identify common chicken and pork salad preparation practices and consumption patterns in Cambodian households.
4. Estimate the daily and annual incidence of salmonellosis resulting from chicken and/or pork salad consumption in Cambodia.

3. Materials and methods

3.1 Study design and frameworks

3.1.1 Study design

The research in this thesis was conducted as part of the Safe Food, Fair Food for Cambodia project (2017-2021), which aimed to identify food safety impacts and barriers using system effects modelling. In the studies described in Papers I-III, QMRA was performed according to the four steps (hazard identification and prioritisation, hazard characterisation, exposure assessment, risk characterisation) in the risk assessment framework established by Codex Alimentarius Commission (CAC/GL-30 1999) (Figure 3). Hazard identification and prioritisation were conducted during a stakeholder meeting in Cambodia in 2018, which identified two common foodborne pathogens, *Salmonella* and *S. aureus* (possibly linked to traditional markets) as priorities. A cross-sectional study was conducted on chicken and pork from traditional markets in Cambodia to determine the prevalence of these two pathogens (Paper I). *Salmonella* was found to have the highest prevalence and was selected for hazard characterisation in QMRA on the risk of *Salmonella* transmission along the Cambodian food chain, from market to consumption at the household level. In exposure assessment, an experiment was conducted on cross-contamination with *Salmonella* when preparing chicken salad (Paper II). A similar study has been performed previously in Vietnam on boiled pork (Dang-Xuan *et al*., 2018). Based on that study, four scenarios were used in Paper II to investigate cross-contamination with *Salmonella* when preparing chicken salad at household level. These scenarios were initially developed for commonly consumed boiled pork salad (Dang-Xuan *et al*., 2018), which has the same procedure of preparation, consumption and likely cross-contamination as chicken salad.

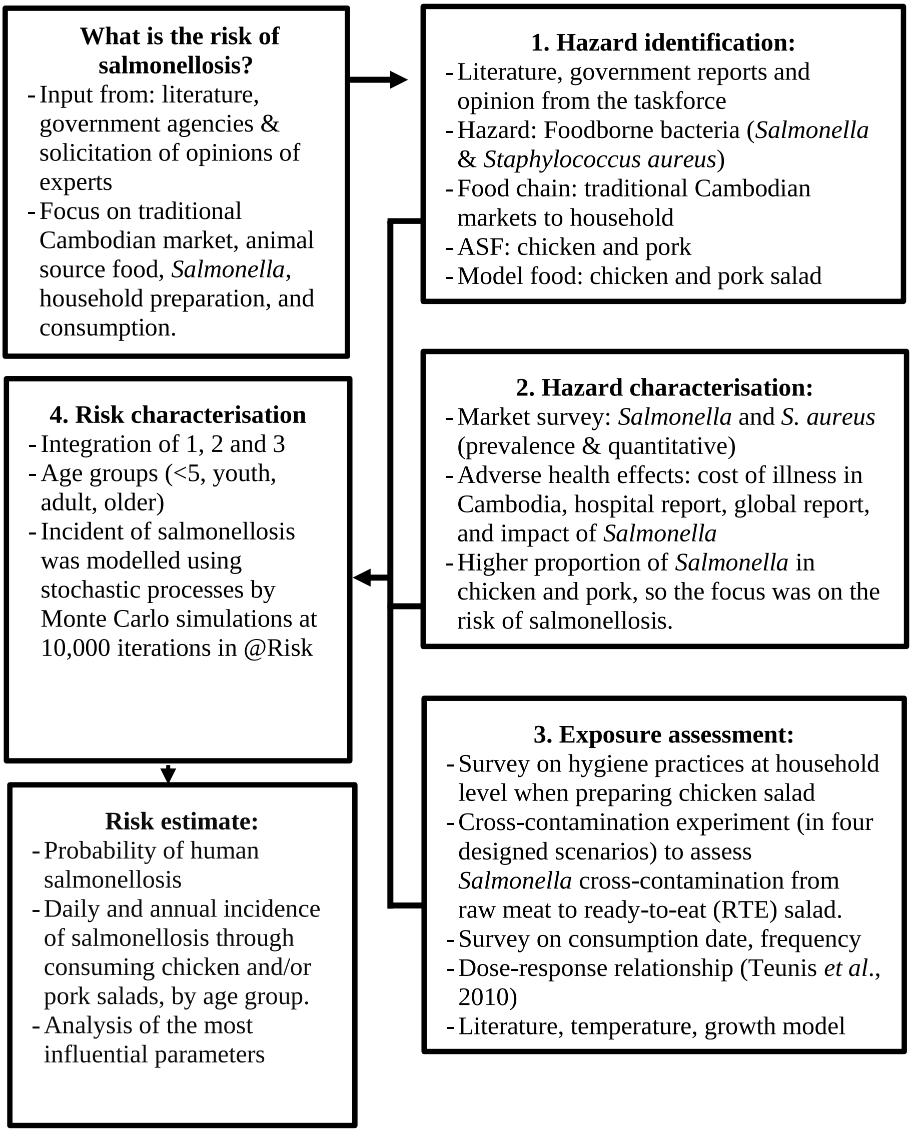

Figure 3. Study framework used in risk assessment of salmonellosis in Cambodia from traditional markets to household consumers.

In risk characterisation, data from the literature and results from Papers I and II were used to build a model for estimating daily and annual incidence of salmonellosis and to assess the influence of various parameters on the risk of contracting salmonellosis after consuming chicken and/or pork salad in Cambodia (Paper III).

3.1.2 Study location and climate

The field work in this thesis was conducted in Cambodia, which is located in the centre of the Mekong sub-region in Southeast Asia (Figure 4). There are 25 provinces/municipalities in Cambodia, all of which were included in this study. Cambodia is influenced by tropical monsoon winds, which define the two main seasons: a dry season from November to April and a rainy season from May to October. In the rainy season, the average temperature in 2019 was 29 °C (range 27-36 °C), with humidity of between 45 and 80 % (DoM, 2019). In 2019, the total population of Cambodia was around 15 million (NIS, 2019).

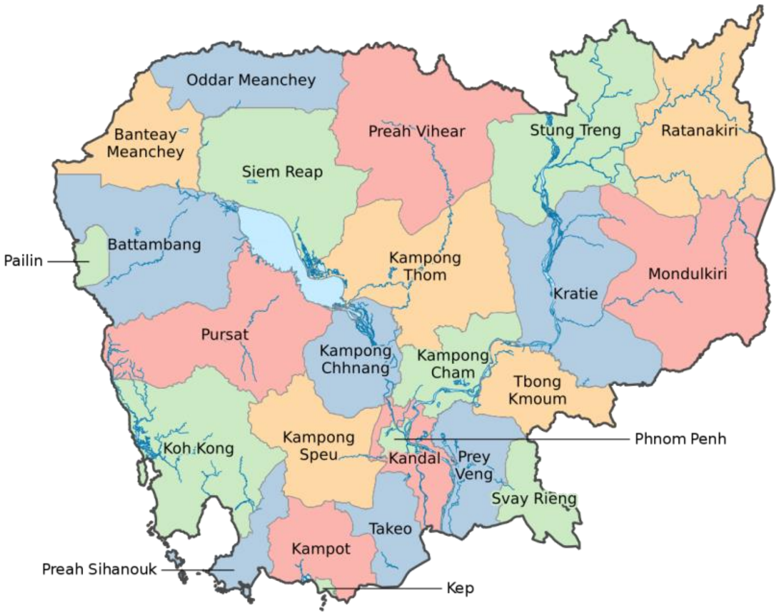

Figure 4. Map of Cambodia and its 25 provinces.

The hazard identification survey was conducted in 52 markets located in all 25 provinces, to represent the entire country (Paper I). Information on how chicken salad is prepared was collected in household surveys conducted in four provinces (Battambang, Preah Sihanouk, Phnom Penh and Siem Reap) (Paper II). These four provinces have the highest population density in Cambodia and can therefore be considered to represent the general Cambodian consumer.

3.2 Hazard identification and prioritisation

3.2.1 Workshop on hazard identification and prioritisation

Hazard identification and prioritisation work involved stakeholders from the government, non-government organisations (NGOs) and academia, and aimed to obtain information about the most relevant bacteria and parasite for food safety in Cambodia. An initial workshop was held in 2018 with the Safety Food Fair Food research team from Cambodia and various stakeholders within the Food Safety Taskforce. There were 43 participants in the Food Safety Taskforce, including Cambodia's Technical Working Group on Food Safety, researchers from ILRI, NAHPRI, CelAgrid, University of Health Sciences, Cambodia (UHS), Royal University of Agriculture, Cambodia (RUA), Food and Agriculture Organization of the United Nations (FAO), WHO, Institute Pasteur du Cambodge (IPC), Department of Communicable Disease Control (CDC) of Cambodia, Directorate General of Consumer Protection, Competition, Fraud Repression (CCF) and other national partners (ILRI, 2018; Lam *et al*., 2019). At the workshop, risk profiles were concisely presented to the participants, including results published earlier (Roesel, 2018). This was followed by another workshop which focused on how to prioritise microbiological hazards for ASF at traditional markets. That workshop provided fundamental principles for risk analysis, with specific experiences in food safety risk communication in Vietnam and Africa. During the workshop, participants were requested to give input on designing the studies included in this thesis, *e.g*. regarding prioritisation of pathogens, geographical setting, types of markets to include and stages to cover in the

ASF value chain (ILRI, 2018; Nguyen-Viet, 2018). Only samples from the traditional market were included in the QMRA model.

3.3 Hazard characterisation

3.3.1 Prevalence of *Salmonella* and *Staphylococcus aureus*

In Paper I, three pork and three chicken meat sellers at each market were selected for sampling between October 2018 and August 2019, using systematic random sampling by shop location in the meat area of the market, beginning from the main entrance gate and ending at the rear end, in order to collect samples representing the entire market (Figure 5). One sample of either chicken or pork was obtained from each selected seller. A total of 532 samples were collected from 52 traditional markets in 25 provinces, and five supermarkets in two provinces. These samples consisted of chicken meat (n=204), chicken cutting board swabs (n=64), pork meat (n=204) and pork cutting board swabs (n=64). Approximately 300-400 grams of meat were purchased from each seller or supermarket. At each market, one cutting board used for chicken and one for pork was swabbed (Paper I).

3.3.2 *Salmonella* culturing

All samples analysed in Paper I and Paper II were subjected to bacteriological culturing according to the ISO procedure ISO-6579:2002/amended:1:2017 (ISO6579-1:2007, 2007; ISO6579, 2002), to identify the presence or absence of *Salmonella*. In Paper I, culturing was performed on chicken meat, swabs from chicken cutting boards, pork meat and swabs from pork cutting boards. In Paper II, *Salmonella* culturing was performed on samples collected at different stages within the cooking procedure in the four scenarios studied (see section 3.4.3). In brief, each sample was subjected to pre-enrichment, selective enrichment and selective culture on XLD agar and second agar (MacConkey agar). Presumptive *Salmonella* colonies were confirmed using biochemical tests (see Paper I for details). All laboratory work was performed at the Bacteriology Laboratory of NAHPRI, Phnom Penh, Cambodia.

3.3.3 Most probable number of *Salmonella*

Analysis of most probable number (MPN) was used to determine the quantity of *Salmonella* per gram of chicken and pork (Paper I) and per gram of RTE chicken salad (Paper II), using a conventional three-tube MPN method described previously (Pavic *et al*., 2010). In brief, 25 g of sample were suspended in 225 mL of Peptone Buffer Water (PBW) and 1 mL portions were taken from this and added serially to each of three tubes containing 9 mL of BPW, thus creating a set of three MPN tubes with dilutions of 10^{-1}, 10^{-2}, and 10^{-3}. *Salmonella* was confirmed using the ISO method referred to in section 3.3.1. MPN index was calculated according to De Man (Blodgett, 2010; De Man, 1983).

3.3.4 Isolation of coagulase-positive staphylococci and *Staphylococcus aureus*

In Paper I, all samples were tested for presence/absence of staphylococci and enumeration was carried out for coagulase-positive staphylococci (CPS). Culturing was performed according to the ISO 6888-1:1999 (which includes amendment A1: 2003) using Baird-Parker (BP; Oxoid, Milan, Italy) agar medium (ISO6888-1, 1999, 2003) and 25 grams of individual samples (chicken meat and pork). Around five typical presumptive colonies of staphylococci were selected for coagulase testing using rabbit serum plasma (BD, USA) and quantity of CPS was calculated according to instruction 10.1.1 of ISO 6888-1:1999 (ISO6888-1, 1999). The presumptive colonies of staphylococci were streaked onto nutrition agar plates and incubated at 37 °C for 24 hours. Presence of *S. aureus* was confirmed using Gram staining (Merck, Germany), oxidase test (Merck, Darmstadt, Germany) and catalase test, and species identification was performed by latex agglutination (Biomerieux SA, Craponne, France) (Essers & Radebold, 1980).

3.4 Exposure assessment

3.4.1 Practices used for preparing chicken and pork salads

The exposure assessment was based on results from quantification of *Salmonella* cross-contamination when preparing chicken RTE salad, the procedure used for cooking chicken salad and data on consumption (frequency and quantity). In 2020, 12 focus group discussions (FGDs) were conducted among 93 households in four provinces (Siem Reap, Preah Sihanouk, Battambang and Phnom Penh) (Figure 6). The discussions focused on hygiene practices during purchasing, storing and washing chicken carcasses and vegetables, using and cleaning kitchen utensils, and hand washing when preparing the traditional chicken salad dish *(ngam sach man sroyong chek*) in participants' homes. There were 6-8 participants in each FDG, including one adult member (aged over 18 years) from each household who frequently purchased and prepared food in the home. The FGDs were conducted in the Khmer language, and each took approximately 1.5 to 2 hours. There were two facilitators and one note-taker at each FGD. Transcripts were translated into English before use in quantitative analysis (Paper II). The survey on cross-contamination when preparing boiled pork (in Vietnam) and chicken salad (in Cambodia) was similar to the previous study in Vietnam on pork RTE salad, which is described in detail by Dang-Xuan *et al*. (2018).

Table 2. The four scenarios for preparing chicken and pork salad

Practices	Scenario			
	1	**2**	**3**	**4**
Cut the meat into pieces	X	X	X	X
Wash vegetables twice with water and slice them into small pieces for salad	-	-	X	X
Debone and cut the boiled meat into small pieces and mix with prepared vegetables using the same (but washed) cutting board, knife and hands.	X	-	X	-
Debone and cut the boiled meat into small pieces and mix with prepared vegetables using a different cutting board and knife, and washing hands	-	X	-	X
Mix and place ready-to-eat salad on the plate	X	X	X	X

Figure 5. Collection of cutting board swabs and meat sampling.

Figure 6. Focus group discussion on practices used in preparation of chicken salad.

3.4.2 Preparation of chicken and boiled pork salads

Cambodian chicken salad was prepared according to the four scenarios described in Table 2, Figure 7 and Figure 8 left. For all scenarios, whole chicken carcasses were bought from traditional markets and immediately brought to the laboratory for analysis. The carcasses were inoculated with *Salmonella* (concentration of 10^4 CFU/mL) using the pipetting technique, dropping inoculum to cover all parts of the carcass (a 1200 gram carcass was inoculated with 1200 µL *Salmonella* medium). This was followed by preparation of chicken salad. Fresh vegetables were always washed and cut into small pieces. The chicken carcass was boiled for 20 minutes, while the approximate time for preparing each chicken salad was 1-2 hours. All four scenarios were carried out on the same day and repeated nine times per scenario (Paper II). *Salmonella* cross-contamination from raw pork to boiled pork (Figure 8 right) via hands and kitchen utensils was examined in a similar way as in the previous study in Vietnam (Dang-Xuan *et al*., 2018).

Figure 7. General procedure for preparing chicken salad considered in this thesis.

Figure 8. Images of (left) chicken salad and (right) boiled pork. m

3.4.3 Culturing and quantifying *Salmonella* in ready-to-eat chicken salad

The four experimental scenarios compared in Paper II were designed to imitate the process of preparing chicken salad. There were two types of sampling, swab sampling and sampling 25 g of RTE salad (meat and vegetables). The scenarios, points of sampling and sample types are listed in Table 3. Similar sampling of pork and RTE was conducted in the study of boiled pork in Vietnam by Dang-Xuan *et al*. (2018).

Table 3. Types of samples collected in the experiment on chicken salad

Practices	Preparation stages in each scenario and no. of samples				Total no. of samples
	1	**2**	**3**	**4**	
Cut chicken carcass into smaller parts[1]	9	9	9	9	36
Wash vegetables twice with water and slice them into small pieces for salad	-	-	9	9	18
Debone and cut the boiled chicken into small pieces and mix with prepared vegetables using the same (but washed) cutting board, knife and hands[2].	27	-	27	-	54
Debone and cut the boiled chicken into small pieces and mix with prepared vegetables using a different cutting board and knife, and washing hands[2]	-	9	-	9	18
Mix and place chicken salad on the plate[3]	9	9	9	9	36
Total no. of samples	45	27	54	36	162

Note: [1] swab of 25 cm^2 of chicken surface; [2] only swabs (from the most contaminated parts of the object) were sampled; [3] 25 g of RTE salad.

3.4.4 *Salmonella* dose-response assessment

For the dose-response assessment (Paper III), the dose-response curve used was from a *Salmonella* outbreak in the USA. The model used in this thesis had an infection ID50 of 7 CFU and an illness dose at ID50 of 36 CFU. In brief, a Beta-Poisson dose-response model (Teunis *et al*., 2010) with alpha equal to 0.00853 and beta equal to 3.14 was selected for the QMRA study (see Paper III for details on the QMRA model).

A survey was conducted to determine consumption of chicken and pork salad, using data from the FGDs described in section 3.4.1. Three additional FGDs (with participants chosen to represent rural, peri-urban and urban areas) in each of the four provinces were conducted by randomly selecting households within one commune. A discussion outline was developed in English, translated to Khmer for the FGD and back-translated into English for analysis. Each FGD was led by trained researchers using flipcharts and notes and lasted about 1.5 h. Information on chicken and pork salad consumption was compiled for young children (<5 years), older children (6-15 years, adults (16-60 years) and the elderly (above 61 years).

3.5 Risk characterisation

3.5.1 Quantitative microbial risk assessment of salmonellosis

Results obtained from the steps described in sections 3.3-3.4 of this thesis were integrated into a QMRA through stochastic modelling (Dang-Xuan *et al*., 2017; Makita *et al*., 2019), including different input variables to reflect the risk pathway that led to risk characterisation in Paper III. The risk outcomes considered were the probability of illness per day (daily incidence) and per year (annual incidence). The parameters, statistics, distribution and data sources used in the QMRA model are presented in Paper III. Data obtained in Paper I on the prevalence of *Salmonella* from samples collected at the traditional markets was used to represent bacteria contamination in fresh chicken meat and pork. The parameters included were the rate of *Salmonella* entering chicken and pork salad estimated at the household level, the temperature at the study site, the duration of storage until cooking, and the level of *Salmonella* in RTE chicken salad (data from Paper II) and boiled pork salad (data from Dang-Xuan *et al*., 2018). Data on consumption rates, including how often people in different age groups consume chicken/pork salad were taken from the survey in Paper III. Sensitivity analysis to determine the most influential factors was conducted using @Risk (Oscar, 2021a; Swart *et al*., 2016).

3.5.2 Data analysis

All data from Papers I-III were entered in MS Excel (Office 365) for storage, management and cleaning before being used in data analysis software. Some of the data from Papers I-III were analysed descriptively (proportion, mean, standard deviation).

In Paper I, the prevalence of the different sample types and bacteria was calculated using Pearson Chi-square. Multi-level logistic regression was used for investigating associations between the prevalence of bacteria and market type, season, sample type and species. The number of colony-forming units (CFU) of CPS was converted to log CFU/g, to generate a more normal distribution, with the value zero replaced with "1", and the CFU/g values were compared using linear regression. All statistical analyses were performed in EpiInfo™, an open-source domain of software tools (CDC, USA) and RStudio (R core team). A *p*-value of 0.05 was used for statistical significance, with no compensation for multiple comparisons.

In Paper II, the Chi-squared test or Fisher exact test was used to evaluate proportion of *Salmonella* cross-contamination for different sample types and scenarios. *Salmonella* concentration in the chicken salad in each scenario was described using the distributions of both non-parametric and parametric bootstrapping techniques. A Bayesian statistic was used to assess variability and uncertainty during simulation of *Salmonella* load and reduction rates. The parameters and functions used to carry out the bootstrapping and simulated sample data distributions followed the procedure described in Dang-Xuan *et al*. (2018). The function fitdist() in the *fitdistrplu*s package in R was used to estimate the mean and standard deviation of *Salmonella* CFU/g (Core, 2015). For visualisation of distributions, kernel density was calculated in the density() function, based on the simulated sample data, and plotted using R.

In Paper III, the QMRA model was developed using a stochastic approach, and Monte Carlo simulation was performed using Microsoft Excel with add-in @Risk software (Version 8.2, Palisade, Corporation, USA) for 10,000 iterations. The sensitivity analysis was conducted by selecting all uncertainty parameters and running 1000 iterations at seven quantile values. Consumption, prevalence and concentration of pathogen were described as mean and median. Final risk estimates were calculated as mean and median with 90% confidence interval (CI) (Paper III).

3.6 Ethical and biosafety approval

The research was conducted with the approval of the General Directorate of Animal Health and Production (the authority of Cambodia's animal health and production sector), dated 12 October 2018. The National Ethical Committee of Cambodia granted ethical approval for interview participants, focus group discussions and data collection from the participants, as stated in a letter coded 300NECHR, dated 26 December 2017. The International Livestock Research Institute approved biosafety compliance for the laboratory testing, in letter ref: ILRI, RC-010-18/IBC/010/CR, dated 5 July 2018. Each FGD was conducted after obtaining written consent from the participants, facilitated by a senior researcher, while another researcher took notes and recorded the discussion.

4. Results and discussion

4.1 Hazard identification and prioritisation

The hazard identification and prioritisation workshop and activities described in Chapter 3 of this thesis resulted in identification of *Salmonella, E. coli, Bacillus*, *Staphylococcus,* and pesticides as the most critical hazards in ASF at traditional markets and slaughterhouses in Cambodia. Final recommendations were to focus on the foodborne bacteria *Salmonella and S. aureus* in chicken meat and pork from traditional Cambodian markets, based on the facts that they have a great public health impact according to available data and that they are frequently present on ASF in traditional markets (Grace, 2015). Another recommendation was to include RTE chicken and pork salads in the analysis, since these are commonly consumed dishes in Cambodia and are often prepared at household level using raw meat and fresh vegetables from traditional markets. As a complementary activity, assessments were conducted on important parasitic foodborne diseases associated with pork (cysticercosis, trichinellosis and the viral disease Japanese encephalitis), but these diseases are not covered in this thesis.

There are many possibilities for bacterial cross-contamination when preparing RTE foods. In addition, there is no step that inactivates bacteria on the vegetables used in RTE chicken and pork salad. Lastly, there are obvious food safety knowledge gaps along the chain from market to household (Brown *et al*., 2022). Following discussions held during the project taskforce meeting, it was agreed that the risk profile should prioritise the part of the meat value chain from the market to the consumer (household level) (ILRI, 2018; Nguyen-Viet, 2018). The findings were expected to help understand the impact and components of resilience, in order to inform the design of food safety strategies.

4.2 Hazard characterisation

4.2.1 Overall prevalence of *Salmonella* and *Staphylococcus aureus* in Cambodian markets (Paper I)

In total, 42.1% (224/532) of the samples analysed (chicken, chicken cutting board, pork, pork cutting board) tested positive for *Salmonella* and 29.1% (155/532) for *S. aureus*, while 14.7% (78/532) of the samples tested positive for both *Salmonella* spp. and *S. aureus*. The prevalence of *Salmonella* was significantly higher than that of *S. aureus* in both chicken and pork samples ($p<0.001$). Overall, at traditional markets in Cambodia the prevalence of *Salmonella* spp. in chicken meat was 42.6% (87/204) and in pork 45.1% (92/204) (Table 4). The *Salmonella* prevalence values were similar to those reported in recent studies in Vietnam, where *Salmonella* prevalences of 45.9% was detected in 900 chicken samples (Ta *et al*., 2012) and of 44.7% in 217 pork samples (Dang-Xuan *et al*., 2019). Other studies have reported *Salmonella* prevalence of 23% in chicken meat from 145 samples collected in the Cambodia-Thailand border region (Trongjit *et al*., 2016) and 88% in 152 poultry carcasses from 10 markets in retail outlets of Phnom Penh (Lay *et al.*, 2011). Due to limited resources, the current inspections for hygiene indicators in Cambodia only screen for *Salmonella* in meat and other ASF (GDAHP, 2021).

Among 36 samples collected from six supermarkets in Cambodia, the prevalence of *Salmonella* spp. was 16.7% (3/18) in chicken and 38.9% (7/18) in pork. *Staphylococcus aureus* was not found in chicken and only in 5.6% (1/18) of pork samples. Only one pork sample tested positive for both *Salmonella* spp. and *S. aureus* (1/18, 5.6%). Thus, chicken and pork meat sold in supermarkets had much lower prevalence of *Salmonella* compared with similar products sold in traditional markets. However, the numbers of samples from supermarkets were much lower, which might have biased the results. In comparison, a study in Bangkok, Thailand, found high prevalence of *Salmonella* spp. in chicken collected from traditional markets (48%, n=61) and supermarkets (57%. n=75), (Minami *et al*., 2010).

Table 4. Prevalence of *Salmonella* in chicken and pork in Cambodian traditional markets (values used risk characterization). MPN = most probable number

Sample type	No. of *Salmonella* positive/Total number	Prevalence (95% CI)	Average MPN/g (min-max)
Chicken meat	87/204	42.6 (35.8–49.7)	16.7 (0.36–120)
Pork	92/204	45.1 (38.2 – 52.2)	17.3 (0.36–120)

4.2.2 Most probable number of *Salmonella* (Paper I)

For the 136 samples that were subjected to quantification, the *Salmonella* MPN/g values obtained were divided into four groups: <0.03, 0.03-3.0, 3.1-30, and ≥30.1. Most of the pork and chicken samples fell into the <0.03 and 0.03-3.0 MPN/g groups and only meat samples from traditional markets showed MPN/g ≥30.1. The average *Salmonella* MPN/g value in chicken meat from traditional markets was 16.7 (0.36-120) and in pork 17.3 (0.36-120) (Table 2). These results can be compared with those in a study in China which also showed high levels of *Salmonella* in chicken, with more than half of *Salmonella* samples having concentrations higher than 0.7 MPN/g (Wang *et al*., 2014). Studies in Vietnam have found a high *Salmonella* concentration (3.0-30 MPN/g) in cut pork from traditional markets (Dang-Xuan *et al*., 2019; Ngo *et al*., 2021). The similarities between the results for Cambodia and Vietnam are likely due to similar slaughterhouse environments, transportation selling periods, and market temperature control (Dang-Xuan *et al*., 2018).

4.3 Exposure assessment

4.3.1 Practices used for preparing salad in Cambodian households (Paper II)

A majority (86%, 80/93) of the households surveyed reported that they first washed chicken carcasses two to three times with water before washing and preparing vegetables. Only 14% (13/93) of households washed and prepared vegetables before washing chicken carcasses. All household participants washed knives and cutting boards at least once, with soap or dishwashing detergent, immediately after cutting fresh chicken carcasses.

However, almost all (97%, 90/93) used the same knife and cutting board to prepare raw vegetables and chicken carcasses, and to prepare raw and boiled chicken, while use of separate knives and cutting boards for raw and cooked chicken was rare (3.2%, 3/93).

It was found that washing chicken carcasses twice in clean water reduced *Salmonella* by 92.2%. However, for a few carcasses the levels of *Salmonella* did not decrease substantially, which is similar to results presented in other studies (Bernstein *et al*., 2020; Dang-Xuan *et al*., 2018; IFIS-USDA, 2021). Rinsing chicken is generally not recommended, as some studies suggest that rinsing contaminated carcasses can spread *Salmonella* to the environment (Carrasco *et al*., 2012; Rouger *et al*., 2017). According to Dang-Xuan *et al*. (2018), household pork handling practices and the prevalence of *Salmonella* in pork in markets are the most important factors contributing to the annual incidence of human salmonellosis from consuming boiled pork in Vietnam. Cambodia and Vietnam have similar food handling and consumption behaviours (Dang-Xuan *et al*., 2018), so it can be assumed that risks associated with meat handling and consumption behaviour in Cambodia are similar to those reported in Vietnam. The prevalence of *Salmonella* in chickens at the retail stage has been found to be predominantly important in determining the probability of illness in South Korea (Jeong *et al*., 2018).

4.3.2 Chicken and pork salad consumption in households (Paper III)

The consumer survey on chicken salad consumption showed that the frequency of eating chicken and pork salad was 0.8 and 0.9 times/per month, respectively (Table 5). The average amount of chicken and pork salad consumed was 141 and 124 grams per person and meal, respectively (Paper III). The results in Paper III revealed that meat consumption in Cambodian households was higher than previously reported (Borin *et al*., 2010; Reinbott & Jordan, 2016). According to a report by GDAHP covering the period 2015-2020, average meat consumption in Cambodia is 18 kg per person and year, with consumption increasing year on year. This increasing demand for meat and other ASF is pushing authorities to pay more attention to the safety and quality of ASF (GDAHP, 2021).

Table 5. Frequency and average amount of chicken and pork salad consumed by different age groups in Cambodian households

Variable	Chicken salad	Pork salad	Either pork or chicken salad
Frequency of consumption by age group [times/month, median (min-max)]			
Young children (<5 years)	0 (0 - 1.6)	0 (0 - 3.3)	0 (0 - 3.3)
Older children (6-15 years)	0.3 (0 - 3.3)	0.8 (0 - 3.3)	1 (0 - 6.6)
Adults (16-60 years)	0.3 (0 - 12)	0.8 (0 - 12)	1 (0 - 24)
Elderly (>61 years)	0.5 (0 - 2.5)	0.6 (0.1 - 2.5)	1.5 (0.2 - 5)
Overall	0.8 (0-12)	0.9 (0-12)	1.6 (0-24)
Average consumption amount [g/person/meal, (mean ± standard deviation)]			
Young children (<5 years)	46 ± 22	46 ± 20	46 ± 20
Older children (6-15 years)	93 ± 62	93 ± 65	92 ± 59
Adults (16-60 years old)	124 ± 71	141 ± 79	134 ± 70
Elderly (>61 years old)	85 ± 62	81 ± 51	83 ± 54
Overall	141 ± 79	124 ± 71	130 ± 75

4.3.3 *Salmonella* contamination from raw meat during preparation (Paper II)

Cross-contamination between raw meat, vegetables, hands, cutting boards and knives while preparing chicken salad was investigated. After washing chicken carcasses twice, *Salmonella* was isolated from 32 out of 36 chicken samples (88.9%, 95% CI: 73.0-96.4). There was slightly lower cross-contamination of *Salmonella* from chicken meat to salad in scenarios that added vegetables (11.1%, 95% CI: 1.9-36.1). Among all 36 samples, the presence of *Salmonella* during chicken salad preparation was found to be on hands after one wash (22.2%), on hands during preparation of salad (23.7%), on knives (50.1%), and on cutting boards (65.2%). Cross-contamination from raw meat along the food chain has been identified in other studies as one of the most critical steps in QMRA (Carrasco *et al.*, 2012; Kusumaningrum *et al.*, 2003; Maffei *et al.*, 2017; Pérez-Rodríguez *et al.*, 2014). In Paper II, clean drinking water was used for rinsing and cleaning, which may not be the case in all households in Cambodia. Several studies have shown that cross-contamination is an important factor behind the transfer of bacteria from raw meat to food ingredients during food preparation, which could contribute to unsafe food (Carrasco *et al*., 2012; Swart *et al*., 2016).

4.3.4 Cross-contamination of *Salmonella* from raw chicken to ready-to-eat chicken salad (Paper II)

Analysis of cross-contamination in the four scenarios showed that most *Salmonella* (7 positive out of 9 experiments) was transmitted to chicken salad in scenario 1. The average quantity of *Salmonella* in that scenario was 37.3 MPN/g. Based on the simulations, the levels of *Salmonella* contamination in scenarios 1, 2 and 3 was 77.8, 11.1 and 22.2 CFU/g, respectively, which exceeded the initial 10 CFU/g from the inoculation. In scenario 4, there was no *Salmonella* cross-contamination to the RTE salad. The increasing numbers of *Salmonella* during salad preparation can have been due to factors such as an increase in time, temperature and co-contamination from other sources (Carrasco *et al*., 2012; Kusumaningrum *et al*., 2003)

In Cambodia, previous foodborne outbreaks have been reported to be associated with contamination during food preparation (Kimsean *et al*., 2017; MOH, 2020, 2021). Chicken meat and pork salad (sometimes also made from other types of meats) are consumed in households, in restaurants or at ceremonies in Cambodia (Baker, 2009; Buntha, 2016; Kimsean *et al*., 2017; Vandy *et al*., 2012), and also in Southeast Asia and Middle Eastern countries (Habib *et a*l., 2020). Foodborne disease cases have been associated with factors such as cooking procedures, hygiene practices and consumption behaviours (Kimsean *et al*., 2017; Vandy *et al.*, 2012). Cross-contamination has also been reported in earlier studies to be caused by inappropriate storage facilities for ASF, poor cleaning and disinfection, and insufficient hygiene practices (Aizaabi & Khan, 2017; Carrasco *et al*., 2012; Nair & Johny, 2019; Nguyen-Viet, 2020). Similarly, Paper II showed that handling and preparing raw meat (including practices related to washing and cutting) increased *Salmonella* contamination on hands, cutting boards, knives and vegetables, and further transmission of *Salmonella* to RTE salad from these items is highly likely (Dang-Xuan *et al*., 2018; Shiowshuh & Cheng-An, 2011; Van Asselt *et al*., 2008). Cross-contamination from raw meat along the food chain is an important step in QMRA (Carrasco *et al*., 2012; Kusumaningrum *et al*., 2003; Maffei *et al*., 2017; Pérez-Rodríguez *et al*., 2014). Clean and *Salmonella*-free water was used for rinsing and cleaning in Paper II. Some other studies have also found that cross-contamination is an important factor in transfer of bacteria from raw meat to food ingredients during food preparation compromising food safety (Carrasco *et al*., 2012; Swart *et al*., 2016).

4.4 Risk characterisation of salmonellosis

4.4.1 Daily incidence of salmonellosis acquired through chicken and/or pork salad consumption in Cambodia

The risk of salmonellosis from salad consumption was higher for chicken than for pork salad. On average, around 4 per 10,000 persons were at risk of contracting salmonellosis on a daily basis due to consumption of chicken salad (90% CI: 2.1-13.4). Table 6 shows the daily incidence of salmonellosis for the various age groups surveyed. The daily incidence was higher for the adult group than for the other age groups, including through consumption of chicken salad (2.1%), pork salad (1.8%) and both chicken and pork salad (6.4%).

Table 6. Daily incidence (by age group) of human salmonellosis acquired through consuming chicken and pork salad in Cambodia

Age group	Estimated daily incidence (%) of human salmonellosis, cases per 10,000 people (mean (90% CI)) (%)		
	Consumption of chicken salad	Consumption of pork salad	Consumption of chicken and pork salad
Young children (<5 years)	1.7 (0-10.5)	0.5 (0-3.6)	2.0 (0-8.0)
Older children (6-15 years)	2.1 (0-12.1)	0.8 (0-5.8)	2.5 (0-9.5)
Adults (16-60 years)	5.2 (1.2-20.0)	1.8 (0-10.9)	6.4 (0-18.0)
Elderly (>61 years)	2 (0-12.2)	0.6 (0-3.8)	2.4 (0-9.3)
Aggregated	3.9 (2.1-13.4)	1.3 (0.1-7.1)	4.8 (0-12.3)

An earlier study in Cambodia reported cases of *Salmonella enterica* serovar Paratyphi infections among humans in Phnom Penh (Vlieghe *et al*., 2013). According to the Ministry of Health of Cambodia, 134 outbreaks were reported between 2014 and 2019, with 5,825 cases not hospitalised, 5,598 hospitalised and 81 deaths. However, there was likely under-reporting of FBD outbreaks, as people with mild symptoms probably did not seek healthcare. The Ministry of Health has acknowledged the importance of FBD and has established and strengthened the Foodborne Disease Outbreak Investigation and Response Team (FORT) to respond to FBD outbreaks (MOH, 2020, 2021). Traceback of FBD outbreaks in Cambodia has generally revealed poor hygiene practices in food preparation, elevated storage temperatures, poor cooking practices, cross-contamination, use of unsafe water and bacterial contamination of the food (Kimsean *et al*., 2017; MOH, 2021; Vandy *et al*., 2012).

Simulation results from Paper III of daily incidence of salmonellosis acquired through consuming chicken salad, pork salad and both these types of salad, including all parameters and their distributions, are shown in Figures 9, 10 and 11, respectively. The graphs in Figure 9 and Figure 10 are skewed to the left, which means that for most people the risk is low, but that a few people are randomly exposed to a higher risk.

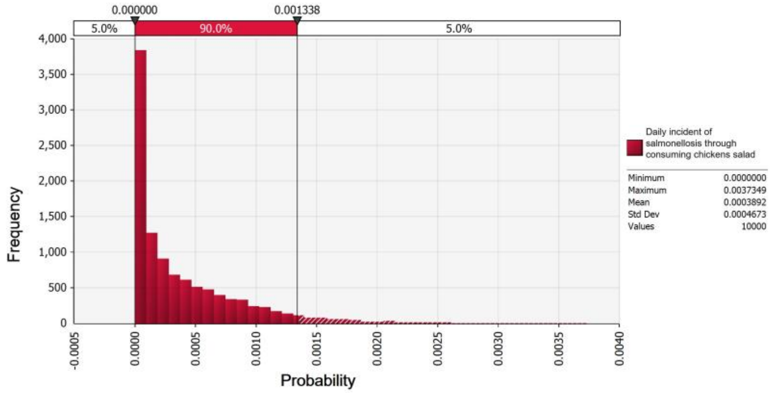

Figure 9. Daily incidence of salmonellosis in Cambodian households acquired through eating chicken salad.

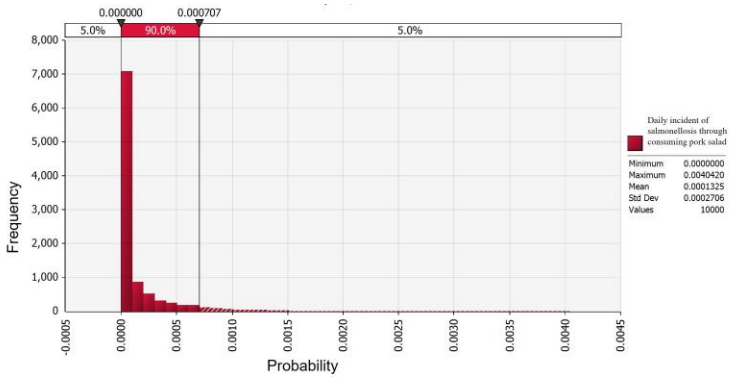

Figure 10. Daily incidence of salmonellosis in Cambodian households acquired through eating pork salad.

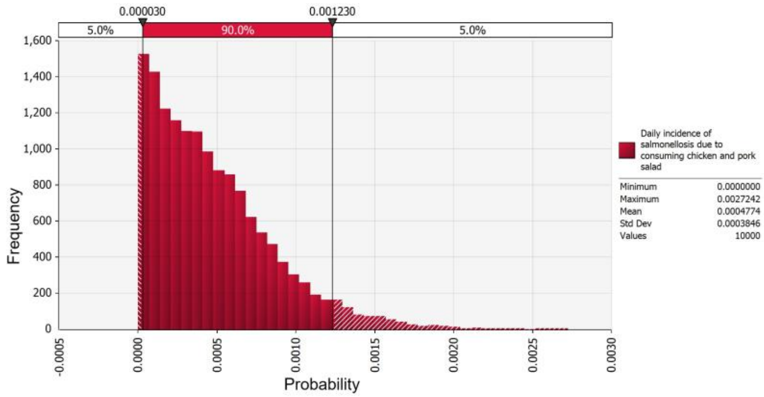

Figure 11. Daily incidence of salmonellosis in Cambodian households acquired through eating both chicken and pork salad.

4.4.2 Annual incidence of salmonellosis following chicken and pork salad consumption (Paper III)

Estimated annual incidence of salmonellosis due to consumption of chicken, pork and both salads were 11.2%, 4.0% and 14.5%, respectively (all confidence intervals are presented in Paper III). On average, about 1 in 10 people were at risk of salmonellosis annually due to consumption of chicken salad, assuming common practices in households identified in Paper II. Adults were found to have higher annual incidence of salmonellosis than the other age groups studied, with an incidence of 14.6%, 5.3% and 19.1% when consuming chicken salad, pork salad and both chicken and pork salad, respectively. A similar pattern was observed for the daily incidence of salmonellosis in adults (Paper III). Among children aged under 5 years, the annual incidence of salmonellosis was 5.3% when consuming chicken salad, 1.7% when consuming pork salad and 6.6% for chicken and pork salad, while among older children the incidence was 6.5% when eating chicken salad, 1.7% when eating pork salad and 8.2% when eating both chicken and pork salad.

A limitation of the study in Paper III was the dose-response of salmonellosis in people of different age groups. In the consumer survey on consumption rates, individuals were categorised into four age groups (<5 years, 6-15 years, 16-60 years, and >61 years). However, available data on dose-response were found only for salmonellosis outbreaks in the USA in 2010, where age of the cases was not considered (Teunis *et al*., 2010). Several studies have attempted to determine the dose-response, but there is a lack of reference data due to ethical concerns about experiments on humans (Oscar, 2019).

4.4.3 Sensitivity analysis for annual incidence

Sensitivity analysis was performed to identify the parameters that most influenced the estimates and assumptions in QMRA (Figure 12). Among the five main factors analysed, that with the greatest influence on estimates was the probability of cross-contamination in preparing salad in scenario 1 (Paper III). The second most influencing factor was the prevalence of *Salmonella* in chickens at the market. Better hygiene practices in scenario 4 (see Table 2) had less influence on daily salmonellosis incidence. This is in line with results from other studies in which cross-contamination has been identified as the most important factor in bacteria transmission along the food chain (Possas *et al.*, 2017; Van Asselt *et al.*, 2008). One important factor contributing to high cross-contamination in RTE foods in Cambodia is the current high prevalence of *Salmonella* in retail meat at markets (Lay *et al.*, 2011; Nadimpalli *et al.*, 2019).

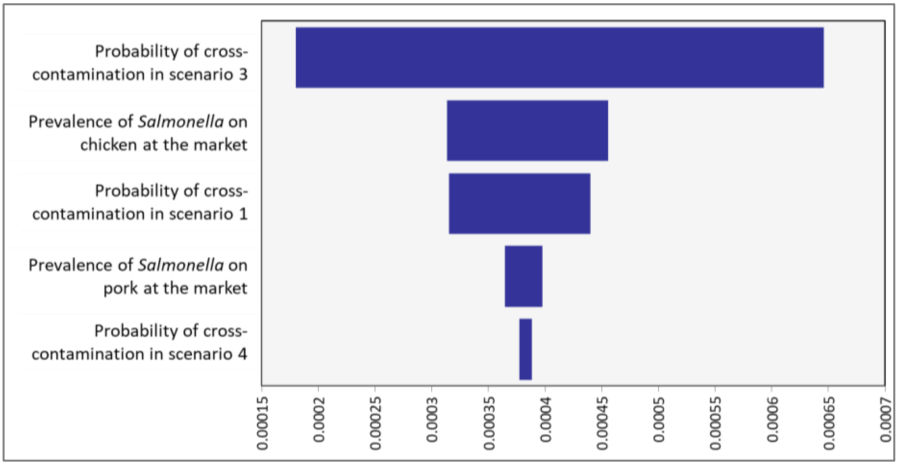

Figure 12. Results of sensitivity analysis of factors influencing daily salmonellosis incidence due to consuming pork and chicken salad (diagram from Paper III).

5. Conclusions

Using chicken and pork salad as a model, this thesis provided new knowledge on the quantitative risk of human salmonellosis along the ASF food chain from traditional markets to consumers at household level. The most important factors contributing to the risk of salmonellosis when consuming ASF purchased from markets and prepared at the household level were identified. The empirical evidence provided in the thesis can support implementation of control measures, especially for improving food safety standards at retail, but also for increasing awareness of hygiene practices at household level. Several food safety concerns related to ASF in Cambodia were identified, including:

- *Salmonella* prevalence in chicken meat and pork was found at 42.6% (87/204) and 45.1% (92/204), which is high in chicken and pork sampled at retail markets. These pathogens can cause severe foodborne diseases in humans and are highly relevant in Cambodia. The levels of bacteria detected were high, and similar levels have been found in other LMIC, including Vietnam. These findings show that foodborne disease is a significant public health issue in Cambodian food safety.

- Median *Salmonella* load in chicken RTE salad was between 0.36 and 12.41 CFU/g, which is associated with a high risk of FBD among consumers.

- Practices identified as enabling transmission of foodborne pathogenic bacteria, in this case *Salmonella*, at household level included using the same cutting board and knife for cutting meat and vegetables, and improper handwashing. Such practices also resulted in elevated levels of *Salmonella* salad contamination.

- A significant risk of consumers in Cambodia contracting salmonellosis after eating chicken and/or pork salads at household level was identified. Estimates showed that around four out of 10,000 consumers in Cambodia were at risk of contracting salmonellosis daily, and one out of 10 was at risk of contracting salmonellosis annually, due to consumption of chicken salad prepared using common household practices.

The empirical evidence provided in the thesis can support implementation of control measures, especially for improving food safety standards at retail, but also for increasing awareness of hygiene practices at household level. These findings should be considered when developing new guidelines and interventions targeting meat retailers, those responsible for household food preparation and RTE food sellers in Cambodia.

6. Future considerations

Based on the results in this thesis, interventions to improve hygiene standards at traditional Cambodian markets are strongly recommended.

Incentive-based and light-touch interventions to improve food safety in traditional markets were conducted in parallel with this study. In brief, a randomised controlled trial to improve hygiene practices and knowledge of pork retailers through packages found low to moderate compliance of pork vendors in cleaning and disinfection of shop equipment and hands in the trial group. There was a significant reduction in the overall level of total bacteria count and *Salmonella* prevalence in the trial group. The knowledge, attitude and practice scores of retailers in the intervention group improved significantly. The light-touch intervention effectively improved pork safety at traditional retail and built upon the data presented in this thesis.

Findings in the thesis can assist policymakers in implementing appropriate and effective intervention strategies to reduce the burden of FBDs in ASF value chain actors. The QMRA provided clear scientific evidence that can lead to prevention of undesirable consequences associated with selected policies relating to microbial contamination in ASF until the point of consumption. The findings in this thesis can also act an evidence base for adapting policies in Cambodia, with suggested changes such as monitoring markets and prepared foods in household and RTE food providers and restaurants. Pilot intervention suggested will hopefully help improve food safety in Cambodia and scaling up these activities is strongly recommended.

There is a need for better public awareness of hygiene in households and at market. Risk communication messages on the need for separate kitchen utensils and frequent and adequate washing and disinfecting of food contact surfaces (cutting board, knife, hands, sink) are necessary to reduce the risk of *Salmonella* cross-contamination to RTE food. The finding that cutting chicken before vegetables resulted in less contamination was not expected and requires further investigation. Data on *Salmonella* levels under different preparation scenarios will be used to support quantitative risk assessments in future studies.

Further studies on how *Salmonell*a and/or *S. aureus* can cross-contaminate RTE food or any typical food in Cambodian households are suggested. As the number of samples from supermarkets analysed in this thesis was small, future wider surveys on foodborne pathogens in chicken meat and pork in supermarkets is recommended. Future monitoring should not be limited to *Salmonella* and *S. aureus* and should cover other harmful bacteria hazards for public health. It is also necessary to formalise the statutory limit for bacteria contamination in ASF approved for consumption. Moreover, antimicrobial resistance in foodborne bacteria and other foodborne pathogens and risk assessment of AMR along the entire food chain should be investigated.

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Popular science summary

This thesis was part of the *Safe Food, Fair Food for Cambodia* project (2017-2021), which sought to identify food safety impacts and barriers in Cambodia. Hazard prioritisation and identification were initially discussed with the research team at Cambodia's Technical Working Group for Food Safety (FSTWG), and with other taskforce members, during a workshop in 2018. These discussions and results from a literature review guided the work to focus on the pork and poultry value chains and to target two key pathogens: *Salmonella* spp. and *Staphylococcus aureus (S. aureus)*. In this thesis, various studies were carried out to assess the impact and components of resilience, in order to inform the design of future food safety interventions. Research activities were carried out in traditional markets in (all) 25 provinces of the country.

A total of 496 samples of pork, chicken and cutting board swabs were collected from traditional Cambodian markets in all 25 provinces. The overall prevalence of *Salmonella* and *S. aureus* in pork and chicken meat was found to be 43-45% and 29-38%, respectively. These levels are high, and similar levels have been found in other low- and middle-income countries in the study region (*e.g*. Vietnam). These findings indicate that foodborne disease is a significant public health issue in Cambodia. In the next step, a laboratory experiment assessed the occurrence and level of cross-contamination of *Salmonella enterica* from raw chicken via kitchen utensils and hands to RTE chicken salad prepared in households. It was shown that there were many possibilities for cross-contamination when preparing chicken salad at household level. It was assumed in the modelling work that people throughout Cambodia generally prepare chicken/pork salad in a similar way, with similar cross-contamination pathways. Quantitative microbial risk assessment was used to estimate the risk of salmonellosis from household consumption of *Salmonella*-contaminated pork and chicken salad. Overall, it was estimated that at least one in 10 chicken salad consumers is at risk of contracting salmonellosis annually in Cambodia.

Populärvetenskaplig sammanfattning

Denna avhandling ingick i forskningsprojektet *Safe Food, Fair Food for Cambodia* (2017 - 2021), vars mål var att identifiera barriärer och risker relaterade till livsmedelssäkerhet i Kambodja. I ett första steg genomfördes ett arbete för att identifiera och prioritera risker kopplade till livsmedelskedjan. Detta gjordes under 2018 av en forskargrupp inom Cambodia's Technical Working Group for Food Safety (FSTWG) och andra experter Resultaten från dessa diskussioner, samt från en genomgång av litteraturen, visade att det finns betydande risker kopplade till värdekedjorna för griskött och kycklingprodukter och att två viktiga sjukdomsframkallande agens är *Salmonella* spp. och *Staphylococcus aureus*. I den här avhandlingen har olika studier genomförts för att få bättre kunskap om vad som påverkar säkerheten i livsmedelskedjan för att på sikt kunna utforma olika rekommendationer och åtgärder för att förbättra livsmedelssäkerhet och därmed folkhälsan. I projektet ingick Kambodjas alla 25 olika provinser.

Totalt analyserades 496 prover från griskött, kyckling, samt svabbar från skärbrädor från traditionella marknader i Kambodjas samtliga provinser. Förekomsten av *Salmonella* och *S. aureus* på griskött och på kycklingprodukter var 43-45% respektive 29-38%. Dessa nivåer är höga och motsvarar resultat från andra liknande studier i andra låg- och medelinkomstländer, till exempel Vietnam. Resultaten från denna studie visar att livsmedelsburna sjukdomar har stor betydelse för folkhälsa i Kambodja. I nästa steg genomfördes ett experiment i laboratoriemiljö för att fastställa förekomst och nivåer av korskontaminering av *Salmonella enterica* från rå kyckling via köksredskap och händer till ätfärdig kycklingsallad som tillagats i hemmiljö. Resultaten visade att det fanns många möjligheter till korskontaminering vid beredning och tillagning av kycklingsallad. I den påföljande studien antogs att människor i hela Kambodja förbereder kyckling/fläsksallad på liknande sätt och därmed med samma korskontamineringsvägar. En kvantitativ mikrobiell riskbedömning genomfördes för att uppskatta risken för salmonellos efter konsumtion av fläsk- och/eller kycklingsallad som tillagats i hemmen. Sammantaget uppskattades det att minst en av 10 personer som äter kycklingsallad årligen drabbas av salmonellos i Kambodja. De empiriska bevisen i avhandlingen kan stödja genomförandet av kontrollåtgärder, särskilt för att förbättra livsmedelssäkerhet på marknader, men också för att öka medvetenheten om betydelsen av god hygien på hushållsnivå.

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We live together under the One Health Approach.

“It always seems impossible until it's done.” Nelson Mandela

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Article

Prevalence of *Salmonella* spp. and *Staphylococcus aureus* in Chicken Meat and Pork from Cambodian Markets

Chea Rortana [1,2,3,*], Hung Nguyen-Viet [2], Sothyra Tum [1], Fred Unger [2], Sofia Boqvist [3], Sinh Dang-Xuan [2,4], Sok Koam [1], Delia Grace [2,5], Kristina Osbjer [6,7], Theng Heng [1], Seng Sarim [1], Or Phirum [1], Roeurn Sophia [1] and Johanna F. Lindahl [2,7,8,*]

[1] National of Animal Health and Production Research Institute, General Directorate of Animal Health and Production, Phnom Penh 12100, Cambodia; sothyratum@gmail.com (S.T.); sok.koam@yahoo.com (S.K.); theng.heng11@gmail.com (T.H.); sengsarim87@gmail.com (S.S.); phirumor@gmail.com (O.P.); roeurnsophai24@gmail.com (R.S.)
[2] International Livestock Research Institute, Nairobi 00100, Kenya; h.nguyen@cgiar.org (H.N.-V.); F.Unger@cgiar.org (F.U.); S.Dang@cgiar.org (S.D.-X.); d.randolph@cgiar.org (D.G.)
[3] Department of Biomedical Science and Veterinary Public Health, Swedish University of Agricultural Sciences, 75007 Uppsala, Sweden; Sofia.Boqvist@slu.se
[4] Center for Public Health and Ecosystem Research, Hanoi University of Public Health, Hanoi 100000, Vietnam
[5] Natural Research Institute, University of Greenwich, Kent ME4 4TB, UK
[6] Emergency Centre for Transboundary Animal Diseases, Food and Agriculture Organization of the United Nations, Phnom Penh 12100, Cambodia; Kristina.Osbjer@slu.se
[7] Department of Clinical Sciences, Swedish University of Agricultural Sciences, 75007 Uppsala, Sweden
[8] Department of Medical Biochemistry and Microbiology, Uppsala University, 75123 Uppsala, Sweden
* Correspondence: r.chea@cgiar.org (C.R.); J.Lindahl@cgiar.org (J.F.L.); Tel.: +855-92-554996 (C.R.)

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Abstract: *Salmonella* spp. and *Staphylococcus aureus* are two of the most common foodborne bacteria in animal-source foods (ASF) that cause illness worldwide. This study aimed to determine the prevalence of *Salmonella* spp. and *S. aureus* in chicken meat and pork in markets in Cambodia. Sampling was done in 52 traditional markets and 6 supermarkets in 25 provinces of Cambodia between October 2018 and August 2019. In total, 532 samples were obtained: chicken meat and pork (n = 408, 204 of each), chicken and pork cutting board swabs (n = 124, 62 of each). All samples were analyzed for the presence of *Salmonella* spp. and *S. aureus*; colony-forming units per gram (CFU/g) of coagulase-positive *Staphylococci* (CPS) were counted, and a subset of samples was also analyzed for the most probable number (MPN, n = 136) of *Salmonella*. The overall prevalence of *Salmonella* spp. and *S. aureus* were 42.1% (224/532) and 29.1% (155/532), respectively, with 14.7% (78/532) of samples containing both bacteria. The prevalence of *Salmonella* spp. in chicken meat was 42.6%, on chicken cutting board it was 41.9%, on pork it was 45.1%, and the pork cutting board 30.6%. Chicken meat had a significantly (p-value < 0.05) higher prevalence of *S. aureus*, 38.2%, compared to the chicken cutting board, 17.7%, pork 28.9%, and pork cutting board 11.3%. Mean MPN-*Salmonella* was 10.6 MPN/g in chicken and 11.1 MPN/g in pork samples. Average Log CFU/g of CPS in chicken and pork samples were 2.6 and 2.5, respectively. The results indicate that chicken meat and pork in Cambodia were highly contaminated with *Salmonella* spp. and *S. aureus*, posing risks to consumers' health. Urgent interventions are necessary to improve hygiene for safer meat in Cambodian markets.

Keywords: animal-source food; Cambodian traditional market; food safety; livestock product; *Salmonella* species; *S. aureus*; wet market

1. Introduction

Foodborne diseases (FBD) are the illness conditions caused by the ingestion of food containing biological, chemical, or physical hazards. Biological hazards such as bacteria, virus, parasites are responsible for most illnesses. FBD constitute a significant threat to health and impediments to social and economic development worldwide, especially in low- and middle-income countries (LMIC) [1–3]. Foodborne disease is one of the leading causes of human mortality and morbidity, comparable to major infectious diseases such as malaria, HIV/AIDS and tuberculosis [4]. Based on a comprehensive review of 31 common microbes causing FBD worldwide, the World Health Organization (WHO) estimated the health burden of 26 priority hazards at 33 million Disability Adjusted Life Years (DALYs); with an exceptionally high (40%) burden in children under five years of age [5]. Older people and people with chronic disease and children under five years of age are the most susceptible to FBD [6,7]. The group most at risk of FBD comprises those living in LMIC, high population density, limited knowledge, and lack of good hygiene practices for fresh meat handling [1,6,7]. A related study, also by the WHO, estimated an additional burden of 9 million DALYs associated with four heavy metals in food [8]. A recent study estimated a loss of more than US$110 billion in productivity and medical expenses each year from unsafe food in LMIC [9]. However, few LMICs monitor the presence of FBD, and thus, data on the burden are limited, while more data are available in high-income countries [1,3,9,10].

Animal-source foods (ASF) provide essential nutrients for humans in palatable and digestible forms; however, they also act as a transmission route for common foodborne pathogens and toxins produced by microbes [11,12]. Bacteria are the leading causes of foodborne illness, particularly diarrheal disease [13,14]. Foodborne bacteria can infect humans by consumption of raw and under-cooked products but may also cross-contaminate ready-to-eat food [15].

Salmonella spp. and *Staphylococcus aureus* are two of the most common foodborne bacteria in ASF. *Salmonella* is a genus of Gram-negative, rod-shaped bacteria with a facultative metabolism. There are two common ASF-associated species of *Salmonella*, including *S. enterica* and *S. bongori*, with almost all *S. enterica* associated with human salmonellosis. *Salmonella* spp. cause a variety of diseases in humans and animals [16]. Non-typhoidal *Salmonella* are among the most important causes of diarrheal in humans, contributing to an estimated 230,000 deaths annually [5,17]. *Salmonella* spp. can contaminate fresh meat during slaughter or processing, handling, and during selling at the markets [18,19]. In livestock, such as pigs and chickens, *Salmonella* spp. colonization can be subclinical and difficult to detect by animal inspectors before slaughter but may contaminate carcasses and infect humans via consumption [18,20].

Staphylococcus aureus is a Gram-positive bacterium regarded as human commensal. It is also an opportunistic pathogen that can cause a broad spectrum of infections, from superficial skin infections to severe, and potentially fatal, invasive disease [21,22]. *S. aureus* and some of the coagulase-positive staphylococci (CPS) species are human pathogens, causing a wide range of clinical signs, including foodborne illness, by its wide range of enterotoxins production [23–25]. Most of the Staphylococcal enterotoxins are mostly heat resistant and can cause human diseases via consumption of contaminated food even if properly cooked [12,23]. *S. aureus* is frequently isolated from meat and ready-to-eat foods [22,24,26,27]. The prevalence of *S. aureus* in meat products needs to be monitored and controlled in LMICs, including Cambodia [1,19,22,28].

Commonly for daily consumption, most Cambodians purchase fresh food, especially fresh meat, from traditional markets, sometimes referred to as wet markets [29]. In these markets, local people buy and sell products, especially ASF, such as fresh pork, poultry, fish, fresh vegetables, and basic household commodities [30,31]. Generally, traditional markets in Cambodia are similar to those in nearby countries such as Lao PDR and Vietnam, where ASF safety is still below satisfactory [19,32,33]. Earlier studies found that hygiene practices in slaughterhouses and among meat retailers in Cambodia were not well, and the methods of handling and slaughtering followed traditional practices that were not always hygienic. For example, the slaughtering process was mainly done on the floor, and the personnel hygiene of workers was not well managed [18,20]. In addition, the basic slaughterhouse facilities and unhygienic handling and transportation of meat could contribute to contamination by microbes through the food chain to both the formal and informal retail market. Several risk factors are contributing to bacterial contamination and growth in carcasses/meat, including poor infrastructure, lack of cleaning and disinfection, unhygienic handling of contaminated materials, and lack of temperature control [30].

Retail meats sold in supermarkets can be safer than meat sold in traditional markets since supermarkets often have access to clean water, cooling systems and appropriate processing, but in Cambodia, supermarkets are uncommon. According to the Cambodian Annual Report of Animal Health and Production in 2019, there were 480 traditional markets that serve and sell fresh meat for most people countrywide, and only a few supermarkets and minimarts selling different types of meat [28]. The objective of this study was to determine the prevalence of *Salmonella* spp. and *S. aureus* in chicken meat and pork and cutting boards for chicken and pork in Cambodian traditional markets and supermarkets, the information needed for food safety management.

2. Results

2.1. Prevalence of Salmonella spp. *and Staphylococcus aureus in Food Samples Collected at Cambodian Markets*

2.1.1. Overall Prevalence

The study comprised 532 samples from 52 traditional markets and 6 supermarkets in 25 provinces/municipalities of Cambodia (Tables 1 and 2). In total, 42.1% (224/532) of the samples were positive for *Salmonella* spp. and 29.1% (155/532) were positive for *S. aureus* (Table 2). Among these, 14.7% (78/532) of the samples were positive for both *Salmonella* spp. and *S. aureus*. The prevalence of both bacteria in meat samples (chicken and pork) was significantly higher than that on cutting boards used for chicken and pork (p-value < 0.001). The bacterial contamination of all sample types (chicken meat and pork) from supermarkets was lower than that from traditional markets (p-value = 0.002). There was a notable variation in microbial contamination between provinces/municipalities (Table 1).

2.1.2. Traditional Markets

The prevalence of both *Salmonella* spp. and *S. aureus* across all samples was 16.3% (68/416), while in chicken meat it was 20.5% (32/156), on chicken cutting boards 9.6% (5/52), in pork 19.2% (30/156) and on pork cutting boards 1.9% (1/52) (Table 2). The prevalence of *Salmonella* spp. in chicken meat was 40.4% (63/156), on chicken cutting boards 42.3% (22/52), in pork 45.7% (70/156), and on pork cutting boards 11.3% (14/52). In comparison between the two species, the prevalence of *Salmonella* spp. in chicken and pork samples (including cutting boards of both sample types) was not significantly different (p-value = 0.15). The prevalence of *S. aureus* in chicken meat was 46.2% (72/156), on chicken cutting boards 21.2% (11/52), in pork 34.6% (54/156), and on pork cutting boards 13.5% (7/52). The prevalence of *S. aureus* was significantly higher in chicken samples than in pork samples (p-value < 0.001).

2.1.3. Supermarkets

Among the 36 samples from six supermarkets (Table 2), the prevalence of *Salmonella* spp. was 16.7% (3/18) in chicken and 38.9% (7/18) in pork. *Staphylococcus aureus* was not found in chicken and only in 5.6% (1/18) of pork samples. Only one pork sample was positive for both *Salmonella* spp. and *S. aureus* (1/18, 5.6%).

2.1.4. Variation in Prevalence within One Year

During the repeated sampling in the dry season, the prevalence of co-contamination with *Salmonella* and *S. aureus* was 20.0% (6/30) in chicken and in pork 10.0% (3/30), no cutting boards being positive for co-contamination. The prevalence of *Salmonella* spp. in chicken meat was 70.0% (21/30), on chicken cutting boards 40.0% (4/10), in pork 50.0% (15/30), and the pork cutting boards 50.0% (5/10) (Figure 1). *S. aureus* was found only in chicken meat and pork at a frequency of 20.0% (6/30) and 13.3% (4/30), respectively (Figure 1).

In four provinces, samples were collected in both dry and wet seasons (Figure 1). In the total number of samples, the prevalence of co-contamination with *Salmonella* spp. and *S. aureus* in the dry season was 21.3% (17/80) and 11.3% (9/80) in the wet season. The prevalence of *Salmonella* spp. in all sample categories in the wet season was 56.3% (45/80), which was significantly higher than in the dry season 38.8% (31/80, *p*-value = 0.01). The prevalence of *S. aureus* in the dry season was 43.8% (35/80), which was significantly higher than in the wet season at 12.5% (10/80, *p*-value < 0.001, Table 2).

2.1.5. Factors Associated with Prevalence of *Salmonella* spp. and *Staphylococcus aureus* Contamination

The multivariable analyses showed significantly lower prevalence in the supermarket when compared to traditional markets regarding the prevalence of both *Salmonella* spp. and *S. aureus* (*p*-value = 0.034) and with only *S. aureus* (*p*-value = 0.002). The prevalence of *Salmonella* was not significantly different between these two market types (*p*-value = 0.09). The prevalence of *S. aureus* was significantly higher (*p*-value < 0.001) in meat samples than in cutting boards. There was also a tendency for higher *Salmonella* spp. prevalence in meat samples (*p*-value = 0.07). The prevalence of *Salmonella* spp. increased during the wet season, while the prevalence of *S. aureus* was the opposite (Table 3).

Of the 136 selected samples, the *Salmonella* MPN/g indexes were divided into four groups: <0.03, 0.03–3.0, 3.1–30, and ≥30.1. Most of the pork and chicken samples ranged from <0.03 to 0.03–3.0 MPN/g. Meat samples from traditional markets had the highest *Salmonella* MPN/g range (≥30.1), which were mainly found in the dry season. While in the wet season, the highest *Salmonella* MPN/g range was only found in pork samples. Both pork and chicken samples collected from supermarkets did not exceed 30.0 MPN/g (Figure 2).

Table 1. Prevalence of *Salmonella* spp. and *S. aureus* in chicken, chicken cutting boards, pork and pork cutting boards in Cambodian traditional markets by province.

Provinces/Municipalities	Markets [1]	Total Sample Collected [2]	Total Positive Samples	Number of *Salmonella* Positive Samples (%)					Number of *S. aureus* Positive Samples (%)				
				Chicken	Cutting Board Chicken	Cutting Board Pork	Pork	Average [4] MPN/g	Total Positive Samples	Chicken	Cutting Board Chicken	Cutting Board Pork	Pork
Phnom Penh	3 (2 times)	48	13 (27.1)	8 (44.4)	1 (16.7)	0 (0%)	4 (22.2)	16.1	12 (25.0)	5(27.8)	1 (16.6)	1 (16.6)	5 (27.8)
Siem Reap	3 (2 times)	48	31 (64.6)	14 (77.8)	3 (50.0)	1 (16.7)	13 (72.2)	2.6	12 (25.0)	8 (44.4)	0	0	4 (22.2)
Battambang	2 (2 times)	32	14 (43.8)	4 (33.3)	2 (50.0)	2 (50.0)	6 (50.0)	5.9	10 (31.3)	5 (41.7)	2 (50.0)	0	3 (25.0)
Preah Sihanouk	2 (2 times)	32	18 (56.3)	9 (75.0)	1 (25.0)	2 (50.0)	6 (50.0)	25.4	11 (34.4)	7 (58.3)	0	0	4 (33.3)
Takeo	2	16	8 (50.0)	3 (50.0)	1 (50.0)	1 (50.0)	3 (50.0)	15.7	5 (31.3)	2 (33.3)	1 (50.0)	0	2 (33.3)
Kampong Cham	2	16	5 (31.3)	1 (16.7)	1 (50.0)	0	3 (50.0)	15.0	10 (62.5)	5 (83.3)	1 (50.0)	1 (50.0)	3 (50.0)
Tboung Khmum	2	16	7 (43.8)	2 (33.3)	1 (50.0)	1 (50.0)	3 (50.0)	8.3	6 (37.5)	3 (50.0)	0	0	3 (50.0)
Kep	2	16	10 (62.5)	3 (50.0)	1 (50.0)	0	6 (100)	58.6	4 (25.0)	1 (16.7)	0	0	3 (50.0)
Kampot	2	16	10 (62.5)	3 (50.0)	1 (50.0)	1 (50.0)	5 (83.3)	55.2	5 (31.3)	4 (66.7)	0	0	1 (16.7)
Kampong Speu	2	16	6 (37.5)	3 (50.0)	0	0	3 (50.0)	3.5	11 (68.8)	6 (100)	0	0	5 (83.3)
Kandal	2	16	6 (37.5)	1 (16.7)	1 (50.0)	2 (100)	2 (33.3)	107.5	3 (18.8)	3 (50.0)	0	0	0
Kampong Chhnang	2	16	9 (56.3)	4 (66.7)	2 (100)	0	3 (50.0)	51.5	10 (62.5)	3 (50.0)	2 (100)	1 (50.0)	4 (66.7)
Oddor Mean Chey	2	16	7 (43.8)	3 (50.0)	0	1 (50.0)	3 (50.0)	1.28	0	0	0	0	0
Koh Kong	2	16	0	0	0	0	0	0	3 (18.8)	2 (33.3)	0	0	1 (16.7)
Paillin	2	16	5 (31.3)	3 (50.0)	1 (50.0)	1 (50.0)	0	4.4	4 (25.0)	2 (33.3)	0	0	2 (33.3)
Bantheay Mean Chey	2	16	2 (12.5)	0	1 (50.0)	1 (50.0)	0	0.29	4 (25.0)	2 (33.3)	1 (50.0)	0	1 (16.7)
Pursat	2	16	5 (31.3)	1 (16.7)	2 (100)	1 (50.0)	1 (16.7)	8.6	2 (12.5)	2 (33.3)	0	0	0
Prey Veng	2	16	6 (37.5)	1 (16.7)	0	1 (50.0)	4 (66.7)	1.3	4 (25.0)	4 (66.7)	0	0	0
Svay Rieng	2	16	3 (18.8)	1 (16.7)	1 (50.0)	0	1 (16.7)	15.0	9 (56.3)	3 (50.0)		1 (50.0)	4 (66.7)
Mundulkiri	2	16	13 (81.3)	5 (83.3)	2 (100)	2 (100)	4 (66.7)	2.6	6 (37.5)	2 (33.3)	0	1 (50.0)	3 (50.0)
Ratanakiri	2	16	7 (43.8)	4 (66.7)	0	0	3 (50.0)	2.0	5 (31.3)	2 (33.3)	0	0	3 (50.0)
Steung Treng	2	16	4 (25.0)	1 (16.7)	0	0	3 (50.0)	10.1	8 (50.0)	3 (50.0)	1 (50.0)	1 (50.0)	3 (50.0)
Kratie	2	16	6 (37.5)	2 (33.3)	0	1 (50.0)	3 (50.0)	5.2	8 (50.0)	3 (50.0)	1 (50.0)	1 (50.0)	3 (50.0)
Kampong Thom	2	16	8 (50.0)	3 (50.0)	2 (100)	0	3 (50.0)	106.1	0	0	0	0	0
Preah Vihear	2	16	11 (68.8)	5 (83.3)	2 (100)	1 (50.0)	3 (50.0)	76.6	3 (18.8)	1 (16.7)	1 (50.0)	0	1 (16.7)
Total [3]	52	496	214 (43.1)	84 (45.2)	26 (41.9)	19 (30.6)	85 (45.7)	23.2	155 (31.3)	78 (41.9)	12 (19.4)	7 (11.3)	58 (31.2)

[1] Three markets were included in Phnom Penh (PP) and Siem Reap (SR), regarded as having the highest population, while two were included in the other 23 provinces. [2] The total number of each specimen was different in Phnom Penh and Siem Reap (18 chicken, 6 chicken cutting boards, 18 pork, and 6 pork cutting boards); Battambang (BB) and Preah Sihanouk (PSH) (12 chicken, 4 chicken cutting boards, 12 pork, 4 pork cutting boards), compared to other provinces (6 chicken, 2 chicken cutting boards, 6 pork, 2 pork cutting boards). [3] The total 496 samples included the 80 repeated samples of the 4 provinces/municipalities (PP, SR, BB, PSH) and excluded 36 samples from supermarkets. [4] Samples with MPN/g < 0.3, negative with *Salmonella* spp. were counted as 0, and not included in the average. MPN/g >110 was assigned randomly between 111 and 250 MPN/g for the calculation.

Table 2. The prevalence of *Salmonella* spp. and *Staphylococcus aureus* in chicken, pork, pork cutting boards and chicken cutting boards from traditional markets, supermarkets, in Cambodia and variation within one year.

Market Types	Total Positive Sample	Chicken (No. of Positive (%))	Chicken Cutting Board (No. of Positive (%))	Pork (No. of Positive (%))	Pork Cutting Board (No. of Positive (%))	*p*-Value [4]
Traditional Market						
Dry season [1] (n = 416)		n = 156	n = 52	n = 156	n = 52	
Salmonella spp. & *S. aureus*	68	32 (20.5)	5 (9.6)	30 (19.2)	1 (1.9)	0.006
Salmonella spp.	169	63 (40.4)	22 (42.3)	70 (44.9)	14 (26.9)	0.150
S. aureus	144	72 (46.2)	11(21.2)	54 (34.6)	7 (13.5)	<0.001
Wet season [2] (n = 80)		n = 30	n = 10	n = 30	n = 10	
Salmonella spp. & *S. aureus*	9	6 (20.0)	0	3 (10.0)	0	-
Salmonella spp.	45	21 (70.0)	4 (40.0)	15 (50.0)	5 (50.0)	-
S. aureus	10	6 (20.0)	0	4 (13.3)	0	-
Supermarkets [3] (n = 36)		n = 18	-	n = 18		
Salmonella spp. & *S. aureus*	1	0	-	1 (5.6)	-	-
Salmonella spp.	10	3 (16.7)	-	7 (38.9)	-	-
S. aureus	1	0	-	1 (5.6)	-	-
Overall (n = 532)		n = 204	n = 62	n = 204	n = 62	
Salmonella spp. & *S. aureus*	78	38 (18.6)	5 (8.1)	34 (16.7)	1 (1.6)	0.166
Salmonella spp.	224	87 (42.6)	26 (41.9)	92 (45.1)	19 (30.6)	0.249
S. aureus	155/532	78 (38.2)	11 (17.7)	59 (28.9)	7 (11.3)	<0.001

[1] The samples were from 2 markets in each of 23 provinces and 3 markets in Phnom Penh and Siem Reap. [2] The 80 repeated samples in the wet season were only from 4 provinces/municipalities, including Phnom Penh, Siem Reap, Battambong and Preah Shihanouk. [3] The samples were from 4 supermarkets in Phnom Penh and 2 supermarkets in Siem Reap and collected only in the dry season. [4] Chi-square test.

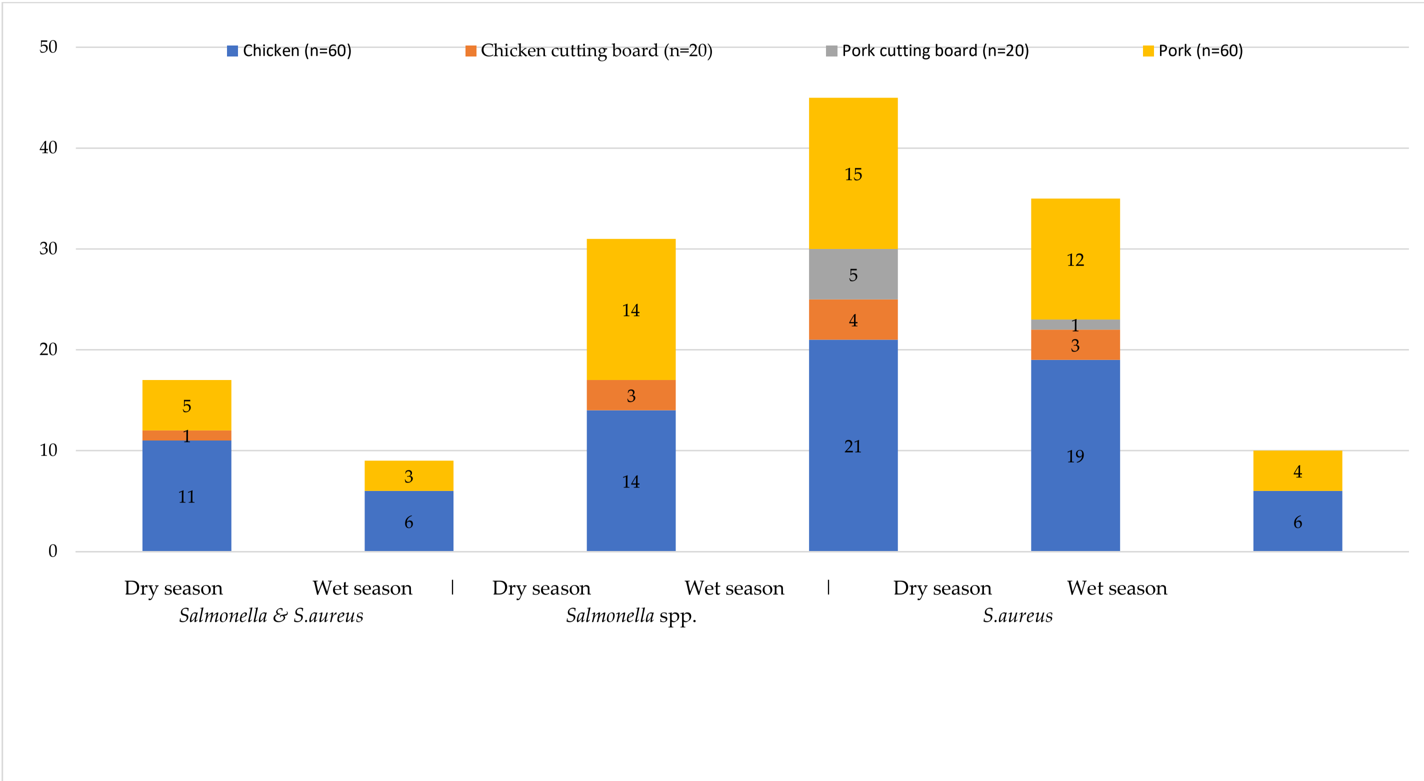

Figure 1. Number of positive samples for *Salmonella* spp. and *Staphylococcus aureus* in the wet and dry seasons in Cambodia. Number of samples (included chicken, chicken cutting board, pork cutting board and pork) per season were 80. The dry and wet seasons in Cambodia are from November to April and May to October, respectively.

Table 3. Factors associated with prevalence of *Salmonella* spp. and *S. aureus* contamination and co-contamination in samples from Cambodian markets using logistic regression.

Pathogens	Variables	Odds Ratio	95% CI	Coefficient	S.E.	*p*-Value
Salmonella spp. & *Staphylococcus aureus*	Species (chicken compared to pork)	1.28	0.78–2.1	0.25	0.25	0.32
	Sample (meat compared to cutting board)	4.66	1.97–11.03	1.54	0.44	<0.001
	Market type (supermarket compared to traditional market)	0.11	0.01–0.84	−2.18	1.02	0.034
	Season (dry compared to wet season)	0.64	0.3–1.36	−0.45	0.38	0.24
	Constant			−3.05	0.44	<0.001
Salmonella spp.	Species (chicken compared to pork)	1.03	0.72–1.46	0.03	0.18	0.86
	Sample (meat compared to cutting board)	1.47	0.96–2.24	0.38	0.22	0.07
	Market type (supermarket compared to traditional market)	0.51	0.24–1.1	−0.67	0.39	0.09
	Season (wet compared to dry season)	1.89	1.16–3.06	0.63	0.25	0.01
	Constant			−0.69	0.21	0.001
Staphylococcus aureus	Species (chicken compared to pork)	1.60	1.07–2.37	0.47	0.2	0.021
	Sample (meat compared to cutting board)	3.55	2.05–6.15	1.27	0.28	<0.001
	Market type (supermarket compared to traditional market)	0.04	0.01–0.3	−3.2	1.02	0.002
	Season (wet compared to dry season)	0.26	0.12–0.51	−1.37	0.36	<0.001
	Constant			−1.89	0.28	<0.001

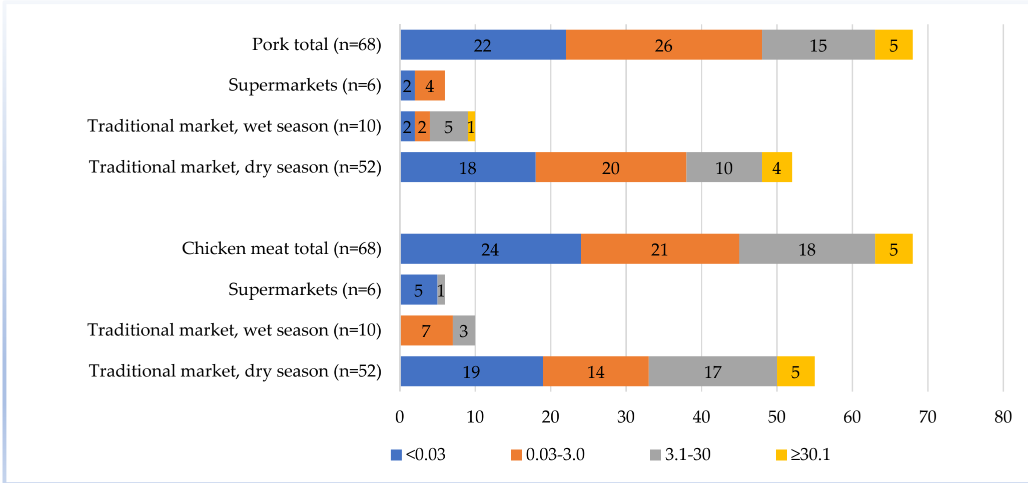

Figure 2. Frequency of *Salmonella* spp. most probable number (MPN/g) ranges in meat samples (n = 136) collected from Cambodian markets.

2.2. Coagulase-Positive Staphylococci

All samples in the traditional market were tested and quantified (CFU/g) for CPS. An average Log CFU/g of CPS from chicken meat and pork samples was higher in wet season compared to dry season, 2.3 (SD 1.0) versus and 2.8 (SD 0.7) in chicken, and 2.1 (SD 0.9) versus 2.2 (SD 0.4) in pork. An average Log CFU/g of CPS contaminated on cutting board was similar in chicken and pork shops (Figure 3). Results from linear regression showed that the CPS contamination in meat in supermarkets was lower than in traditional markets (p-value < 0.001; Table 4). Regarding meat types, the load of CPS in chicken was significantly higher than in pork (p-value = 0.017), whereas the load of CPS in meat was significantly higher than in cutting board (p-value < 0.001, Table 4).

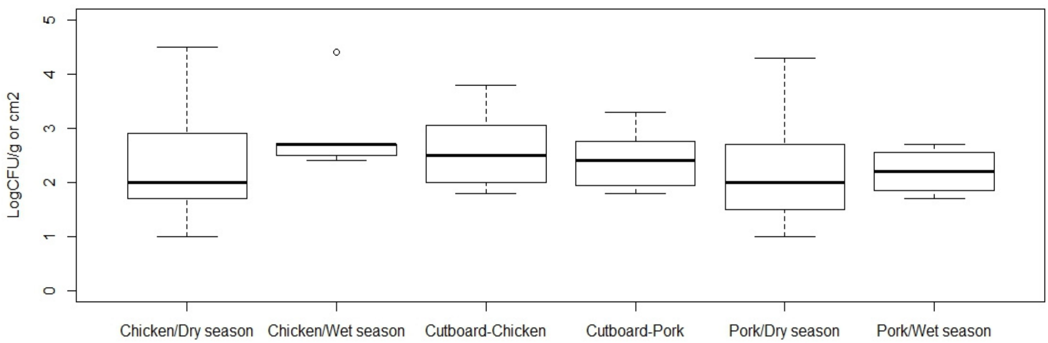

Figure 3. Contamination of coagulase-positive staphylococci (Log CFU/g or cm^2) in samples collected from Cambodian traditional markets in dry and wet seasons. Cutting board samples in chicken and pork shops were only collected in the dry season.

Table 4. Variables associated with Log CFU/g of coagulase-positive staphylococci in samples collected from Cambodian markets.

Variable	Coefficient	95% Confidence Interval	Std Error	*p*-Value
Market type (supermarket compared to traditional market)	−1.054	−1.471–−0.638	0.212	<0.001
Meat type (chicken compared to pork)	0.250	0.044–0.456	0.105	0.017
Sample type (meat compared to cutting board)	0.648	0.402–0.894	0.125	<0.001
Season (dry compared to wet)	−0.590	−0.880–−0.300	0.147	<0.001
Constant	0.927	0.516–1.338	0.209	<0.001

3. Discussion

The main objective of this research was to assess the prevalence of two important human pathogens in meat sold in Cambodia, mostly in traditional markets, to understand the risks for consumers and inform interventions for improving hygiene practices for safer ASF retail. This is the first nationwide survey in traditional markets in all 25 provinces/municipalities and in supermarkets of Cambodia. Our study found a high prevalence of both *Salmonella* spp. and *S. aureus* in all market types.

The overall prevalence of *Salmonella* spp. of this study was 42.1%, with similar contamination rates in both chicken and pork. *Salmonella* spp. is one of the most common foodborne pathogens in fresh meat in Southeast Asia [2,14]. The *Salmonella* spp. prevalence found in this study in Cambodia is similar to that in Vietnam, where some recent studies reported a *Salmonella* spp. prevalence of 45.9% out of 900 chicken samples [34] and 44.7% out of 217 pork samples [33]. An earlier study from the border of Cambodia–Thailand reported a 23% prevalence of *Salmonella* spp. in chicken meat from 145 samples [35]. Another study reported a much higher prevalence of *Salmonella* spp. of 88.2% from 152 poultry carcasses, randomly selected from 10 markets in retail outlets of Phnom Penh between March 2006 and February 2007 [36]. However, our study found a large variation among 25 provinces/municipalities, with some having less than 20% of chicken samples contaminated, and others more than 75%. This result indicates that the prevalence may vary considerably among provinces. We also found seasonal variation in prevalence. Another study conducted in Bangkok, Thailand, found that the prevalence levels of *Salmonella* spp. in chicken collected from open markets and supermarkets were 48% (n = 61) and 57% (n = 75), respectively [31]. Although our study indicated that supermarkets had a lower prevalence of *Salmonella* spp. contamination than in traditional markets, the supermarket prevalence was still at an unacceptable level. Moreover, as the number of samples from supermarkets was small, expanding future surveys on the foodborne pathogen in chicken meat and pork in the supermarket is recommended.

The present study showed that the MPNs for *Salmonella* spp. in fresh chicken meat and pork mainly ranged from <0.03 to 30 MPN/g. An earlier study in Phnom Penh, Cambodia, found a varied concentration of *Salmonella* from 10 to 10^4 CFU/g [36]. Comparable to our results, a study in China on the *Salmonella* quantity in chicken meat showed that more than half of *Salmonella* samples had higher than 0.7 MPN/g [37]. In Vietnam, *Salmonella* concentration in cut pork from traditional markets was mainly lower than 3.0-30 MPN/g, which is at similar contamination ranges compared to our findings in pork [33]. There was a similar concentration of *Salmonella* spp. in Cambodia and Vietnam, which might be due to a similar slaughterhouse environment and transportation. The fact is that bacteria are more likely to grow well during the selling period without temperature control [38].

S. aureus was found among both meat samples and cutting boards of both meat types, which shows that the pathogen is present in fresh meat and its environment in Cambodian markets. CPS are among the major foodborne pathogens that produce enterotoxins which could persist even when products are well cooked and are the etiological agents of staphylococcal food poisoning [24]. There was a slight difference in the prevalence of bacteria found in chicken and pork in traditional markets in 25 provinces/municipalities of Cambodia, which could be due to different hygiene practices. The contamination of *S. aureus* was more common in the dry season, which could be explained by the fact that the wet markets have high moisture and temperature, stimulating the growth of this pathogen in meat. In 2014, *S. aureus* was reported as the cause of gastroenteritis from the FBD outbreak in rural Cambodia. Those cases were due to poor personal hygiene and handwashing, and cross-contamination from other raw animal products [27]. Another study presented a high *S. aureus* contamination rate in Vietnamese ready-to-eat food, ranging from 12.5% to 35.4%, and the contamination in milk was the highest [26]. Another study found that about 40% (18/45) of these isolates having classical *S. aureus* and staphylococcal enterotoxins pose threats to human health [39]. These results indicate the importance of CPS for human health, not only in Cambodia but the whole region. A previous study found an acceptable number of *S. aureus* in beef products in supermarkets in Cambodia [40] but did not test pork or chicken. However, in general, this pathogen has only been little studied in food in Southeast Asia [14].

This study indicates that *Salmonella* spp. contamination was more common during the wet season when increased moisture and water on handling equipment could facilitate *Salmonella* spp. contamination of meat. In the wet season, Cambodia has a high humid condition, which could increase the survival of *Salmonella* in the market, where there is a tradition of selling meat at the shop without temperature control. A study from Denmark in the past decades also found that seasons with higher rainfall can support the survival of *Salmonella* spp. and increase contamination of meat carcass during slaughtering, transportation, and at retail [41].

Previously, FBDs were known collectively as "diarrheal diseases" rather than caused by specific foodborne pathogens. Recently, however, the Foodborne Disease Burden Epidemiology Reference Group (FERG) of WHO reviewed FBD as a distinct category based on secondary data, but the exact source of microbial contamination in food remains limited available in many LMIC [5]. The FERG found that around half the burden of FBD was due to diarrhea, the rest being caused by less common but more severe illnesses such as epilepsy, congenital disabilities, and arthritis. The current Cambodian food safety standard for animal-source food such as chicken and pork requires less than 50,000 CFU/g of total bacteria count, *Salmonella* spp. free in 25 g of meat, and <100 CFU/g of CPS [42]. However, due to lack of resources, the current inspection practices are based on hygienic indicators, TBC and *Salmonella* spp., but not limited to other pathogens such as *S. aureus*. The present study found that 42.1% of meat contained *Salmonella* and 29.1% contained *S. aureus*, showing meat contamination higher than the current standards. The results suggest the need of improving hygienic practice at markets, as well as food safety awareness of meat sellers, to reduce the risk of FBD. Successful interventions in retailer markets have been reported in Vietnam, Malaysia, and African countries [32,43–45]. An example from Vietnam shows that food safety research and evidence of bacterial contamination can attract much attention from media and scientists and inform the government, leading them to adopt a risk-based approach to manage food safety [32,46]. In addition, the study in Malaysia suggests the need for enforcement of legislation and regulations and improvement of public–private partnership in the food system [45]. According to studies in African countries, a powerful method for improving food safety in the informal market was applying risk-based approaches and intense collaboration of local and international institutions [1,43,44]. Our study provides local data on microbial contamination in chicken and pork in both traditional and modern markets, which will help inform consumers about the public health risks. The result will also be an important message to food safety policy makers to improve risk management and risk communication.

Finally, this study focused mainly on sampling in traditional markets where more than 90% of the food was traded for Cambodia and collected only a few samples from supermarkets in the two largest cities. Recent discussion at the global level on market types showed that ASF from supermarkets was not necessarily safer than traditional markets [32]. However, in this study, pork and chicken from supermarkets had lower levels of samples contaminated with *S. aureus* or *Salmonella* spp. and even both pathogens. Although the number of samples from supermarkets is small (36 samples versus 416 from traditional markets), this shows a promising trend in food safety correlated with the formalization of markets in demand for food in Cambodia. Interestingly, supermarkets were relatively better performing with *S. aureus* than *Salmonella* spp.; the former is often associated with poor handling and hygiene, while the latter may be more related to contamination at production. In contrast, the low prevalence of *S. aureus* in the supermarket may be associated with appropriate temperature control, a clean water system, and handling practices.

In conclusion, this study found a high prevalence of both *Salmonella* spp. and *S. aureus* in chicken meat and pork samples, which could cause serious FBD in humans. Vulnerable people who consume fresh chicken meat and pork purchased from the traditional market might be at risk of contracting FBD. These pathogens may contribute to common food-borne illness in Cambodia, and interventions to improve hygienic practices in markets are strongly recommended. Policies engagement of local government is vital for the success of intervention and reduction of FBD.

4. Materials and Methods

4.1. Study Design and Sampling Frame

This cross-sectional study was carried out between October 2018 and August 2019. The first part of sampling was conducted during the dry season, October 2018 to May 2019, at Cambodian traditional markets selling meat products. This included two medium (i.e., having 15 to 50 meat sellers) or large (i.e., having more than 50 meat sellers) traditional markets in each of the 25 provinces/municipalities of Cambodia, except for the two cities with the largest population (Phnom Penh and Siem Reap), where three markets were included. The two traditional markets were the largest markets in each province identified by provincial veterinary authorities. In total, 52 traditional markets were included in the study. At each market, three pork and three chicken meat sellers were selected for sampling using systematic random sampling by the shop's location in the meat selling area at the market, beginning from the main entrance gate, middle, and around the end. Among the three shops where chicken or pork was sampled, only one shop was selected for sampling cutting board swabs. A total of 416 samples were collected in this first part of sampling, representing the dry season.

The second part was a repeated sampling approximately five months after the first part of sampling and was conducted during the wet season from July to August 2019. The sampling was done only in four provinces/municipalities: Battambang, Phnom Penh, Siem Reap, and Preah Sihanouk. This repeated sampling targeted the same number of samples as in the first part of sampling (in the dry season) and generated a total of 80 samples.

The third part of sampling was conducted in supermarkets in October 2018, including four supermarkets in Phnom Penh and two in Siem Reap. Three chicken and three pork samples were purchased from each supermarket. A total of 36 meat samples, but no cutting board samples, were collected. The detailed sampling frame is shown in Table 5.

Table 5. Number of samples collected from traditional markets and supermarkets in Cambodia.

Sampling Round	Chicken Meat	Chicken Cutting Board	Pork Cutting Board	Pork
Traditional market, dry season [1]	156	52	52	156
Traditional market, wet season [2]	30	10	10	30
Supermarkets [3]	18	-	-	18
Total specimen	204	62	62	204
			Total specimen = 532	

[1] Three markets were included in Phnom Penh and Siem Reap, while two markets were included in the other 23 provinces.[2] The total 80 samples were re-sampled from Battambang, Phnom Penh, Siem Reap, and Preah Sihanouk. [3] Four supermarkets in Phnom Penh and two supermarkets in Siem Reap.

4.2. Sample Collection

This study aimed to assess the consumer exposure risk by obtaining samples following the ways customers would buy. Chicken meat and pork were purchased from the selected shops with approximately 300–400 g of each. The vendors used their knife and cutting board to cut the meat and their scale for weighing before placing it into the sterilized sampling bag. In addition, for one pork vendor and one poultry vendor per market, 100 cm^2 of cutting board surface (the most common site used to cut meat) were swabbed. Swab samples were collected using a pre-moisturized sterilized cotton bandage compress, a 10 × 10 cm stainless frame, and a sterilized pincer and were placed in a sterilized plastic zip-lock bag containing 10 mL normal saline. The study excluded the co-contamination of bacteria from hand retailers and all their equipment attached with meat at the shop. The samples were stored in cooling boxes and transported to the laboratory within 24 h by field staff. All the tests were done at the bacteriology laboratory at the National Animal Health and Production Research Institute, General Directorate of Animal Health and Production, Phnom Penh, Cambodia.

4.3. Bacteriological Analysis

4.3.1. *Salmonella* spp. Isolation

Salmonella spp. isolation followed the ISO procedure ISO-6579:2002/amended: 1:2017 [47,48]. Each of the meat (chicken meat and pork) samples was sliced into small pieces aseptically, and 25 g were diluted in 225 buffered peptone water (BPW; Merck, Darmstadt, Germany) and homogenized using stomacher (Seward Limited, West Sussex, UK) for 2 min. For cutting board swab samples, which already contained 10 mL of liquid samples, 90 mL BPW were added and then homogenized manually. The suspensions of the meat sample and cutting board swabs were incubated for 16–20 h at 37 °C for pre-enrichment. Selective enrichment step was done by pipetting 1 mL aliquot in 9 mL Muller Kauffmann Tetrathionate (MKTT; Merck, Darmstadt, Germany) incubated for 16–20 h at 37 °C, and 0.1 mL aliquot in 10 mL Rappaport-Vassiliadis Soya (RVS; Merck, Darmstadt, Germany) incubated for 16–20 h at 41.5 °C. The selective plating was performed by one loop full (approx. 10 μL) of each MKTT and RVS onto Xylose-Lysine Deoxycholate Agar (XLD; Hi-Media, Mumbai, India) and MacConkey agar (Merck, Darmstadt, Germany) as the second plating-out medium. Five presumptive *Salmonella* colonies, with darker pink center or yellow with or without blackening, were subcultured on nutrition agar at 37 °C overnight for biochemical tests. Biochemically, *Salmonella* spp. were confirmed using lactose, indole production, lysine decarboxylase, H_2S production, and urease.

4.3.2. Most Probable Number of *Salmonella*

One-third of total meat samples (n = 124), including pork (n = 62) and chicken meat (n = 62), were selected for quantification of *Salmonella* spp. using a traditional 3-tube MPN method described previously [49]. In brief, each of the 25 g samples was suspended in 225 mL of PBW. From each dilution, 1 mL was added serially to each of 3 × 9 mL of BPW, thus creating a set of three MPN tubes with the dilutions of 10^{-1}, 10^{-2}, and 10^{-3}. Pre-enrichment was followed by incubated (37 °C for 24–48 h) and transferred (one drop) to a corresponding 24-well plate containing 2.5 mL Modified Semi-Solid Rappaport-Vassiliadis (MSRV; Merck, Germany) and then incubated (41.5 °C for 24 h). *Salmonella* was confirmed by subculturing onto XLD agar (37 °C for 24 h), and biochemical tests were followed as mentioned above. MPN index was recorded according to De Man [50] and the bacteriological analytical manual [51].

4.3.3. Isolation of Coagulase-Positive Staphylococci and *Staphylococcus aureus*

All samples were tested for the presence/absence and enumeration of coagulase-positive staphylococci (CPS) following the ISO 6888-1:1999 (includes amendment A1: 2003) using Baird-Parker (BP; Oxoid, Milan, Italy) agar medium [52,53]. In brief, each of the 25 g of pork or chicken meat samples was weighed, cut, and homogenized in 225 mL BPW. Each of the swab samples, approximately 10 mL, was added to 90 mL of BPW to produce the 10^{-1} dilution. Then, the diluted samples were aliquoted to a new 15 mL tube to produce the series of 10-fold dilution from 10^{-1} to 10^{-3}. Then, 0.1 mL aliquoted suspension was transferred and streaked on to two BP agar plates. The plates were then incubated at 37 °C in aerobic atmosphere. After 48 h, plates were examined to find the typical presumptive colonies with opaque and atypical without opaque. Both typical and atypical colonies were counted and calculated for the number of presumptive CPS. About 5 typical colonies were selected for the coagulase test using rabbit serum plasma (BD, USA). An equation [$Ne = Suma/(V(n1 + 0.1n2)d$] from ISO-6888-1-1999 for calculation of the number N of identified CPS present in the test proportion. After confirmation as coagulase-positive, the number of CPS were calculated according to the instruction in 10.1.1 of ISO 6888-1:1999. Colonies of CPS were streaked on to nutrition agar plates for growth at 37 °C for 24 h for further *S. aureus* confirmation using gram stain (Merck, Germany), oxidase test (Merck, Darmstadt, Germany), catalase test, and latex agglutination (Biomerieux SA, Craponne, France) [54].

4.4. Data Management and Analysis

All data were entered in Microsoft Excel. The relation of prevalence of the different sample types and bacteria were calculated using Pearson Chi-square. Multi-level logistic regression was the method for comparison between prevalence of bacteria with market type, seasons, sample types and species. The prevalence of *Salmonella* spp. in chicken meat, chicken cutting board, pork meat and pork cutting board by provinces/municipalities was analyzed using logistic regression. The number of colony-forming units for CPS were converted to Log CFU/g with the value zero substituted with 1, to generate a more normal distribution, and compared between CFU/g of CPS using linear regression. All statistical analyses were performed in EpiInfo^{TM}, an open-source domain of software tools (CDC, USA) and RStudio (R core team). A p-value of 0.05 was used for statistical significance, with no compensation for multiple comparisons.

4.5. Ethical Consideration

Ethnical approval for meat specimen collection was received from the General Directorate of Animal Heath and Production, dated 12 October 2018. Ethical approval for retailer interviews was received from the National Ethical Committee of Cambodia, coded 300NECHR, dated 26 December 2017. Compliance for testing of sample and biosafety was approved by International Livestock Research Institute in letter ref: ILRI, RC-010-18/IBC/010/CR, dated 5th July 2018.

5. Conclusions

The study found a high prevalence of *Salmonella* spp. and *S. aureus* in chicken and pork samples, which can cause severe foodborne diseases in humans. These pathogens may contribute to common foodborne illness in Cambodia. Interventions to improve hygienic standards in Cambodian markets are strongly recommended in the traditional markets in provinces/municipalities with higher contamination levels. Further studies on how *Salmonell*a spp. and/or *S. aureus* could cross-contaminate to ready-to-eat food or any typical food in Cambodian households are suggested.

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II

PLOS ONE

RESEARCH ARTICLE

Experimental cross-contamination of chicken salad with *Salmonella enterica* serovars Typhimurium and London during food preparation in Cambodian households

Rortana Chea[1,2,3]*, Hung Nguyen-Viet[2], Sothyra Tum[1], Fred Unger[2], Johanna Lindahl[2,4,5], Delia Grace[2,6], Chhay Ty[7], Sok Koam[1], Vor Sina[7], Huy Sokchea[7], Son Pov[7], Theng Heng[1], Or Phirum[1], Sinh Dang-Xuan[2,8]*

1 National Animal Health and Production Research Institute, General Directorate of Animal Health and Production, Phnom Penh, Cambodia, **2** International Livestock Research Institute, Nairobi, Kenya, **3** Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Uppsala, Sweden, **4** Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden, **5** Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden, **6** Natural Resources Institute, University of Greenwich, Kent, United Kingdom, **7** Livestock Development for Community Livelihood Organization, Phnom Penh, Cambodia, **8** Center for Public Health and Ecosystem Research, Hanoi University of Public Health, Hanoi, Vietnam

* rortana.chea@slu.se (RC); s.dang@cgiar.org (SDX)

Abstract

Non-typhoidal *Salmonellae* are common foodborne pathogens that can cause gastroenteritis and other illnesses in people. This is the first study to assess the transfer of *Salmonella enterica* from raw chicken carcasses to ready-to-eat chicken salad in Cambodia. Twelve focus group discussions in four Cambodian provinces collected information on typical household ways of preparing salad. The results informed four laboratory experiments that mimicked household practices, using chicken carcasses inoculated with *Salmonella*. We developed four scenarios encompassing the range of practices, varying by order of washing (chicken or vegetables first) and change of chopping utensils (same utensils or different). Even though raw carcasses were washed twice, *Salmonella* was isolated from 32 out of 36 chicken samples (88.9%, 95% CI: 73.0–96.4) and two out of 18 vegetable samples (11.1%, 95% CI: 1.9–36.1). *Salmonella* was detected on cutting boards (66.7%), knives (50.0%) and hands (22.2%) after one wash; cross-contamination was significantly higher on cutting boards than on knives or hands (*p*-value < 0.05). The ready-to-eat chicken salad was contaminated in scenario 1 (wash vegetables first, use same utensils), 2 (wash vegetables first, use different utensils) and 3 (wash chicken first, use same utensils) but not 4 (wash chicken first, use different utensils) (77.8%, 11.1%, 22.2% and 0%, respectively). There was significantly higher *Salmonella* cross-contamination in scenario 1 (wash vegetables first, use same utensils) than in the other three scenarios. These results show how different hygiene practices influence the risk of pathogens contaminating chicken salad. This information could decrease the risk of foodborne disease in Cambodia and provides inputs to a quantitative risk assessment model.

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Introduction

Food safety is a major concern worldwide [1]. Animal-source food (ASF) provides essential nutrients but is a common source of pathogens. The World Health Organization estimated that, in 2010, more than 600 million illnesses were caused by 31 common foodborne hazards: most were diarrhoeal and caused by zoonoses [2]. Foodborne disease (FBD) affects humans of all age groups, but children under five years are among the most vulnerable. According to the Foodborne Disease Burden Epidemiology Reference Group (FERG), one in 10 children worldwide suffer from FBD annually [3]. FBD also decreases human capital, entails prevention and treatment costs, and hinders trade [4]; economic losses are estimated at more than USD 100 billion a year across developing countries [1]. In Cambodia, over 5,000 people fell sick from FBD in 371 outbreaks in 2019 [5], but this is a huge underestimate as there is inadequate surveillance of FBD [6, 7].

Salmonella spp. is one of the most important causes of FBD and is often associated with ASF consumption [8]. Non-typhoidal *Salmonella* (NTS) was estimated to cause 59,000 deaths out of 420,000 annual deaths globally from foodborne hazards in 2010 [3].

Chicken is popular in Cambodia; it is affordable, widely available and provides protein and micronutrients essential for growth and health [9, 10]. In 2020, total meat consumption was 301,000 tons per year, of which poultry meat was 62,000 tons [11]. Poultry meat consumption is expected to increase by 5.5% annually due to increased demand driven by population growth, urbanization and increasing incomes [12]. Cambodians prefer local, backyard chicken, but the modern industrial sector, which uses exotic chicken, has higher productivity and is growing rapidly [13]. High and rising consumption of chicken meat is a health concern because it is a common source of *Salmonella* and other pathogens [14, 15]. *Salmonella* can persist in the chicken intestinal tract without causing clinical signs and is not detected by meat inspection. Therefore, *Salmonella* can contaminate carcasses at the slaughterhouse, especially if facilities and hygiene are poor: for example, if floors are dirty, or if the same tank filled with dirty water is used for all washing steps. In addition, transport and sale under humid tropical conditions may further facilitate *Salmonella* contamination and growth [16–19]. A recent nationwide survey in Cambodian markets reported that 42.6% of chicken meat samples were contaminated with *Salmonella*, with an average most probable number (MPN) of 10.6 MPN/g [20]; previous studies on retailed chicken meat samples also showed high *Salmonella* prevalences which ranged from 20–60% [21–24].

Chicken is widely used in Cambodian cuisine, including traditional salad, consisting of boiled chicken mixed with raw vegetables, herbs, spices and banana flowers [25]. Chicken salad is commonly prepared in households and restaurants and for wedding banquets. Salad is prone to contamination because it includes raw ingredients, is served cold, and its preparation requires equipment and several handling steps [16]. A common cause of bacterial cross-contamination of ready-to-eat (RTE) foods is poor hygiene practices allowing pathogens from raw meat to contaminate hands, knives and cutting boards and hence be transferred to other food [17, 25, 26]. Unfortunately, food hygiene often receives insufficient attention since Cambodian consumers are more concerned about chemicals than microbes [27]. Yet, considering the high *Salmonella* prevalence in chicken meat, consumers could be at risk from chicken salad. To quantify the risk of foodborne illness from eating chicken salad, information on microbial load and cross-contamination in this typical dish is needed. However, the levels and mechanisms of contamination of Cambodian salad with *Salmonella* have not been investigated. Therefore, this study aimed to investigate how *Salmonella* from raw chicken could cross-contaminate RTE chicken salad, given the usual practices in Cambodian households. These experimental results can model exposure assessment steps in quantitative microbial risk assessment of *Salmonella* in chicken salad and recommend reducing cross-contamination while preparing food.

Materials and methods

Household survey on hygiene practices when cooking chicken salad

In 2020, 12 focus group discussions (FGD) with consumers were held in four provinces (Siem Reap, Preah Sihanouk, Battambang and Phnom Penh) in Cambodia. In each province, three areas representing different geographical and social contexts, covering rural, peri-urban and urban areas, were purposely selected based on information from local authorities. In each area, individuals who were mainly in charge of purchasing and preparing food for their families, from six to eight households, were invited by the communal authorities to participate in the FGD. A total of 93 participants discussed food safety practices during purchasing, storing and washing of chicken carcasses and vegetables, use and cleaning of kitchen utensils, and hand washing when preparing the traditional chicken salad *('ngam sach man sroyong chek')*. After gaining written consent from the participants, the FGD was facilitated by a senior researcher while another researcher took notes and recorded the discussion. The FGD was conducted in the Khmer language and lasted approximately 1.5 to 2 hours. Transcripts were then translated into English for quantitative analysis.

Cooking chicken salad scenarios

Cambodian chicken salad is a mix of boiled chicken meat with sliced banana flowers, cucumber, tomato, lemon juice, basil, fresh chili, herbs, and spices (hereafter, these plant ingredients are referred to as 'vegetables'). Fresh vegetables are washed and cut into small pieces. A whole chicken carcass bought from the market is washed, cut into pieces, boiled, deboned, then torn and sliced into shreds. Boiling lasts around 20 minutes, and the time for total preparation is one to two hours.

Four experimental scenarios were designed to imitate the process of preparing the chicken salad in the household as reported in FGDs (that is, washing vegetables either before or after washing the chicken carcass and using the same knife and cutting board (utensils) for chicken and vegetables or different utensils). All four experimental scenarios were carried out on the same day and repeated nine times per scenario. The main steps of cooking chicken salad in each scenario are described in Table 1 and Fig 1.

Scenario 1. *Wash vegetables first and use the same utensils (WVF-SU)*. The process was washing (twice) and chopping vegetables; washing and cutting the raw chicken carcass; washing the cutting board, knife and hands (once with dish detergent); boiling the chicken and using the same (washed) knife, cutting board and hands to debone, tear and slice the boiled chicken and, finally, mixing the salad.

Scenario 2. *Wash vegetables first and use different utensils (WVF-DU)*. The process was washing (twice) and cutting vegetables; washing and cutting raw chicken carcass; washing the cutting board, knife and hands (once with dish detergent); boiling the chicken; using a separate knife and cutting board, and washing hands to debone, tear and slice boiled chicken and mixing the salad.

Scenario 3. *Wash chicken first and use the same utensils (WCF-SU)*. The process was washing (twice) and cutting raw chicken carcass; washing and chopping vegetables; washing the cutting board, knife and hands (once with dish detergent); boiling the chicken; using the same (washed) knife, cutting board and hands to debone, tear and slice boiled chicken, and mixing the salad.

Table 1. Chicken salad preparation steps in each experimental scenario and the number of samples collected and analyzed.

Practices	Preparation steps in each scenario and number of samples collected (n)				Total samples	*Salmonella* analysis	
	Scenario 1	Scenario 2	Scenario 3	Scenario 4		Qualitative (Yes/No)	Quantitative (MPN/g)
1. Wash vegetables twice with water* and slice into small pieces for salad	●	●					
2. Wash chicken carcass twice with water*	●	●	●	●			
3. Cut chicken carcass into smaller parts[1]	● (9)	● (9)	● (9)	● (9)	36	●	
4. Wash vegetables twice with water and slice into small pieces[2] for salad			● (9)	● (9)	18	●	
5. Wash used cutting board, knife and hands once with dish detergent and water	●	●	●	●			
6. Boil chicken carcass (20 min) and take out and wait to cool down (40–45 min)	●	●	●	●			
7. Debone and cut the boiled chicken into small pieces and mix with prepared vegetables using the same, but washed, cutting board, knife and hands[3].	● (27)		● (27)		54	●	
8. Debone and cut the boiled chicken into small pieces and mix with prepared vegetables using a different** cutting board and knife and also washing hands[4]		● (9)		● (9)	18	●	
9. Mix and place ready-to-eat chicken salad on the dish[5]	● (9)	● (9)	● (9)	● (9)	36	●	●
Total samples	45	27	54	36	162		

Note:

* Water for all steps was clean and *Salmonella*-free;

** The cutting board and knife were disinfected to be *Salmonella*-free prior to use in each experiment;

[1] swab of 25 cm^2 of chicken surface;

[2] approximately 50 g of mixed-prepared vegetable was collected;

[3] a set of surface swab samples including cutting board (25 cm^2 in the centre), knife (both sides of the blade, 25 cm^2 each) and hands (palms, fingers and interdigital folds of two hands) was collected right before slicing boiled chicken;

[4] only swabs of hands (palms, fingers and interdigital folds of two hands) were sampled;

[5] approx. 50 g of ready-to-eat salad comprising both chicken meat and vegetable was sampled.

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Scenario 4. *Wash chicken first and use different utensils (WCF-DU).* The process was washing (twice) and cutting the raw chicken carcass; washing and chopping vegetables; washing the cutting board, knife and hands (once with dish detergent); boiling the chicken; using a separate knife and cutting board, and washing hands to debone, tear and slice boiled chicken, and mixing the salad.

Chicken carcass preparation and inoculation

Preparation of the chicken carcass. Four whole chicken carcasses (approximately 1.2 ± 0.2 kg each) were purchased from a shop that had tested negative for *Salmonella* in a recent Cambodian market survey [20]. The butcher was supervised to ensure hygienic processing, including thorough washing and disinfection of hands and equipment (knives and buckets). After cleaning, each chicken carcass was washed thoroughly twice with clean and *Salmonella*-free bottled drinking water (Vital, Phnom Penh, Cambodia) to minimize bacterial contamination. Immediately after that, the washed carcasses were placed into separate sampling bags. The packed carcasses were placed in a cool box and transported immediately to the laboratory to start the experiment within 1 hour.

Preparation of *Salmonella* inoculum. According to recent studies in Cambodia and Vietnam, *Salmonella* Typhimurium and *Salmonella* London were commonly found in retailed

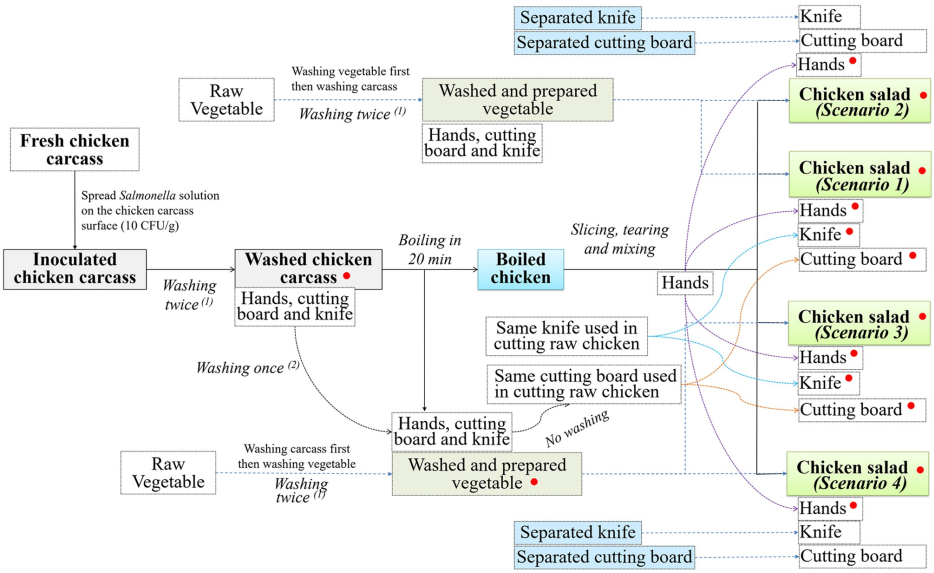

Fig 1. The scenario diagram of the preparation and practice steps of cooking chicken salad, including the sampling points. Red dots indicate the sampling types and stages collected during the experiment of each scenario: [1]washing with clean, *Salmonella*-free water; [2]washing with clean, *Salmonella*-free water, dishwashing detergent and clean dishcloth.

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ASF, including chicken meat and pork [24, 28]. This study utilized two *Salmonella* strains (*S.* Typhimurium and *S.* London) isolated from Vietnam [16] to prepare an inoculation culture. Both strains were cultured separately in a 100 mL glass bottle (Schott Duran, Mainz, Germany) consisting of 50 mL Buffered Peptone Water (BPW; Merck, Darmstadt, Germany) for 12–14 h at 37˚C with gentle agitation. Next, the *Salmonella* load in each strain culture was enumerated using a spread plate count method on Xylose-Lysine Deoxycholate Agar (XLD; Hi-Media, Mumbai, India). Based on the determined concentration in each initial cultured strain, a final suspension of 10^4 *Salmonella* CFU/mL medium was made of appropriate mixed-volume proportions of the two strains. The final *Salmonella* suspension was then used to inoculate the chicken carcass.

Inoculation of chicken carcass. Immediately after preparing the *Salmonella* medium (concentration of 10^4 CFU/mL), each chicken carcass was weighed (in grams) and inoculated with a corresponding volume (in microlitres) of *Salmonella* medium; for instance, a 1,200-gram carcass was inoculated with 1,200 μL *Salmonella* medium. *Salmonella* medium was dropped over the entire chicken carcass surface using 10–200 μL tips and pipette (Corning, NY, US), which created a 10 CFU *Salmonella* per one gram of chicken. After the inoculation, carcasses were kept at room temperature for 30 min for stable absorption, following the methodology of previous experiments [16, 29].

Washing of vegetables, chicken carcasses, hands and equipment, and preparation of vegetables and chicken carcasses. Vegetables comprised banana flowers, lemon, fresh chilli, cucumber, tomato and basil bought from a shop in the early morning. Before using, the researchers washed them twice with clean water containing 1% sodium chloride and immersed them in saline water for 30 min to minimize contamination and then cut them into small slices using a washed cutting board, knife and hands.

The inoculated chicken carcass was washed twice in a basin using clean, *Salmonella*-free water (approximately 5 L per chicken) with clean, bare hands. The washed carcass was put on the cutting board and cut into parts (neck-head, two wings, two drumsticks, two thighs and two breasts). The chicken parts were then boiled in a pot with 2–2.5 L of water for 20 min, after which a spoon was used to remove and place them on a sterile plate to cool down for approximately 40–45 min. The boiled chicken was then deboned, sliced and torn into small pieces using clean bare hands, knives and a cutting board. The cutting board, knife and hands used to cut the fresh carcass were washed once in a different basin with clean water using dish-washing detergent (Sunlight, Unilever, Vietnam) and dishcloth (Sunlight, Unilever, Vietnam) for about 3–4 min as described in an earlier study [16]. The cutting board and knife were kept at ambient temperature to dry at least 15 min before the next step. Vegetables were prepared and processed according to four pre-defined scenarios (Table 1).

Sampling. In each scenario, just before chopping for boiling, the chicken carcass was sampled using a 5 x 5 cm stainless steel frame and a pre-moistened gauze to swab the breast surface. In scenarios 3 and 4, after washing and cutting, but before mixing with meat, vegetables were sampled by taking approximately 50 g of the mixed vegetables with sterile forceps. In scenario 1 (WVF-SU) and 3 (WCF-SU), just before slicing the boiled chicken, a set of three surface swabs and pre-moistened gauze samples were collected from the cutting board (25 cm^2 in the centre), knife (both sides of the blade, 25 cm^2 each) and two hands (palms, fingers, interdigital folds). In scenarios 2 (WCF-SU) and 4 (WCF-DU), the surface of two hands (palms, fingers and interdigital folds) was swabbed just before slicing boiled chicken. After finishing the last step (mixing salad) in each scenario, samples of RTE chicken salad were collected by taking approximately 50 g each of chicken meat and vegetables. The number of samples taken in each step by scenarios and *Salmonella* analyses is presented in Table 1.

Microbiological test. Following the ISO procedure, all samples (n = 162, Table 1) underwent *Salmonella* isolation [30]. A sample of 25 g chicken salad or vegetables or swab was homogenized with appropriate BPW volume and incubated for 18±2 h at 37˚C as a pre-enrichment step. Selective enrichment step was done by adding 1 mL of suspension into 9 mL Muller Kauffmann Tetrathionate (MKTT; Merck, Germany) incubated for 24 ± 3 h at 37˚C and 0.1 mL into 10 mL Rappaport-Vassiliadis Soya (RVS; Merck, Germany) incubated for 24 ± 3 h at 41.5˚C. Selective plating was performed by streaking one loopful (approximately 10 μL) each of MKTT and RVS onto XLD agar. MacConkey (Merck, Darmstadt, Germany) was the second selective plating agar. Two presumptive *Salmonella* colonies per plate were selected to test biochemically (lactose, indole, lysine and hydrogen sulfide) for *Salmonella* confirmation.

A 3-tube MPN method was used to quantify *Salmonella*, as described previously [31]. In brief, the sample was diluted first at 10^{-1} by adding 25 g of chicken salad in 225 mL BPW. Three tubes containing 10 mL of this 10^{-1} dilution were prepared, after which 1 mL of the 10^{-1} dilution was added to three tubes containing 9 mL BPW to make the second series of 10^{-2}; then 1 mL of 10^{-2} was added to the last three tubes containing 9 mL of BPW to make the third series of 10^{-3}. The three-tube set of three consecutive dilutions was incubated at 37˚C for 18 ± 2 h. The steps to detect *Salmonella* in each tube were followed according to the isolation procedures mentioned above. The presence of *Salmonella* in the three tube-set was used to calculate the MPN index according to the method described earlier [32, 33].

Data analysis and modelling. Data were entered into Excel spreadsheets (Microsoft, 2016) and analyzed descriptively (proportion, mean, standard deviation). A chi-squared test or Fisher exact test was used to evaluate the *Salmonella* cross-contamination proportions among sample types and scenarios. R version 3.3.2 (R Core Team, 2020) was used to compute testing and bootstrapping [34]. To describe the distributions of *Salmonella* concentration in the chicken salad in each scenario, both non-parametric and parametric bootstrapping techniques were used. *Salmonella* was quantified as MPN/g and thus followed log-normal distribution. A Bayesian statistic was used to assess variability and uncertainty during the simulation of *Salmonella* load and reduction rates. The parameters and functions used to carry out the bootstrapping, and simulated sample data distributions followed the steps described earlier [16]. The function fitdist() in the *fitdistrplus* package in R was used to estimate the mean and standard deviation of *Salmonella* CFU/g [34]. For the presentation of distributions, kernel density was calculated in density() function based on the simulated sample data and plotted using R. We model the reduction of *Salmonella* CFU/g by using the equation: *Reduction rate = (10—Salmonella CFU/g in RTE chicken salad)/10 x 100*, where 10 was an inoculated CFU/g in raw chicken carcass from the beginning of the test. The distribution of the reduction rate was calculated using iterations and presented in a histogram. The experiments showed four MPN values (110, 110, 15 and 15 MPN/g in scenario 1) which were higher than the inoculation level. The simulation performed without and with these four values was named Scenario 1 and the worst-case Scenario 1, respectively. The simulation was not carried out for Scenario 4 since no *Salmonella* positive salad samples were found in this scenario.

Ethical statement. The experiments were conducted at the National Animal Health and Production Research Institute (Phnom Penh, Cambodia). Participants invited to the focus group discussions were asked for consent before starting (S1 File). All information on the participants was used among the research team only and not shared with any third party. Written consent was obtained from researchers participating in the experiment, including instructions on safety procedures (S2 File). The chicken salad was sterilized and hygienically discarded after finishing the experiment. Ethical approvals of this study were under the Safe Food, Fair Food Cambodia project and granted by the National Ethical Committee of Cambodia (S3 File), No. 300NECHR, dated 26th December 2017, and the International Livestock Research Institute Institutional Research Ethics Committee (S4 File), No. ILRI-RC010 18/IBC/010/CR, dated 5th July 2018.

Results

Hygiene practices when preparing and cooking chicken salad in Cambodian households

Most (86%, 80/93) households reported that they first washed chicken carcasses two to three times with water before washing and preparing vegetables; only 14% (13/93) washed and prepared vegetables before washing chicken carcasses. All participants washed knives and cutting boards at least once, with soap or dishwashing detergent, immediately after cutting fresh chicken carcasses. However, almost all (97%, 90/93) used the same knife and cutting board to prepare raw vegetables and chicken carcasses, as well as to prepare raw and boiled chicken, while the use of separate knives and cutting boards between raw and cooked chicken was less common (3.2%, 3/93, Table 2).

Salmonella contamination from the raw chicken after washing to vegetables/herbs, hands, cutting board and knife during chicken salad preparation

After washing the chicken carcasses twice, *Salmonella* was isolated from 32 out of 36 samples (88.9%, 95%CI: 73.0–96.4). Two out of 18 vegetable samples were cross-contaminated with

Table 2. Food safety practices for preparing chicken salad in Cambodian households.

Practice steps	No. of households (n = 93)	Steps in experiment scenarios
Store or process after buying raw chicken from the market (Yes*, %)		
Start cooking immediately after getting home	53 (57.0)	Keep at room temperature for 30 to 45 mins during preparation in all scenarios
Keep at room temperature	31 (33.3)	
Keep in the refrigerator	9 (9.7)	
The sequence of washing vegetables/herbs and raw chicken (Yes, %)		
Wash vegetables first, then wash the chicken carcass	13 (14.0)	Applied in scenarios 1, 2
Wash chicken carcass first, then wash vegetables	80 (86.0)	Applied in scenarios 3, 4
Number of times to wash chicken carcass before processing (time, *mean (min-max)*)	2.9 (1–5)	Applied to wash carcass two times in all scenarios
Wash knives, cutting board and hands with soap/ dish detergent after cutting chicken carcass *(Yes, %)*	93 (100)	Applied to wash hands, equipment in all scenarios
The average number of times when washing knives, cutting board, hands after cutting raw chicken *(times, mean (min-max))*	1.3 (1–3)	Applied to wash one time in all scenarios
Length of boiling chicken for salad dish counted from boiling stage (minutes, *mean (min-max)*)	29 (15–60)	Applied to boil chicken in 20 mins in all scenarios
Use the same knife and cutting board with washing once in between when preparing raw and cooked chicken *(Yes, %)*	90 (96.8)	Applied in scenarios 1, 3
Use the same knife and cutting board with washing once in between when preparing raw vegetable and chicken carcass *(Yes, %)*	90 (96.8)	Applied in scenarios 3, 4

* Yes versus No *("No" means using separate knives and cutting boards between vegetable and meat, or between raw and cooked meat, but hands were washed once with soap or detergent).*

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Salmonella (11.1%, 95%CI: 1.9–36.1, Table 3). Eight out of 36 hand swabs (palms, fingers and interdigital folds of two hands) (22.2%, 95%CI: 10.7–39.6), which had been washed once after handling contaminated chicken carcasses, were positive for *Salmonella*, which was significantly lower compared to 66.7% of washed cutting boards being contaminated (12/18, $\chi2 = 8.35$, df = 1, p-value = 0.004) and lower compared to contamination on washed knives (50.0%, 9/18, $\chi2 = 3.10$, df = 1, p-value = 0.07, Table 3). *Salmonella* from chicken carcasses was most often transferred to the cutting boards, followed by knives and hands, even though hands and equipment were washed once with clean water and dishwashing detergent. From the simulated data, *Salmonella* cross-contamination to cutting boards was significantly higher than to knives and hands and was higher to knives than to hands (p-value < 0.001); average contamination on hands, knives and cutting boards was 23.7% (95%CI: 5.1–28.3), 50.1% (95%CI: 42.5–57.8) and 65.2% (95%CI: 58.3–72.6), respectively (Table 3, Fig 2).

Cross-contamination of *Salmonella* to ready-to-eat chicken salad

In Scenario 1 (WVF-SU), seven out of nine (77.8%) chicken salads were positive with *Salmonella*, while the number of salad samples positive with *Salmonella* in Scenarios 2 (WFV-DU) and 3 (WCF-SU) was one out of nine (11.1%) and two out of nine (22.2%), respectively. Scenario 4 (WCF-DU) showed no positive *Salmonella* in salad in all nine experiments. Scenarios 1 and 3 used the same knife, cutting board, and hands for handling salad, while in Scenarios 2

Table 3. *Salmonella* contamination from the raw chicken after washing twice and vegetables, hands, knives and cutting boards during chicken salad preparation.

Sample types	Experimental data		Simulated data*	
	No. of *Salmonella* positive (Scenario 1/2/3/4)/No. of experiments	Contamination percentage (%, 95%CI)	No. of *Salmonella* positive/No. of iterations	Contamination percentage (%, 95%CI)
***Salmonella* contamination in raw chicken and vegetables** ** **after washing twice**				
Washed raw chicken carcasses	32 (8/7/8/9)/36	88.9 (73.0–96.4)	4340/5000	86.8 (83.5–90.9)
Washed and prepared vegetables	2 (na/na/1/1)/18	11.1 (1.9–36.1)	745/5000	14.9 (9.1–19.6)
Cross-contamination of *Salmonella* to hands, cutting boards and knives* **				
Washed hands after handling contaminated chicken carcasses	8 (3/1/3/1)/36	22.2 (10.7–39.6)[a]	1185/5000	23.7 (5.1–28.3)[a]
Washed knives after handling contaminated chicken carcasses	9 (3/na/6/na)/18	50.0 (29.0–70.9)[a]	2505/5000	50.1 (42.5–57.8)[b]
Washed cutting boards after handling contaminated chicken carcasses	12 (5/na/7/na)/18	66.7 (41.2–85.6)[b]	3260/5000	65.2 (58.3–72.6)[c]

Note: "na": Not applicable; Different superscript letters in the same column indicate significant difference; CI: confidence interval;

* Simulated data were generated from random sampling 5000 times, in which initial values were based on experiment samples and positive numbers using beta distribution in RStudio: [rbeta(5000, positive+1, n-positive+1)];

** Vegetables (included banana flower, lemon, fresh chilli, cucumber, tomato and basil) were washed twice with clean Salmonella-free water using the same knives, cutting boards and hands for preparation (i.e. cutting);

*** Knives, cutting boards and hands were washed once using clean Salmonella-free water and dishwashing detergent after cutting the raw chicken carcasses.

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and 4, a separate knife and cutting board were used. There was a significantly higher cross-contamination rate in Scenario 1 compared to the other three scenarios (Fisher exact test, *p*-value = 0.05). The average *Salmonella* contamination in the salad was highest in Scenario 1 (37.3 MPN/g) and 0.36 MPN/g in both Scenarios 2 and 3. The overall proportion of *Salmonella* contamination in all four scenarios was 27.8% (95%CI: 14.8–45.4, Table 4).

Based on simulated data (5000 iterations), the average *Salmonella* contamination in salad was higher in Scenario 1 (8.58 CFU/g) (including 4 values that exceeded the initial value of 10 CFU/g) with a mean of 77.78 CFU/g. In contrast, the average *Salmonella* contamination in Scenarios 2 and 3 was 0.8 and 0.78 CFU/g, respectively (Table 5 and Fig 3). (*Salmonella* was absent in Scenario 4).

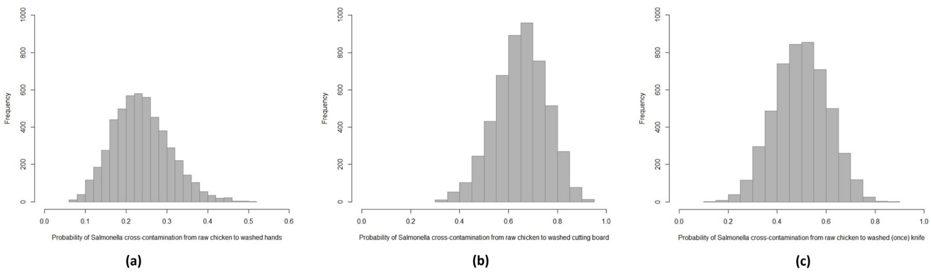

Fig 2. Simulated probability distribution of *Salmonella* cross-contamination from raw chicken to hands (a), cutting board (b) and knife (c) after washing once after washing and cutting the fresh chicken carcass.

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Table 4. Contamination of *Salmonella* in ready-to-eat chicken salad during preparation and handling in four different experiment scenarios.

Scenario	No. of *Salmonella* positive/Total samples	Proportion of contamination	95%CI	*Salmonella* MPN/g (mean, min-max)
Scenario 1 (WVF-SU)	7/9	77.8[a]	40.2–96.1	37.3 (0.1–110)
Scenario 2 (WVF-DU)	1/9	11.1[b]	0.6–49.3	0.36 (0.36–0.36)
Scenario 3 (WCF-SU)	2/9	22.2[b]	3.9–39.8	0.36 (0.36–0.36)
Scenario 4 (WCF-DU)	0/9	0.0[b]	0.0–37.1	NA
Overall	**10/36**	**27.8**	**14.8–45.4**	**26.2 (0.1–110)**

Note: Different superscript letters in the same column indicate significant differences; CI: confidence interval; NA: not available.

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The reduction rate of *Salmonella* contamination from raw chicken to ready-to-eat chicken salad

Most *Salmonella* was transmitted from raw chicken to salad in Scenario 1 (WVF-SU). In this scenario, four out of seven positive samples had higher levels of *Salmonella* after processing. On the other hand, in Scenarios 2 (WVF-DU) and 3 (WCF-SU) *Salmonella* was reduced by 92%. The proportion of the simulated values that exceeded initial CFU/g in Scenarios 1, 2 and 3 were 53.8, 18.6, 3.8 and 3.6, respectively (Table 6 and Fig 4).

Discussion

This study examined how food handling might affect the risk of cross-contamination by *Salmonella* in households under different preparation scenarios. The results indicate interventions to reduce risk. In addition, findings can be used to model exposure assessment steps in conducting a quantitative microbial risk assessment of *Salmonella* in chicken salad. In Cambodia, several foodborne outbreaks have been associated with contamination during food preparation [5, 35]. The FGDs of the 93 households described the common practices that were used to develop scenarios for the experiment. Chicken and pork salad are typically consumed in households, restaurants or ceremonies in Cambodia [36–38], Southeast Asia and Middle Eastern countries [39]. FBD cases have been associated with these [35, 36]. *Salmonella* in raw chicken carcasses sold in the market is a source of contamination, especially when using the same hands, knife, or cutting board without adequate washing [40]. Several factors could contribute to the current high prevalence of *Salmonella* in retailed chicken and pork in Cambodian markets (exceeding 40%) [20]. These include reused or unsafely used water for cleaning (for example, washing intestines in the same basin as carcasses), absence of appropriate storage facilities for food, low frequency of cleaning and disinfection of the shop, etc. [6, 14, 41].

Table 5. *Salmonella* (CFU/g) concentration was simulated in each scenario in the ready-to-eat chicken salad, based on the experiment values with 5000 iterations.

Scenario	The concentration of *Salmonella* (CFU/g)* contaminated with chicken salad			
	Mean	Median	Lower limit	Upper limit
Scenario 1 (WVF-SU)**	8.58	1.31	0.002	59.15
Scenario 1***	77.78	12.41	0.008	577.0
Scenario 2	0.80	0.36	0.032	4.09
Scenario 3	0.78	0.36	0.029	4.03

* CFU: Colony-forming unit;

** Scenario 1 had only values below the initial concentration (10 CFU/g);

***Worse-case Scenario 1 included four MPN/g values (two 110 CFU/g and two 15 CFU/g) which exceeded the initial concentration (10 CFU/g).

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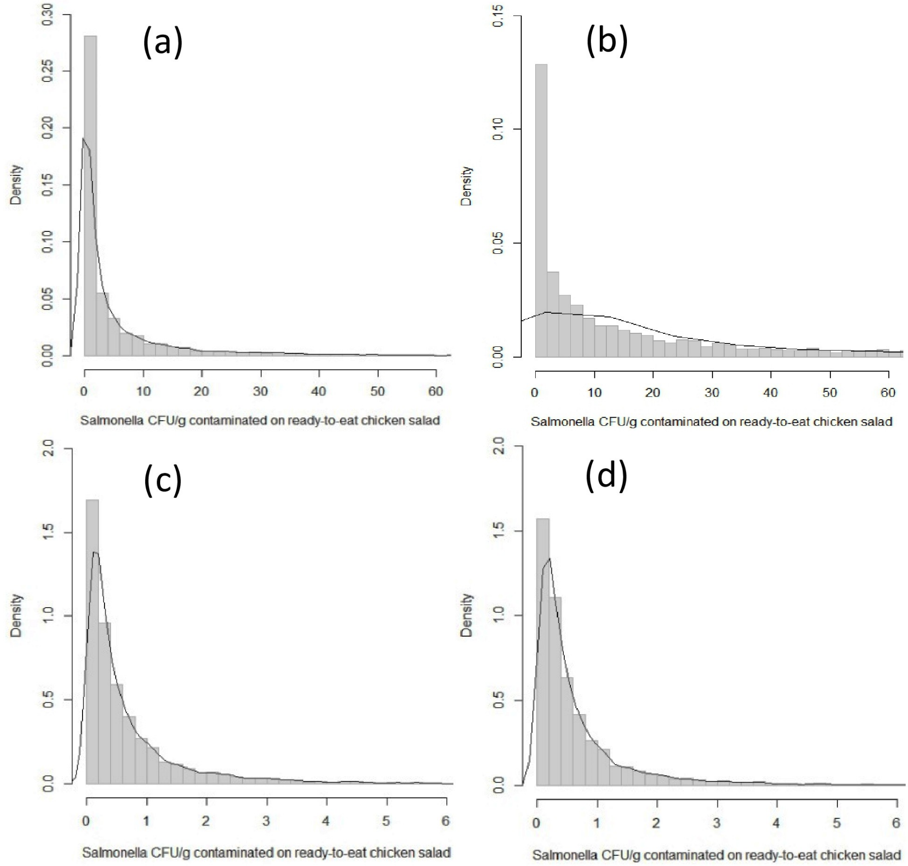

Fig 3. *Salmonella* concentration (CFU/g) on contaminated RTE chicken salad in Scenario 1* (a), Scenario 1 (b), Scenario 2 (c) and Scenario 3 (d) based on the experiment values simulated 5000 times.** * Scenario 1 had only values below the initial concentration (10 CFU/g); ** worst-case Scenario 1 included four MPN/g values (two 110 CFU/g and two 15 CFU/g), which exceeded the initial concentration (10 CFU/g).

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Despite washing the chicken carcasses twice in water, this study found that 88.9% (95% CI: 73.0–96.4) of experimental carcasses still harboured *Salmonella*, similar to a finding in an experiment of washing contaminated pork in Vietnam, but the washing steps significantly reduced the number of *Salmonella* bacteria by up to 92.2% (Table 5) [16, 42, 43]. In this study, handling and preparing raw meat (including washing and cutting) increased *Salmonella* contamination of hands, cutting boards, knives and vegetables. Washing of food products before preparation is often observed in many low- and middle -income countries [39] and was also a common practice in the households interviewed in the present study.

Table 6. Simulated reduction rate (percentage) of *Salmonella* concentration (CFU/g) in the RTE chicken salad in each scenario based on the experiment values simulated 5000 times.

Scenario	The reduction rate of *Salmonella* concentration, CFU/g*				
	Mean	Median	Lower limit	Upper limit	Exceeded initial CFU/g
Scenario 1	14.2	86.9	-504	99.9	18.6
Worst-case Scenario 1**	-728.2	-24.1	-6241.1	99.9	53.8
Scenario 2	92.0	96.4	58.5	99.7	3.8
Scenario 3	92.2	96.4	59.7	99.7	3.6

* CFU: Colony-forming unit;

**Worse-case Scenario 1 included four MPN/g values (two 110 CFU/g and two 15 CFU/g) which exceeded the initial inoculated concentration (10 CFU/g).

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In high-income countries, washing raw meat before cooking is usually not recommended because the meat is often contaminated with bacteria, and washing raw meat can spread pathogens around the kitchen. On the other hand, in developing countries, it is often believed that washing meat before cooking removes dirt, slime and blood and makes it safer. In Cambodia, only a few supermarkets or minimarts in urban areas provide packed and cooled or chilled meat and the level of hazards present in these is not well established. Indeed, studies have found that contamination levels are not always lower in modern retail shops in developing

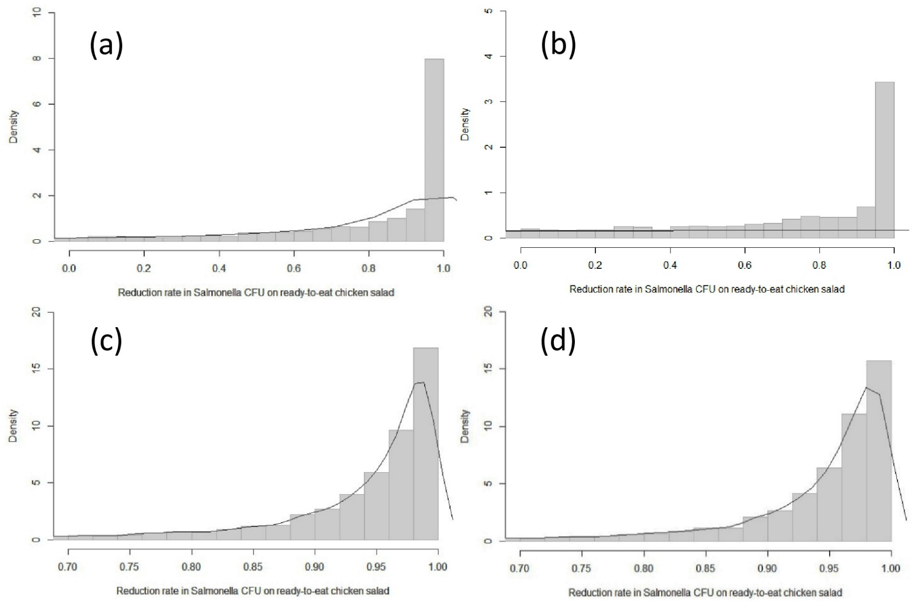

Fig 4. The reduction rate of *Salmonella* concentration (CFU/g) in the contaminated RTE chicken salad in Scenario 1* (a), worst-case Scenario 1 (b), Scenario 2 (c) and Scenario 3 (d) based on the experiment values to bootstrap 5000 times.** * Scenario 1 had only values below the initial concentration (10 CFU/g); ** Worst-case Scenario 1 included four MPN/g values (two 110 CFU/g and two 15 CFU/g), which exceeded the initial concentration (10 CFU/g).

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countries [20, 43]. Nevertheless, separating equipment used for raw and RTE foods is strongly recommended in all contexts. Cleaning and disinfection of hands, knives and cutting board surfaces right after contact with raw meat/chicken can minimize contamination with bacteria [43].

The current study detected *Salmonella* cross-contamination from chicken meat to RTE Cambodian chicken salad, with 27.8% of all salads being contaminated. This is the first study to quantify cross-contamination of bacteria during simulated home preparation of ASF products in Cambodia. Studies in other countries on cross-contamination from pork [16] and chicken [29] found a similar trend of *Salmonella* cross-contamination to RTE food. *Salmonella* from ASF can contaminate hands, equipment and containers and cross-contaminate RTE chicken salad during preparation, consequently causing foodborne illness in consumers. Several reports show that FBDs, especially in developing countries, are often underreported, and there is a lack of food safety surveillance and traceability systems [1, 3, 5–7, 44]. This implies there are more foodborne illness cases, including salmonellosis, in Cambodia than the number officially reported by the health authority (5000 cases of FBD in 2019) [5, 35].

Scenarios 1 (WVF-SU) and 3 (WCF-SU), using the same utensils for chopping chicken and vegetables and assumed to be the scenarios with the poorest hygiene practices, had the highest proportion of *Salmonella* contamination of salad (77.8% and 22.2%, respectively) and the highest quantity of *Salmonella* (37.3 MPN/g, Scenario 1). About 90% of the surveyed households practiced these sub-optimal procedures for preparing chicken salad in their homes. Other studies have also found that unwashed knives, cutting boards and hands increased the risk of cross-contamination in frequency and CFU/g [16, 41, 45]. For example, a Cambodian study of a foodborne outbreak revealed that the unhygienic practices could have led to cross-contaminated food [36]. Cross-contamination of bacteria to RTE food was also reported in studies in China, Indonesia, Malaysia, Thailand and Vietnam [17, 46], where daily food preparation practices were examined. In a similar experiment comparing *Salmonella* contamination when using the same or different utensils to prepare boiled pork in Vietnam, the *Salmonella* prevalence varied from 22.2–77.8%, and average *Salmonella* concentrations were from 0.12 to 5.79 CFU/g in cooked pork [16].

This study shows that washing did not eliminate *Salmonella* from the chicken. In addition, washing vegetables after washing the raw chicken carcasses resulted in the transfer of *Salmonella* to the vegetables via hands or equipment, or both. However, we did not expect that levels of *Salmonella* in the chicken salad in scenario 1 (vegetables washed first) would be worse than in scenario 3 (chicken washed first). In addition, in Scenarios 3 and 4 of our experiment, two out of eighteen washed and prepared vegetables were positive with *Salmonella* (Table 3) would explain the less involvement of hands and sink in transferring *Salmonella* to vegetables during washing contaminated chicken first. Practices of washing chicken carcass before washing vegetables were reported in 86% of interviewed households (Table 2); however, actual of using the sink, basket, hands, or contact with rinsed water in washing vegetables can be different by households thus, the level of cross-contamination to vegetables could be higher than in our experiment. Washing meat could decrease bacterial contamination; nonetheless, the varied practices also result in different levels of bacteria remaining in food [42]. Cutting boards have been reported to contribute the most to the cross-contamination of bacteria from raw ASF to other food [28]. A study in China using *Campylobacter* spp. as an indicator of in-home cooking procedures also found that cutting boards were an important source of cross-contamination [47].

Scenarios 2 (WVF-DU) and 4 (WCF-DU) were more hygienic as a separate knife and cutting board were used to prepare raw and cooked chicken. A lower proportion of *Salmonella*-contaminated chicken salads was observed in scenarios 2 and 4 (11.1% and 0.0%, respectively) than in scenarios 1 (WVF-SU) and 3 (WCF-SU). However, there was only one *Salmonella*-positive vegetable sample in scenario 3 and one in scenario 4 (WCF-DU) when vegetables were washed and chopped after the chicken. It is generally agreed that washing vegetables before handling and preparing raw meat would significantly reduce the risk of cross-contamination [42, 43]. Our study did not find any evidence of this, with scenario 1 having the highest contamination despite washing vegetables first and scenario 4, with chicken, washed first, resulting in no contamination of the chicken salad.

Information on *Salmonella* prevalence, load and reduction rate in each hygiene practice scenario, will be helpful in exposure assessment; a step often inadequately addressed in risk assessment [48]. This is the first study investigating cross-contamination by *Salmonella* in Cambodian households when preparing salad. The findings show different ways that bacteria can contaminate RTE food and may be generalized to other types of salad prepared by similar procedures and used to assess the risk of cross-contamination in other types of raw meat and seafood. Furthermore, the results can be used to design and disseminate more targeted, evidence-based food safety practice messages, such as the need to use a separate cutting board and knife for raw and RTE food and adequately clean and disinfect hands and equipment surfaces after contact with raw meat.

This study had some limitations. The experiment used raw chicken and 'vegetables' (cucumber, tomato, basil, fresh chili and banana flower) purchased from hygienic slaughterhouses and shops; however, these might not always have been *Salmonella*-free during all nine experimental days. In addition, during the experiment, the variation in contact time, pressure and moisture and the nature (surface) of meat, vegetables and equipment between replications might have affected *Salmonella* (cross) contamination [49–51]. Future studies should also assess the risk of washing instead of not washing chicken carcasses before cooking.

Conclusions

Our finding that the median *Salmonella* load in the chicken salad was between 0.36 and 12.41 CFU/g raises health concerns. We described the usual practices of preparing chicken salad in Cambodia and examined how these could lead to *Salmonella* being transferred from chicken carcasses to salad, identifying risky practices including the use of the same cutting board and knife for meat and vegetables and inadequate handwashing. Different salad preparation practices result in very different contamination levels, with washing vegetables before the chicken and using the same utensils for chopping chicken and vegetables resulting in higher levels of salad contamination. Risk communication messages should focus on the need for separate kitchen utensils and frequent and adequate washing and disinfecting of food contact surfaces (cutting board, knife, hands). The finding that washing chicken carcass before the vegetables resulted in less contamination was not expected and requires further investigation. Data on *Salmonella* levels under different preparation scenarios will be used to support quantitative microbial risk assessments through eating salad.

Supporting information

S1 File. Consent form for participant in research: Household.
(PDF)

S2 File. Consent form for participant in research: Laboratory research team.
(PDF)

S3 File. Ethical approvals of this study granted by the National Ethical Committee of Cambodia.
(PDF)

S4 File. Biosafety check by International Livestock Research Institute Institutional Research Ethics Committee.
(PDF)

S5 File. Funding statement.
(PDF)

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Author Contributions

Conceptualization: Rortana Chea, Hung Nguyen-Viet, Sothyra Tum, Fred Unger, Johanna Lindahl, Delia Grace, Chhay Ty, Huy Sokchea, Sinh Dang-Xuan.

Data curation: Rortana Chea, Johanna Lindahl, Sinh Dang-Xuan.

Formal analysis: Rortana Chea, Johanna Lindahl, Sinh Dang-Xuan.

Funding acquisition: Hung Nguyen-Viet, Sothyra Tum, Fred Unger, Delia Grace.

Investigation: Rortana Chea, Sothyra Tum, Fred Unger, Chhay Ty, Sok Koam, Vor Sina, Son Pov, Theng Heng, Or Phirum, Sinh Dang-Xuan.

Methodology: Rortana Chea, Hung Nguyen-Viet, Fred Unger, Johanna Lindahl, Delia Grace, Chhay Ty, Sok Koam, Sinh Dang-Xuan.

Project administration: Hung Nguyen-Viet, Fred Unger, Delia Grace.

Resources: Rortana Chea.

Software: Rortana Chea, Sinh Dang-Xuan.

Supervision: Hung Nguyen-Viet, Sothyra Tum, Fred Unger, Johanna Lindahl, Delia Grace.

Validation: Rortana Chea, Sinh Dang-Xuan.

Visualization: Rortana Chea, Sinh Dang-Xuan.

Writing – original draft: Rortana Chea.

Writing – review & editing: Rortana Chea, Hung Nguyen-Viet, Sothyra Tum, Fred Unger, Johanna Lindahl, Delia Grace, Sinh Dang-Xuan.

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EDITED BY
M. Leonor Faleiro,
University of Algarve, Portugal

REVIEWED BY
Ian Jenson,
University of Tasmania, Australia
Peter S. Evans,
Food Safety and Inspection Service (USDA), United States

*CORRESPONDENCE
Chea Rortana
✉ rortana.chea@slu.se
Sinh Dang-Xuan
✉ S.Dang@cgiar.org

†These authors have contributed equally to this work and share first authorship

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Quantitative risk assessment of salmonellosis in Cambodian consumers through chicken and pork salad consumption

Chea Rortana[1,2,3*†], Sinh Dang-Xuan[2*†], Hung Nguyen-Viet[2], Fred Unger[2], Johanna F. Lindahl[2,4,5], Sothyra Tum[1], Chhay Ty[6], Delia Grace[2,7], Kristina Osbjer[5,8,9] and Sofia Boqvist[3]

[1]General Directorate of Animal Health and Production, National Animal Health and Production Research Institute, Phnom Penh, Cambodia, [2]International Livestock Research Institute, Nairobi, Kenya, [3]Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Uppsala, Sweden, [4]Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden, [5]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden, [6]Livestock Development for Community Livelihood Organization, Phnom Penh, Cambodia, [7]Natural Resources Institute, University of Greenwich, London, United Kingdom, [8]Emergency Centre for Transboundary Animal Diseases, Food and Agriculture Organization of the United Nations, Phnom Penh, Cambodia, [9]International Centre for Antimicrobial Resistance Solutions, Copenhagen, Denmark

Salmonella is a globally important foodborne bacterial pathogen that poses a high risk to human health. This study aimed to estimate the risk to Cambodian consumers from acquiring salmonellosis after consuming chicken and pork salad, using a quantitative microbial risk assessment (QMRA). Chicken and pork salads are typical Cambodian dishes containing raw vegetables and boiled chicken meat or pork. As previously described, chicken meat and pork samples ($n = 204$ of each) were collected from traditional markets in 25 Cambodian provinces to generate data on *Salmonella* contamination. Salad preparation and consumption practices were surveyed in 93 Cambodian households and this information was used to design an experiment to assess *Salmonella* cross-contamination from raw meat to ready-to-eat salad. In the part of the study reported here, data on consumption, *Salmonella* in salad, dose-response, and predicted salmonellosis were modeled using Monte Carlo simulations at 10,000 iterations. The prevalence of *Salmonella* in chicken meat and pork were set to 42.6 and 45.1%, respectively, with average most probable number (MPN) per gram of *Salmonella* in chicken meat was 10.6 and in pork 11.1 MPN/g, based on an earlier study. Half of the interviewed households cooked meat for the salad directly after purchase. The QMRA model showed that the modeled annual risk of salmonellosis from consuming chicken salad, pork salad and both chicken and pork salad were 11.1% probability of illness per person per year (90% CI 0.0–35.1), 4.0% (90% CI 0.0–21.3), and 14.5% (90% CI 0.0–33.5), respectively. The factors most influencing the estimate were cross-contamination while preparing the salad, followed by the prevalence of *Salmonella* in chicken meat and pork at the market. The wide confidence interval for the incidence was mainly due to the variability in reducing bacteria concentration by cooking and salad consumption. The predicted risk of salmonellosis due to chicken and pork salad consumption is high, and the study provides evidence supporting control measures of improving the safety of retailed chicken and pork obtained from markets to households and improving food preparation methods in the household.

KEYWORDS

ASF consumption, Cambodia, QMRA, cross-contamination, Cambodian chicken and pork salad, traditional market

1. Introduction

The World Health Organization (WHO) has estimated that foodborne diseases (FBD) cause 33 million disability adjusted life years (DALYs) globally, and a loss of more than US$110 billion in productivity and medical expenses each year in low- and middle-income countries (LMIC) (Havelaar et al., 2015; Devleesschauwer et al., 2018). Annually, around 200 different types of foodborne pathogens cause disease in 600 million people, and FBD has been reported to result in around 420,000 deaths every year (World Health Organization Regional Office for South-East Asia., 2016). People living in LMIC are at particular risk for contracting FBD due to challenges related to insufficient hygiene practices, poor knowledge and reduced access to safe food (Grace, 2015; Varijakshapanicker et al., 2019). FBD thus constitute a significant health challenge globally, and non-typhoid *Salmonella* serovars have been reported as the most common foodborne bacteria causing FBD (Havelaar et al., 2015; World Health Organization Regional Office for South-East Asia., 2016; Boqvist et al., 2018).

Most *Salmonella* serovars are human pathogens and may cause a wide range of symptoms, of which diarrhea is the most common (Oscar, 2004; Majowicz et al., 2010; Crump and Wain, 2017). Animal-source food (ASF) is often implicated in human salmonellosis. It is estimated that globally *Salmonella* causes approximately 230,000 deaths annually, mainly in elderly and children under 5 years (Majowicz et al., 2010; Havelaar et al., 2015; World Health Organization Regional Office for South-East Asia., 2016; Devleesschauwer et al., 2018). *Salmonella* is carried by many animal species and can be transmitted by ASF, contributing to food safety concerns in LMIC (Unger et al., 2020).

In Cambodia, ASF (especially chicken meat and pork) are essential parts of the diet eaten by all age groups (General Directorate of Animal Health Production of Cambodia, 2021) and contribute important micronutrients (Tum, 2008; Sary et al., 2019). Chicken and pork are commonly sold at traditional markets where most people buy fresh food. Meat is commonly stored without chilling at the markets and in most households (People in Need, 2015; Rortana et al., 2022). Several popular dishes are prepared from boiled chicken meat or pork mixed with raw vegetables (Baker, 2009; Saorath, 2019; Cambodia Recipe, 2020).

In LMIC, chicken and pork are easily contaminated with *Salmonella*, which can occur at slaughterhouse facilities, markets, and storage facilities with insufficient cooling (Cliver, 2006; Carrasco et al., 2012; Aizaabi and Khan, 2017; Possas et al., 2017). Storing of meat in warm temperatures provides good conditions for the growth of *Salmonella* (Possas et al., 2017; Dang-Xuan et al., 2019). Improper handling and poor practices also contribute to the transmission of bacteria along the food chain, especially from markets to ready-to-eat (RTE) foods (Kristina and Sophal, 2018)). In addition, recent studies have found that poor handling of meat before and during cooking causes bacterial cross-contamination to RTE food, including chicken salad in Cambodia (Rortana et al., 2022) and boiled pork in Vietnam (Dang-Xuan et al., 2018). In Cambodia, a recent study detected 43% prevalence of *Salmonella* in chicken meat, 41.9% on chicken cutting board, 45% on pork, and 30% on pork cutting board; and the mean MPN of *Salmonella* per gram was 10.6 MPN/g in chicken and 11.1 MPN/g in pork samples (Rortana et al., 2021).

Quantitative microbial risk assessment (QMRA) can estimate health consequences and help in food safety management and communication. In Cambodia, QMRA has been conducted on *Salmonella* and different hazards and food type, but there are, to our knowledge, no publications on the risk of salmonellosis related to chicken meat or pork (Tum, 2008; Kristina and Sophal, 2018; Walia et al., 2018; Food and Agriculture Organization of the United Nations, 2021) although QMRA models of salmonellosis have been developed in other countries (Dang-Xuan et al., 2016; Perez-Rodriguez, 2020; Oscar, 2021a,b).

In Cambodia, there is a lack of comprehensive and solid evidence on the impact of FBD that can guide policymakers on health hazards related risks, and support meat production and donors to tackle food safety issues and public health notices (Tum, 2008; Public Health of Canada, 2017; Lam et al., 2019). Moreover, the household knowledge of FBD is low in Cambodia, and most people associate food safety challenges mainly with chemical contamination (Brown et al., 2022; Duong et al., 2022). This study aimed to estimate the risk of consumers acquiring

Salmonella infection through consuming contaminated pork and chicken salad at the household level to generated new and actionable information.

2. Materials and methods

2.1. Study location

The study was conducted in Cambodia, located in the Mekong sub-region in Southeast Asia. In 2019, the total population of Cambodia was around 15 million (National Institute of Statistics of Cambodia, 2019). The country is influenced by tropical monsoon winds and has two seasons, the dry season (November–April) and the rainy season (May–October). In the rainy season, the average temperature in 2019 was 29°C, ranging between 27 and 36°C, with a humidity between 45 and 80 % (Department of Meteorology of Cambodia, 2019). Data for *Salmonella* contamination used in this QMRA was collected from all 25 provinces in Cambodia.

2.2. Study design

The QMRA model was built according to the Codex Alimentarius Commission quantitative microbial risk assessment framework (Codex Alimentatius Commission, 1999), consisting of hazard identification, hazard characterization, exposure assessment, and risk characterization (CAC/GL-30, 1999). This QMRA model was designed using data published earlier. Firstly, a cross-sectional market survey of the *Salmonella* prevalence in chicken meat and pork had been conducted in traditional markets in all 25 provinces in Cambodia (Rortana et al., 2021). Secondly, a household survey had been carried out in four provinces and cities (Battambang, Preah Sihanouk, Phnom Penh and Siem Reap) to explore handling and consumption patterns of chicken and pork salad in Cambodian (Rortana et al., 2022). Thirdly, experiments to identify cross-contamination scenarios during the preparation of chicken and pork salad at the household level had been done at the National Animal Health and Production Research Institute (Phnom Penh, Cambodia) (Rortana et al., 2022) and in Vietnam (Dang-Xuan et al., 2018). Lastly, data for the hazard characterization, of bacteria growth and dose-response model were obtained from the literature (Teunis et al., 2010; Velugoti et al., 2011). These surveys and experiments were conducted from November 2018 to June 2020 and are described briefly below.

2.3. Salmonellosis risk assessment model

2.3.1. Hazard identification

In a publication from WHO on the global burden of FBD, salmonellosis was identified as the most important bacteria hazard in DALYs (Havelaar et al., 2015). Salmonellosis is also considered one of Cambodia's most critical FBD (Rortana et al., 2021). The hazard identification of this study was made using data from a systematic literature review (Kristina and Sophal, 2018), from key stakeholder meetings in Cambodia (including representatives from a national food safety working group, policymakers, and international partners) (Nguyen-Viet, 2018), from a multi-hazard survey (Rortana et al., 2021), and from the cost of hospitalization for FBD performed by the Ministry of Health in Cambodia (Srey, 2019).

2.3.2. Hazard characterization

Non-typhoid *Salmonella* serovars are the most common foodborne bacteria causing FBD (Havelaar et al., 2015; World Health Organization Regional Office for South-East Asia., 2016; Boqvist et al., 2018). Most *Salmonella* serovars are human pathogens and may cause a wide range of symptoms, of which diarrhea is the most common (Oscar, 2004; Majowicz et al., 2010; Crump and Wain, 2017). Moreover, invasive *Salmonella* infection has been reported in Cambodia (Emary et al., 2012; Vlieghe et al., 2012; Kimsean et al., 2017; Kristina and Sophal, 2018; Kuijpers et al., 2018). *Salmonella* contamination in ASF is often implicated in human salmonellosis infection (Botteldoorn et al., 2003; Carrasco et al., 2012; Havelaar et al., 2015). In this study, the Beta-Poisson dose-response model developed from *Salmonella* outbreak data was used (alpha = 0.00853; beta = 3.14) (Teunis et al., 2010). That dose-response model presented an infection ID50 of 7 colony forming unit (CFU) and an illness ID50 of 36 CFU.

2.3.3. *Salmonella* exposure assessment

2.3.3.1. Meat sampling at market

A previous study investigated the prevalence and concentration of *Salmonella* in chicken meat and pork sold in traditional Cambodian markets (Rortana et al., 2021). In brief, samples from chicken meat ($n = 204$) and pork ($n = 204$) from markets in 25 provinces in Cambodia were included. The prevalence of *Salmonella* from all the markets in chicken meat was 42.6% and in pork 45.1%. The mean MPN of *Salmonella* was 10.6 MPN/g in chicken meat and 11.1 MPN/g in pork samples.

2.3.3.2. Cross-contamination study

Cross-contamination of *Salmonella* has been described in two published papers on chicken salad (Rortana et al., 2022) and boiled pork (Dang-Xuan et al., 2018). According to a recent study, cross-contamination of *Salmonella* in chicken salad was common in the four scenarios or sets of household practices used for salad preparation in Cambodia (Rortana et al., 2022). Briefly, *Salmonella* occurrence on cutting boards, knives and hands under four preparation scenarios (Table 1) was assessed. Similarly, *Salmonella* cross-contamination from raw pork to boiled pork *via* a hands and kitchen utensils was examined in

Vietnam (Dang-Xuan et al., 2018). The similarity of the four scenarios is described in detail in Table 1. The proportion of households using each scenario, as well as the probability of contamination, were part of the modeling.

2.3.3.3. Chicken and pork salad consumption

Chicken and pork salad consumption were assessed using focus group discussion (FGD) among 93 households in four provinces (Siem Reap, Preah Sihanouk, Battambang, and Phnom Penh) in Cambodia (Rortana et al., 2022). Three FGDs (with participants chosen to represent rural, peri-urban, and urban areas) in each of the four provinces were conducted by randomly selecting households within one commune. A discussion outline was developed in English and translated to Khmer language for FGD and back translated into English for analysis. The FGD was led by trained researchers using flipchart and notes, and lasted about 1.5 h. The information of chicken and pork salad consumption was determined for children below 5 years, youth (6–15 years old), adults (16–60 years old), and the elderly (above 61 years old).

2.3.4. Risk characterization of *Salmonella* infection

The data presented above was integrated into a stochastic risk model, including different input parameters (Table 2). The risk of salmonellosis (health outcome) was defined as the probability of illness per year per person, simulated by combining different transmission pathways through chicken and pork salad consumption. The parameters, statistics, distribution, and data sources used in the QMRA model are presented in Table 2 and Figure 1. In step 1, the prevalence of *Salmonella* from samples collected at the markets was used as representative of bacterial contamination in fresh chicken meat and pork (Rortana et al., 2021). In step 2, the rate of *Salmonella* entering chicken and pork salad was estimated at the household level, the temperature at the study site, duration of storage until cooking, and the laboratory experiment to measure the level of *Salmonella* in RTE chicken salad (Rortana et al., 2022) and boiled pork (Dang-Xuan et al., 2018). In step 3, the consumption rate was assessed including how often people consume chicken/pork salad and age groups (result from this study).

2.4. Data management and analysis

Data were managed and processed using MS Excel (Office 365). Descriptive statistical analysis was used to describe *Salmonella* prevalence using RStudio version 3.2.2 (R Core Team). The risk model was developed, and Monte Carlo simulation was performed using @Risk (Version 8.1, Palisade, Corporation, USA) for 10,000 iterations. The sensitivity analysis was conducted by selecting all the uncertainty parameters and running 1000 iterations at seven quantile values. Consumption data, prevalence and concentration of pathogen were described as mean and median. Final risk estimates were presented as mean and median with 90% confidence interval (CI).

2.5. Ethical considerations

Ethical approval of this study was done under the Safe Food, Fair Food Cambodia project and granted by the National Ethical Committee of Cambodia, coded 300NECHR, dated 26th December 2017. The participating researchers were informed and instructed on the safety procedures and provided their signed informed consent prior to starting the experiment. For the FGDs, participants invited to the discussion were asked for their written consent agreement prior to starting.

3. Results

3.1. Exposure assessment

The consumer survey on consumption of chicken and pork salad was conducted among 93 households in 12 FGDs. Detailed salad eating frequency (times/month) and amount of salad consumed (gram/meal) by age groups are presented in Table 3. In brief, the median frequency of consuming either chicken or pork salad was 1.6 times per month, ranging from 0–24, and the average amount consumed per meal was 130 grams per person (Table 3).

3.2. Risk characterization

The modeled annual incidence rate of salmonellosis was higher for chicken salad (11.1% probability of illness per person per year; 90% CI: 0–35.1) than for pork salad (4.0%, 90% CI: 0–21.3); considering consumption of both chicken and pork salad the annual incidence rate was 14.5% (90% CI: 0–33.5, Table 4; Figure 2). Adults had the highest modeled annual incidence rate (19.1%; 90% CI: 0–48.3): incidence by age categories and types of salad are shown in Table 4.

3.3. Sensitivity analysis

The sensitivity analysis found the most important influencer of the annual incidence rate of salmonellosis was the probability of cross-contamination in preparing salad in scenario 3 (wash chicken and pork first, use same utensils). This was followed by the prevalence of *Salmonella* on chicken at the market; probability of cross-contamination in scenario 1 (wash vegetables first, use same utensils for cutting salad and raw chicken and pork); prevalence *Salmonella* in chicken and pork

TABLE 1 Description of the four scenarios where cross-contamination of *Salmonella* may occur when preparing chicken and pork salad.

Description of scenarios	Procedure when preparing chicken and pork salad	Probability of cross contamination to RTE salad (%)		Concentration of *Salmonella* on RTE salad (CFU/g)	
		Chicken [a]	Pork [b]	Chicken [a]	Pork [b]
Scenario 1	- Wash and chop vegetables; then wash and cut the raw chicken or pork; and wash the cutting board, knife and hands (once with detergent). - Use the same washed cutting board, knife and hands to debone and slice cooked chicken and pork and mix the salad.	77.8	77.8	37.3	0.71
Scenario 2	- Wash and chop vegetables; then wash and cut the raw chicken or pork; and wash the cutting board, knife, and hands (once with detergent). - Use separate cutting board and knife to debone and slide cooked chicken and pork, and mix the salad.	11.1	0.0	0.36	0.0
Scenario 3	- Wash and cut raw chicken or pork; then wash and chop vegetables; wash the cutting board, knife and hands (once with detergent). - Use the same washed cutting board, knife to debone and slide cooked chicken and pork, and mix the salad.	22.2	22.2	0.36	0.12
Scenario 4	- Wash and cut raw chicken or pork; then wash and chop vegetables; wash the cutting board, knife and hands (once with detergent). - Use separate cutting board, knife to debone and slide cooked chicken and pork, and mix the salad.	0.0	66.7	0.0	2.49

This table is adapted from authors (Dang-Xuan et al., 2018[b] and Rortana et al., 2022[a]) which aimed to model the cross-contamination rate of bacteria entering ready-to-eat (RTE) food from raw animal-source food. There were only slight differences between the experiments for chicken and pork and therefore they are described as similar.

from the market; and probability of cross-contamination in scenario 4 (wash chicken and pork first, use different utensils, Figure 3; Table 5). The scenarios are described in detail in Table 1.

4. Discussion

This study developed a QMRA model from retail-to-table pathways predicting the likelihood of salmonellosis owing to consumption of chicken and pork salad in the Cambodian setting. The two most crucial factors for bacterial contamination of consumed food were the probability of cross-contamination during preparation in scenario 3 and the *Salmonella* prevalence in meat from markets. According to Rortana et al. (2022), most (86–96%) households practice preparing salad according to scenario 3, which had less contamination than scenario 1, but the fact that this practice is so common, gave it a larger influence in the model. The high influence of this common practice in the model also shows that there is a great scope of improvements. If the risk of cross-contamination at household level could be reduced, or people could change their habits completely to scenario 2 or 4, the risks could be reduced. All scenarios included rinsing the chicken, since it was the common practice, even if this step should be completely discouraged, as it increases the risks for salmonella contamination.

Most of the meat in Cambodia is sold in traditional markets where temperatures are suitable for bacterial growth (Sary et al., 2019; General Directorate of Animal Health Production of Cambodia, 2021; Rortana et al., 2022). Earlier studies found that meat and vegetables were frequently contaminated with *Salmonella* at this level (Rortana et al., 2021; Schwan et al., 2021). People in urban and peri-urban areas commonly purchase meat in the morning and cook it the same day (Brown et al., 2022; Rortana et al., 2022), while people in rural areas often keep meat longer before cooking (Duong et al., 2022). The focus of this QMRA was on meat purchased in the traditional value chain, as this is still the most common source of food in Cambodia, and where the prevalence was found the highest (Rortana et al., 2021). The model was built according to how people handle meat in their daily life. Another study in Cambodia used QMRA of *Salmonella* for risk assessment, specifically on the consumption of cricket powder to treat undernutrition in infants and children (Walia et al., 2018), while our study is the first to build

TABLE 2 Summary of the parameters, statistics, distribution, and data sources used in the QMRA model to estimate the risk of salmonellosis through chicken and pork salad consumption in Cambodia.

Parameters (symbol)	Distribution	Unit	Source
Traditional market			
Salmonella prevalence in chicken meat (P_{ck}) and pork (P_{po}) at market	P_{ck}: 87/204 (42.6%); described as Beta (87+1, 204-87+1) P_{po}: 92/204 (45.1%); described as Beta (92+1; 204-92+1)	-	Dang-Xuan et al., 2018; Rortana et al., 2021
Salmonella concentration in chicken meat and pork at the market (Cck and Cpo)	LogNormal (mean, 0.32) Chicken meat 16.7 CFU/g (0.36-120) Pork 17.3 CFU/g (0.36-120)	Log CFU/g	Dang-Xuan et al., 2018; Rortana et al., 2021
Status of *Salmonella* contamination in chicken meat (Sck) and cut pork (Spo)	Chicken meat: Binomial (1, P_{ck}) Pork: Binomial (1, P_{po})	-	Dang-Xuan et al., 2018; Rortana et al., 2021
Growth model at household			
The temperature when storing raw pork in the refrigerator at household (Tre)	Fixed at °C	°C	Present survey
The temperature when storing raw pork at ambience condition at household (Tro)	Normal (28, 4)	°C	Department of Meteorology of Cambodia, 2019
Duration of storage of raw pork at household before cooking (Hst)	Actual data (mean, min, max)	hour	Present survey
Salmonella grow rate in food matrices (h0)	Normal (2.14, 0.71)	Log CFU/g	Baranyi and Roberts, 1994
Cooking and consumption at household			
Probability of *Salmonella* cross-contamination after preparing chicken or pork salad in each cooking scenario (P_{sc})	Scenario 1: P_{sc1} =Beta(7+1, 9-7+1) Scenario 2: P_{sc2} =Beta(1+1, 9-1+1) Scenario 3: P_{sc3} =Beta(2+1, 9-2+1) Scenario 4: P_{sc4} =Beta(0+1, 9-0+1)	-	Dang-Xuan et al., 2018; Rortana et al., 2022
Status of *Salmonella* cross-contamination after boiling chicken/pork in cooking scenarios (C_{sc})	Scenario 1: C_{sc1} =Binomial(1, P_{sc1}) Scenario 2: C_{sc2} =Binomial(1, P_{sc2}) Scenario 3: C_{sc3} =Binomial(1, P_{sc3}) Scenario 4: C_{sc4} =Binomial(1, P_{sc4})	-	Dang-Xuan et al., 2018; Rortana et al., 2022
Probability of Cambodian consumer eating chicken/pork salad every meal (P_{eat}, $0< P_{eat} \leq 1$)	Non-parametric bootstrapping from household data (using DUniform)	-	Present survey and Dang-Xuan et al., 2018
Status of eating chicken/pork salad in the meal by Cambodian consumer (S_{eat})	Binomial (1, P_{eat})	-	Present survey and Dang-Xuan et al., 2018
Quantity of chicken/pork salad consumed per meal by Cambodian consumer (Qty)	Non-parametric bootstrapping from household data (using DUniform)	gram/meal	Present survey and Dang-Xuan et al., 2018
Illness (salmonellosis) probability from dose response model (I_{pro})	Beta Poisson (α,β) equation, α =0.00853 and β = 3.14	-	Teunis et al., 2010

CFU, Colony Forming Unit.

a QMRA on commonly consumed meat. In the future, as supermarkets get more common, it would be interesting to include the origin of the meat in the model, but this was not done here.

This study found that prevalence of *Salmonella* in the market was the major driver of risk of salmonellosis to salad consumers. As already described, salmonellosis is one of the leading foodborne diseases globally, as well as in Cambodia (Shiowshuh and Cheng-An, 2011; Yates, 2011; Nair and Johny, 2019). This study also found that the prevalence of *Salmonella* in food sold in markets was an important determinant of the incidence of *Salmonella* infection, adding insight to discussions on which points in the value chain food safety interventions should target. Recent studies have detected high prevalence of *Salmonella* in chicken meat and pork in markets in Cambodian (Rortana et al., 2021) and Vietnam (Dang-Xuan et al., 2019; Ngo et al., 2021). Moreover, other studies in Cambodia have found *Salmonella* in chicken meat (Nadimpalli et al., 2019) and vegetables that are in contact with meat during the selling period at the market (Schwan et al., 2021). In the current study, the prevalence of *Salmonella* in meat was relatively high compared to studies in nearby countries, including studies from Vietnam and Thailand (Dang-Xuan et al., 2019; Poomchuchit et al., 2021). Pathogenic *Salmonella enterica* have also been isolated

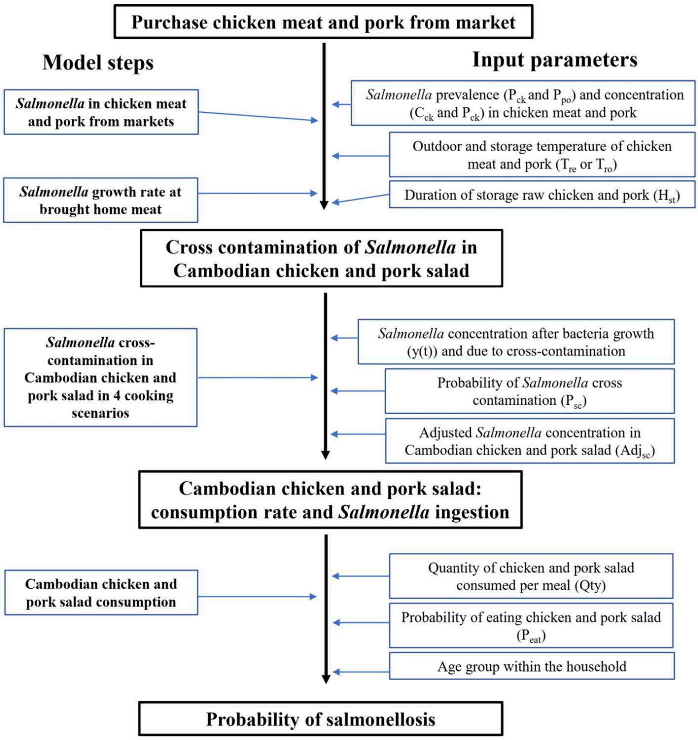

FIGURE 1
Model steps and input parameters in *Salmonella* QMRA from market to household related to chicken and pork salad preparation and consumption.

from multiple sources, including humans, animals, and food in Cambodia (van Cuyck et al., 2011; Schwan et al., 2021). Even *S. enterica* serovar Paratyphi infections have been found earlier in Phnom Penh, Cambodia (Vlieghe et al., 2013). The study found that the average incidence of salmonellosis in adults was higher than in children, youth, and the elderly, which is probably because adults more commonly consume chicken salad. Chicken salad and other similar salads are common foods in Asian countries, including Cambodia (Rortana et al., 2022) and Vietnam (Dang-Xuan et al., 2016). Even though most *Salmonella* does not cause severe disease in humans, regular exposure to these bacteria could be harmful over long time periods (Bollaerts et al., 2008; Perez-Rodriguez, 2020). Earlier studies also support that the cooking conditions and procedures such as moisture, contact time and pressure could result in higher transfer between the surface of contaminated objects (Cliver, 2006; Pérez-Rodríguez et al., 2008; Van Asselt et al., 2008). Chicken salads and similar salads (e.g. with beef, fish, shrimp, octopus) are very common at ceremonies such as traditional weddings in Cambodia. There is also an earlier report that a group of people got sick from a foodborne pathogen after eating salad in a wedding reception in Kampong Speu province (Vandy et al., 2012).

Two previous studies found that Cambodian people worry more about chemical food safety than microbial contamination (Duong et al., 2021; Brown et al., 2022). Most households also believe that chemicals (additives substances) used to make ASF products look good is the only cause of foodborne illness, leading them to care less of microbially contaminated in ASF (Brown et al., 2022). They tend to pay more attention to purchasing chemical free food than to proper storage, cooking, and good practice to reduce

TABLE 3 Frequency and the average amount of chicken and pork salad consumption by age groups in Cambodian households.

Information	Eating chicken salad	Eating pork salad	Eating either pork or chicken salad
Frequency of consumption by age group [times/month, *median (min-max)*]			
Children (under 5 years old)	0 (0–1.6)	0 (0–3.3)	0 (0–3.3)
Youth (6-15 years old)	0.3 (0–3.3)	0.8 (0–3.3)	1 (0–6.6)
Adult (16-60 years old)	0.3 (0–12)	0.8 (0–12)	1 (0–24)
Elder (over 61 years old)	0.5 (0–2.5)	0.6 (0.1–2.5)	1.5 (0.2–5)
Overall	0.8 (0–12)	0.9 (0–12)	1.6 (0–24)
Average consumption amount [g/person/meal, *(mean ± standard deviation)*]			
Children (under 5 years old)	46 ± 22	46 ± 20	46 ± 20
Youth (6–15 years old)	93 ± 62	93 ± 65	92 ± 59
Adult (16–60 years old)	124 ± 71	141 ± 79	134 ± 70
Elder (over 61 years old)	85 ± 62	81 ± 51	83 ± 54
Overall	141 ± 79	124 ± 71	130 ± 75

TABLE 4 The annual incidence rate of human salmonellosis due to chicken and pork salad consumption by age groups in Cambodia.

Age groups	Estimated annual salmonellosis incidence rate (%, Mean, 90% Confidence interval)		
	Consume chicken salad only	Consume pork salad only	Consume chicken and pork salad
Children (under 5 years old)	5.3 (0–31.8)	1.7 (0–12.3)	6.6 (0–25.3)
Youth (6–15 years old)	6.5 (0–35.7)	2.6 (0–19.0)	8.2 (0–29.4)
Adult (16–60 years old)	14.6 (0–51.9)	5.3 (0–32.9)	19.1 (0–48.3)
Elder (over 61 years old)	5.9 (0–35.9)	1.8 (0–12.9)	7.9 (0–28.8)
Overall	**11.1 (0–35.1)**	**4.0 (0–21.3)**	**14.5 (0–33.5)**

microbial contamination (Kristina and Sophal, 2018; Brown et al., 2022). Yet this study shows a high risk of FBD from a bacterial hazard. Changing perceived risks requires awareness raising data. This in turn, demands a food surveillance system, which is not yet in place in Cambodia (Thompson et al., 2021). Slaughterhouse hygiene improvements are still under development in the country, and the government has only started to monitor microbial contamination in slaughterhouses (Tum, 2008; Thompson et al., 2021) and markets (Rortana et al., 2021) to identify critical control points and prevent cross-contamination.

As consumption varies with age, different age categories were used in this study. However, the dose-response model used did not take differences in susceptibility between age groups into account (Teunis et al., 2010). Therefore, the separate dose-response model of *Salmonella* according to the categories of age and health condition was uncertain and could not be used as a formal analysis (Bollaerts et al., 2008; Marks and Coleman, 2017; Sanaa, 2021).

Food safety regulations vary between countries, but the usual goal is to combat foodborne diseases (Kunthear, 2022). In Cambodia the government recently adopted the National Plan on Food Safety. Six ministries are currently involved in governing national food safety, and coordinated by a multi-ministries team, the Technical Working Group for Food Security and Nutrition, which included representatives from each ministry. In June 2022, the law on food safety which addresses the entire food chain was adopted and brings Cambodia in line with international food safety standards (Food and Agriculture Organization of the United Nations, 2022; Kunthear, 2022). The law authorizes food inspection and provides a legal basis for action where food safety hazards are identified. This study provides scientific evidence that cross-contamination of *Salmonella* in the food chain (in this case from market to preparation of RTE salads) is a significant factor for human salmonellosis. This data is of relevance for local and national authorities and could be used to guide future policies, surveillance, and intervention to improve food safety along the food chain.

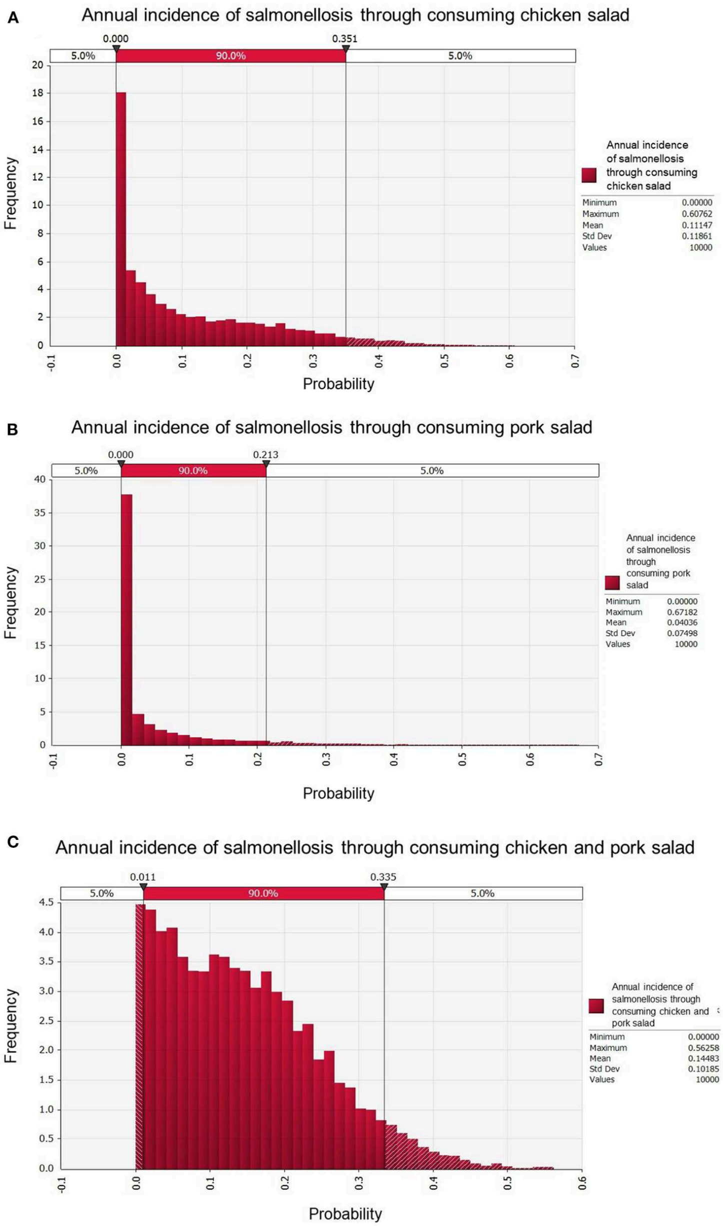

FIGURE 2
Annual incidence rates of salmonellosis in Cambodian households eating chicken salad **(A)**, pork salad **(B)**, and both chicken and pork salad **(C)**.

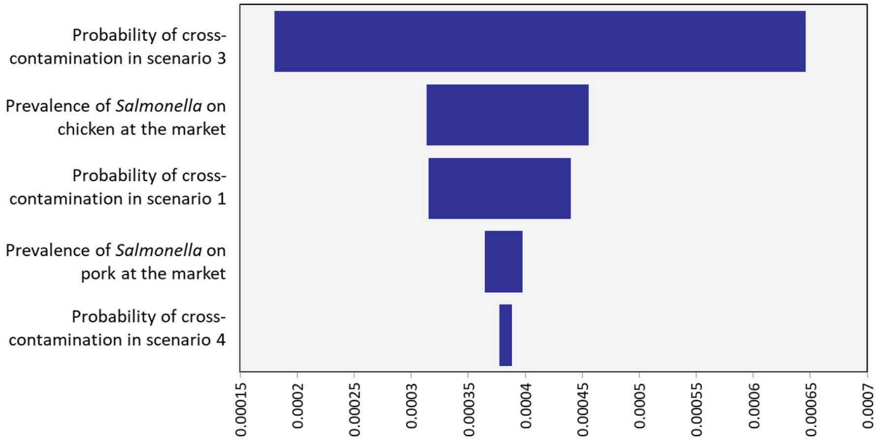

FIGURE 3
Sensitivity analysis of influence factors on annual salmonellosis incidence due to consuming both pork and chicken salad.

TABLE 5 Sensitivity analysis of influence factors on daily salmonellosis incidence due to consuming pork and chicken salad.

Rank	Influence factors	Values at 50th (1st–99th) percentiles	Mean (90% CI) daily incidence of salmonellosis per 10,000 people
1	Probability of cross-contamination in scenario 3	0.24 (0.07–0.5)	4.70 (0.35–12.57)
2	Prevalence of *Salmonella* on chicken at the market	0.43 (0.35–0.51)	4.76 (0.32 –12.3)
3	Probability of cross-contamination in scenario 1	0.76 (0.5–0.93)	4.77 (0.3–12.21)
4	Prevalence of *Salmonella* on pork at the market	0.45 (0.37–0.53)	4.75 (0.31–12.39)
5	Probability of cross-contamination in scenario 4	0.04 (0.00–0.22)	4.73 (0.32–12.45)

CI, Confidence interval.

5. Conclusions

The study presents new results on the risks of contracting salmonellosis after eating chicken and pork salad in Cambodia. It describes household practices that may facilitate *Salmonella* contamination of RTE food and estimates the probability of salmonellosis caused by consumption of this food. The QMRA suggests that changing meat storage and handling practices from market to household can reduce the likelihood of foodborne disease. The results are evidence for use as a basis for adapting policies in Cambodia. The new knowledge can guide implementation of appropriate and effective intervention strategies to prevent and control the undesirable consequences associated with microbial contamination in animal source food until RTE. Through enhancing food safety practices and responsibility among actors across the value chain, targeting markets, households and RTE food providers and restaurants, the findings can contribute to reduce the burden of FBDs in ASF in Cambodia and elsewhere.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved under the Safe Food, Fair Food Cambodia project and granted by the National Ethical Committee of Cambodia, coded 300NECHR, dated 26th December 2017. The participants provided their written informed consent to participate in this study.

Author contributions

Conception: DG, HN-V, ST, FU, JL, SD-X, SB, and CR. Data curation and writing—original draft: CR and SD-X. Formal analysis: CR, SD-X, and JL. Investigation: CR, SD-X, CT, and ST. Methodology: CR, CT, and SD-X. Supervision: SB, HN-V, ST, SD-X, DG, and JL. Writing—review and editing: SD-X, SB, HN-V, FU, ST, DG, KO, and JL. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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This thesis has estimated the risk of salmonellosis through quantitative microbial risk assessment from market to household. The annual risk estimate of salmonellosis from consuming chicken salad, pork salad and mixtures of chicken and pork salad were 11.2%, 4.0%, and 14.5%, respectively. The factors with most influence on incidence were cross-contamination while preparing the salad and the prevalence of *Salmonella* on chicken meat and pork at the market. The results suggest risk communication, exepecially improve hygiene of practices at traditional market and household.

Rortana Chea received his doctoral education at the Department of Biomedical Sciences and Veterinary Public Health, Master of Animal Health and Biomedical Sciences (in Thailand) and Bachelor of Science of Veterinary Medicine (in Cambodia).

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