Université de Montréal

Prevalence of *Salmonella* in retail whole chicken carcasses in Hanoi, Vietnam

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Ce mémoire intitulé

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Résumé

Au Vietnam, les informations sur la contamination de la viande de volaille par les salmonelles sont presque limitées. L'étude cherche à comparer la prévalence des salmonelles entre les marchés traditionnels et les supermarchés ainsi qu'entre les carcasses fraîches et congelées en plus de mesurer la température interne au moment de l'achat. Deux cent quarante-cinq carcasses de poulets entiers ont été achetées des marchés et des supermarchés dans sept arrondissements de la ville de Hanoi au Vietnam de juin à juillet 2011. L'échantillonnage a inclu 110 carcasses fraîches de marchés traditionnels (F/M), 109 carcasses fraîches des supermarchés (F/SM) et 26 carcasses congelées des supermarchés (FZ/SM). La température intérieure des carcasses a été évalué au moment de l'achat des carcasses. *Salmonella* a été isolé à partir de rinçage de carcasses et les isolats ont été sérotypés. La prévalence de carcasses positives pour *Salmonella* était de 66,5% (163/245) et variait entre les trois catégories : 84,55% (93/110) de F/M, 59,63% (65/109) de F/SM et 19,23% (5/26) de FZ/SM ($P<0.05$). Pour un total de 25 sérovars détectés, le sérovar principal fut Agona (24,78%) suivi de Albany (20,43%) et enfin Corvallis (10%). Deux des sérovars repérés se retrouvaient sur les mêmes carcasses pour 66 échantillons (26,9%). La température interne des carcasses des marchés traditionnels et des supermarchés était associé une différence significative ($P < 0.05$) avec une température moyenne de 27,3°C et 15,8°C respectivement. Cette étude dévoile une prévalence élevée de *Salmonella* spp. des carcasses de poulets à Hanoi et démontre une difficulté partagée par tous les types de marchés à maintenir une température adéquate des carcasses.

Mots-clés : *Salmonella*, carcasses de poulet, prévalence, sérovar, température interne des carcasses, Vietnam.

Abstract

In Vietnam, the data on the prevalence of *Salmonella* contamination in retail chicken meat is limited. We wanted to compare that prevalence at traditional and modern supermarkets, as well as in fresh versus frozen carcasses, and to verify the inner carcass temperatures at time of purchase. A collection of 245 whole chicken carcasses were purchased from traditional markets and supermarkets, in seven urban district areas of Hanoi in June and July, 2011. Sampling plan included 110 fresh chickens from traditional markets (F/M), 109 fresh chickens from supermarkets (F/SM) and 26 frozen chickens from supermarkets (FZ/SM). The inner carcass temperature was measured at the time of purchase. *Salmonella* was isolated from carcass rinses and isolates were serotyped. The overall prevalence of *Salmonella*-positive carcasses was 66.5% (163/245). The *Salmonella* prevalence in the three types of chickens varied significantly, 84.55% (93/110) from F/M, 59.63% (65/109) from F/SM and 19.23% (5/26) from FZ/SM ($P< 0.05$). A total of 25 serovars were recovered. The predominant serovars were Agona (24.78%), Albany (20.43%) and Corvallis (10%). Two different serovars were isolated and coexisted on the same carcass in 66 samples (26.9%). The inner carcass temperatures of fresh samples from traditional markets and supermarkets were significantly different ($P < 0.05$) with a mean inner carcass temperature of 27.3^{o}C and 15.8^{o}C respectively. This study revealed a high prevalence of *Salmonella* spp. from retail chickens in Hanoi and uncovered the difficulty encountered by all market types to store broiler chicken carcasses at a safe temperature.

Key words: *Salmonella*, chicken carcass, prevalence, serovar, inner carcass temperature, Vietnam.

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List of abbreviation

BAM: Bacteriological Analytical Manual

BGA: Brilliant Green Sulfa agar without sulfa pyridine

BGS: Brilliant Green Sulfa agar

BPW: Buffered Peptone Water

CDC: Centers for Disease Control and Prevention

DOC: day old chick

ECDC: European Centre for Disease Prevention and Control

EFSA: European Food Safety Authority

EU: European Union

FAO: Food and Agriculture Organization of the United Nation

FAPQDCP: Food Agricultural Production Qualification and Development Control Project

FDA: U.S. Food and Drug Administration

F/M: fresh chicken carcasses sold at traditional markets

F/SM: fresh chicken carcasses sold at supermarkets

FZ/SM: frozen chicken carcasses sold at supermarkets

GGP stocks: great grandparent stocks

GP stocks: grandparent stocks

ICT: inner carcass temperature at time of purchase

ISO: International Organization for Standardization

LB: Lactose Broth

LPS: Lipopolysaccharide

MARD: Ministry of Agriculture and Rural Development of Vietnam

MFHPB-20: HPB Method for Isolation and Identification of *Salmonella* from food and environmental samples

$MKTT_n$: Muller-Kauffmann Tetrathionate/novobiocine broth

MSRV: Modified semi-Solid Rappaport-Vassiliadis medium with novobiocin

RVS: Rappaport-Vasiliadis Soy broth

TT: Tetrathionate Broth

USAID: U.S. Agency for International Development

USDA: U.S. Department of Agriculture

VietGAHP: Vietnamese Good Animal Husbandry Practices

WHO: World Health Organization

XLD: Xylose Lysine Desoxycholate agar

To my mother, who brought to me the spirit support

To my father and my spouse, who gave me
their love and encouragement

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> And my friends, who are always beside me…

CHAPTER 1. INTRODUCTION

In recent years, a lot of foodborne human illnesses caused by *Salmonella* infection have been reported and salmonellosis has become one of the most prevalent foodborne diseases worldwide (Altekruse et al., 2006; CDC, 2009, 2010, 2011; Cogan & Humphrey, 2003; Kennedy et al., 2004; WHO, 2005). Although each country has their way to collect data regarding foodborne diseases, reported *Salmonella* infection is still predominant. In Canada, based on the data collected at hospitals nationwide, *Salmonella* ranked the second most common bacterial pathogen with about 5000 to over 6000 cases reported annually from 2004 to 2009 (Public Health Agency of Canada, 2009). Collecting data from several national surveillance systems, *Salmonella* is the most commonly reported cause of bacterial foodborne illness in the United States (CDC, 2010, 2011; Mead et al., 1999), with an estimated 1.4 million cases of *Salmonella* infections annually (Kennedy, et al., 2004; Voetsch et al., 2004). In Europe, *Salmonella* is one of the most important causes of human illnesses which has been considered as a priority in an extended control program for zoonoses since 2003. Data of *Salmonella* infectionhave been collected from foodborne disease database and from the report of Annual EU Summary Reports prepared by EFSA and ECDC, in which 131,468 human cases in 2008 and 108,614 in 2009were reported (EFSA, 2011; Pires et al., 2010). In Asia, China's surveillance program indicated that *Salmonella* is the number one bacteria in foodborne disease outbreaks (Lu, 2010). In Thailand, *Salmonella* accounted for 56.1% of acute diarrhea among children in one hospital from 1994 to 1996 (Moolasart et al., 1997). In Singapore, a total of 1480 laboratory-confirmed cases of non-typhoidal salmonellosis were reported in 2010, an increase of 1.3-fold from the 1144 cases reported in 2009 (Ministry of Health of Singapore, 2011).

Most human *Salmonella* outbreaks are associated with the consumption of contaminated products from animal origin (Wray and Wray, 2000a; Domingues et al., 2011; Pires et al., 2010). This bacterium is primarily associated with chicken – chicken meat and eggs have been recognized as vehicles of human infection (Altekruse et al., 2006; Baumler et al., 2000; Capita et al., 2003; FAO/WHO, 2009; Kim et al., 2007; Madden et al., 2011; Pires, et al., 2010; Uyttendaele et al., 1998; Yang et al., 2011).Proportions of 31% and 33% of *Salmonella* isolates recovered from retail skin-off and skin-on breast samples were also documented in the Region of Waterloo, Ontario, Canada (Cook et al., 2012).Similar results were seen in Australia, as*Salmonella* was found in 47.7% and 35.5% of retail chicken samples in New South Wales and South Australia, respectively (2005) (Pointon et al., 2008). . In the European Union, using outbreak data in 2005 and 2006 for source attribution of human salmonellosis cases, meat and poultry-meat were estimated to be the second most important food sources (15%) (Pires, et al., 2010). Even in developed countries, prevention and control of *Salmonella* contamination is challenging – a high prevalence of *Salmonella* contamination in retail raw chicken meat has been reported for over ten years (Capita et al., 2003; Dominguez et al., 2002; Jerngklinchani et al., 1994; Mikoajczyk et al., 2002; Uyttendaele et al., 1998). High prevalence of *Salmonella* contamination in raw poultry were also reported in several Asian countries, with an overall *Salmonella* prevalence of 52.2% in China (2011) (Yang, et al., 2011) and 61% in Thailand (Vindigni et al., 2007).

In Vietnam, based on results of the Meat Quality Supervision Program carried out by the Department of Animal Health – Ministry of Agriculture and Rural Development, the prevalence of *Salmonella* contamination in fresh meat samples (chicken and pork) sold at

traditional markets was 31% (n = 254) in 2011 but this report did not mention serogroups and serovars. Besides this data from the national program, there have been few studies with the same objective that obtained different results of prevalence and distribution of serovars. The prevalence of *Salmonella* in raw chicken in these studies varies from 8.3% (n = 60) (Ha & Pham, 2006), to 48.9% (n = 262) (Luu et al., 2006) and 53.3% (n = 30) (Van et al., 2007). No study has used whole-carcass fresh and frozen chicken as samples.

In Vietnam, consumers believe that free grazing broilers are more fresh and of higher quality than the conventional industrial ones. In addition to their habit of purchasing chickens at traditional markets rather than buying the "industrial" broiler meat sold at supermarkets, Vietnamese consumers have a perception that there are no harmful effects of bacteria growth if they consume well-cooked meat.

The objectives of this study are: (1) to determine the prevalence of *Salmonella* contamination in retail raw whole chicken carcasses and to compare the incidence of *Salmonella* contamination of three groups of chicken carcasses (fresh meat from traditional markets and both fresh and frozen meat from supermarkets); (2) to examine the diversity and distribution of *Salmonella* serovars; and (3) to measure and compare the inner carcass temperature at various purchasing sites at the time of purchase. Data from this study will provide more detailed information to the competent authorities and help them establish policies and strategies for controlling *Salmonella* contamination in retail chicken in Hanoi and also on a nationwide scale.

CHAPTER 2. LITERATURE REVIEW

2.1. The bacterium *Salmonella*

2.1.1. Taxonomy and Nomenclature

The genus name *Salmonella* was suggested by Lignières in 1900 from the first isolation in pigs by Salmon and Smith (1886), and was considered to be the cause of swine fever (hog cholera).

In the 1920s and 1930s, analysis of O (the somatic or outer membrane antigens), H (the flagella antigens), and Vi (the capsular antigens) led to the creation of an antigenic formula, which is unique to each *Salmonella* serotype (Old, 1992). This resulted in the description of many serovars and each serovar was considered as a species (Kauffmann, 1961). By the 1960s, the name *Salmonella* was widely accepted as belonging to the family *Enterobacteriaceae* and was listed in the *Approved Lists of Bacterial Names* published in 1980.

Since the 1970s, the application of newer methods to the taxonomy of *Salmonella*, such as DNA-DNA relatedness studies, has indicated that all *Salmonella* serovars are probably a single bacterial species. By international agreement, the nomenclature of genus *Salmonella* is based on recommendations from the WHO Collaborating Centre (Grimont & Weill, 2007a).It contains two species, *S. enterica* and *S. bongori*, each of which contains multiple serovars (Brenner, et al., 2000). *S. enterica* is further divided into six subspecies: *S. enterica* subsp. *enterica, S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae* and *S. enterica* subsp. *indica*. More than 2500 serovars have been described(Grimont & Weill, 2007a).

Serotype names designated by antigenic formula include the following: (1) subspecies designation (subspecies II through VI); (2) O (somatic) antigens followed by a colon; (3) H (flagellar) antigens (phase 1) followed by a colon; and (4) H antigens (phase 2, if present). For formula of serotypes in *S. bongori,* V is still used for uniformity to avoid confusion with serovar names of *S. enterica* subsp. *enteric*. For *Salmonella* subspecies I, a name was used instead of "I"(Brenner, et al., 2000).

2.1.2. Characteristics and classification of serogroups and serovars

2.1.2.1. Characteristics

Salmonella are facultative anaerobic, gram-negative, straight, non-spore forming rods (0.7-1.5 x x2.-5.0 μm), which have motility due to their peritrichous flagella (except for *S.* Gallinarum and *S.* Pullorum) and share some same biochemical characteristics.

The organism is mesophilic with an optimum growth temperature in the range of 32ºC to 37ºC but is capable of growth within a wide temperature range of 6ºC to 46ºC. It can survive at a pH between 4.1 and 9.0 and is able to grow with water activities above 0.94 (Brands, 2006). Although the cooling time and values for temperature and time can change depending on the serotype and the food matrix, *Salmonella* is heat-labile and can be inactivated at ordinary cooking temperature (> 70ºC). Also, *Salmonella* are not destroyed by freezing (Dominguez & Schaffner, 2009; Dykes & Moorhead, 2001; Niemira et al., 2003; Sorrells et al., 1970).

TABLE1. Biochemical characteristics of *Salmonella*, adapted from Brenner (1984) and Le Minor (1984)

Characteristic	**Usual reaction**
Catalase	+
Oxidase	-
Acid produced from lactose	-
Gas produced from glucose*	+
Indole	-
Urease produced	-
Hydrogen sulphide produced from triple-sugar iron agar	+
Citrate utilised as sole carbon source*	+
Methyl red	+
Voges-Proskauer	-
Lysine decarboxylase	+
Ornithine decarboxylase	+

+ positive reaction, - negative reaction
* *An important exception is S.* Typhi *which is negative in these tests.*

2.1.2.2. Classification of serogroups and serovars

Salmonella serotyping is a subtyping method that has proven valuable in differentiating isolates of the two species of *Salmonella*, particularly for public health purposes such as surveillance and sometimes in outbreak investigation. The typing system for *Salmonella* species based on antigenic formulae was created in the 1930s by White, Kauffmann and Le Minor. This classification system was designated as the White-Kauffmann-Le Minor scheme (Popoff et al., 1998). The antigenic structure was revealed mostly by cross absorption of antisera, which subdivided antigens into different factors(Wray & Wray, 2000a). As mentioned previously, identification of various serovars of *Salmonella* is the result of determining the presence and characterization of three typesof antigens: somatic (O), flagellar (H) and (mostly for serovar Typhi) surface (Vi) antigens.

The O-antigen is one part of the lipopolysaccharide (LPS) which is a key component of the outer membrane of Gram-negative bacteria. The O-antigen is made of a number of oligosaccharide repeats (O-units) which usually contain between two and eight sugar residues. Due to the extensive variation in types of sugar present, its position, and the combination of O-units, O-antigen in particular (or LPS in general) is one of the most variable cell surface constituents, leading to major antigenic variability. In *Salmonella* species, a number of studies have established an important role for O-antigen side chains in bacterial virulence (Carroll et al., 2004; Duerr et al., 2009; Valvano, 2003). O-antigens are identified by agglutination with specific agglutinating antibodies in serum, usually prepared in rabbits, to reveal the serogroup. For example, *S*. Typhimurium is a group B *Salmonella* due to the presence of the O4 somatic antigen. Historically, O-groups were designated by letters but due to the limited number of letters compared to the number of serogroups, O-groups are now designated by numbers, continuing with number 51 to 67, using the characteristic O factor with letters in brackets.

H-antigens are carried by flagella. H-antigens play an important role in taxonomy of *Salmonella* and are thought to have little influence on bacterial virulence (Lockman & Curtiss, 1990). H-antigens are typically diphasic in *Salmonella*. But some serovars have only one phase of flagellar antigen and are described as monophasic. Two serovars, *Salmonella* Pullorum and *Salmonella* Gallinarum, are exceptions in that they have no flagella and are non-motile strains.

The combination of O-antigens and H-antigens (and the uncommon Vi-antigens) is described as antigenic formulae of *Salmonella* serovars. Serotyping of *Salmonella* strains is carried out

by identification of O, H and Vi antigens using a list of 2579 *Salmonella* serovars (Grimont & Weill, 2007b), maintained and updated by the World Health Organization Collaborating Centre for Reference and Research on *Salmonella* (White-Kauffmann-Le Minor scheme).

2.1.3. *Salmonella* detection

A great number of methods for the detection of *Salmonella* have been developed over the last few decades and a significant improvement in sample preparation techniques has been made for isolation and detection of *Salmonella* in food and food ingredients. (Day et al., 2009; FDA, 2011; Gilbert et al., 2003; Gonzalez-Escalona et al., 2009; Gracias & McKillip, 2004; Jiang et al., 2009; Kim et al., 2006; Kruy et al., 2011; Kuhn et al., 2002; Ribot et al., 2006; Shaw et al., 1998). Generally, these methods are based on physiological and biochemical markers of the organism (Williams, 1981). Conventional culture methods are based on nutrient acquisition, biochemical characteristics, and metabolic products unique to *Salmonella* spp. (Ricke et al., 1998). More rapid immunological and molecular screening methods of detection have been devised to detect cell surface markers and nucleic acids, respectively (Odumeru & León-Velarde, 2012).

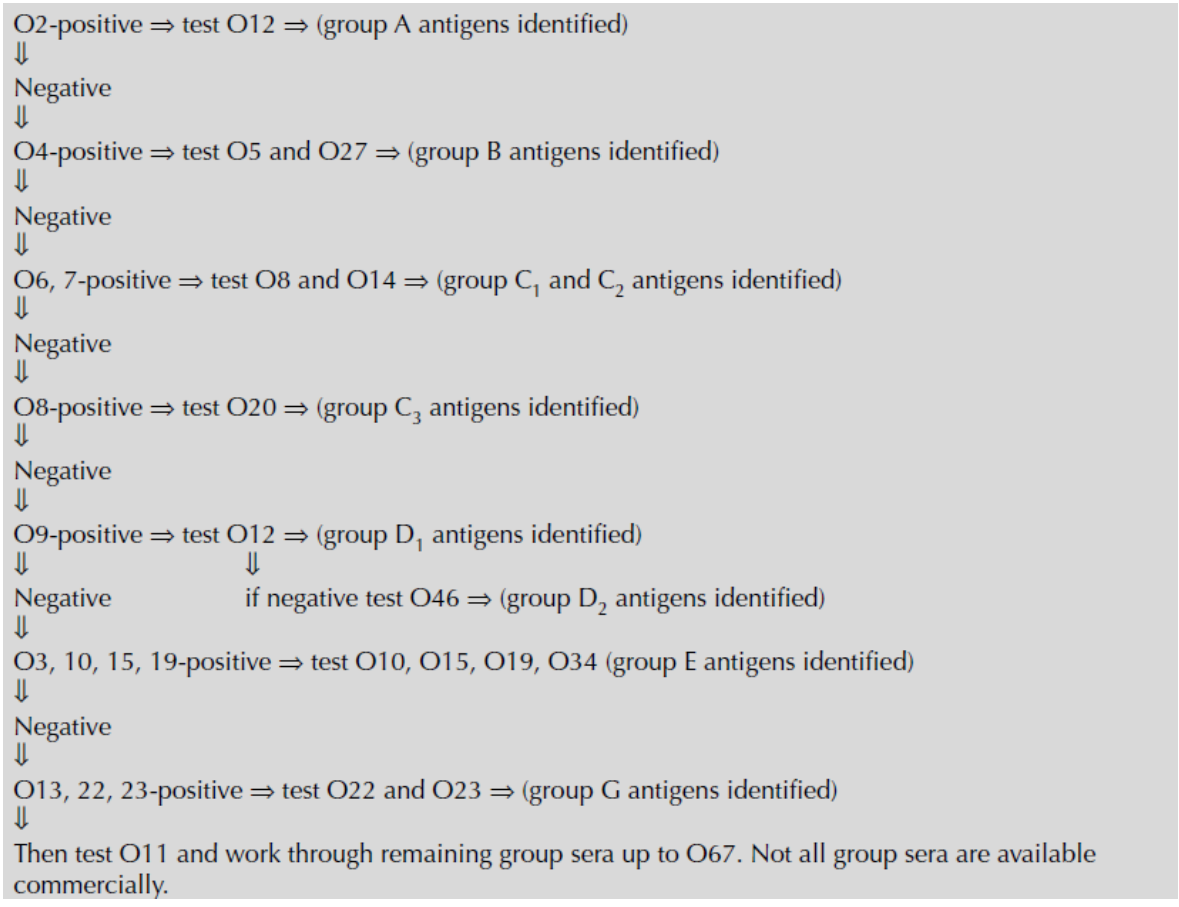
Culture based methods remain the most widely used by many labs, especially by regulatory agencies, because they are harmonized methods, looked at as the "gold standards" in food diagnostics and thus overall accepted. The standard culture methods typically require 5 to 7 days to obtain presumptive positive or negative results along with biochemical and serological confirmations, in four stages to recover injured

Salmonella cells from a food matrix (ISO 6579:2002; MFHPB-20 (Government of Canada, 2009); BAM – Chapter 5: *Salmonella*(FDA, 2011)). First, there is pre-enrichment in a non-selective liquid medium, with the aim to proliferate and regenerate damaged cells. The culture is performed in buffered peptone water (BPW) and incubated at 37ºC for 18 ± 2 hours. The use of lactose broth (LB) in the pre-enrichment stage was advocated but because of fermentation of lactose after incubation and resulting acidity, several studies have found that BPW was better than LB for isolating *Salmonella*(Fricker, 1987). After the non-selective pre-enrichment stage, the incubated culture is transferred to selective secondary enrichment broths to selectively inhibit other bacteria while allowing *Salmonella* to multiply to levels that may be detected after plating, such as Rappaport-Vasiliadis Soy broth (RVS), Tetrathionate Broth (TT), Muller-Kauffmann Tetrathionate/novobiocine broth (MKTTn) (ISO 6579:2002; MFHPB-20 (Government of Canada, 2009); BAM – Chapter 5: *Salmonella*(FDA, 2011)) and incubated at elevated temperatures as a further selective part of enrichment (42ºC for 24h ± 3h). Besides enrichment broths, another method involves a modified semi-solid Rappaport-Vassiliadis (MSRV) medium with novobiocin in a plate format, first developed by Goossens et al. (1984) and De Smedt and Bolderdijk (1987) (Wray & Wray, 2000b). It is used to rapidly detect motile *Salmonella* in feces and food products. Based on RVS selectivity, MSRV plate is incubated at 41.5ºC and the motility of *Salmonella* (except for non-motile *Salmonella*, such as *Salmonella* Pullorum and *Salmonella* Gallinarum) further selects and differentiates *Salmonella* from other microorganisms. The third stage is to plate on selective media with the aim of obtaining

typical *Salmonella* colonies. There are several media suggested by published standard methods utilizing the combinations of at least two media, such as Brilliant Green Sulfa (BGS) Agar (MFHPB-20 (Government of Canada, 2009) or without the addition of sulfapyridine (BGA), Bismuth Sulfite Agar (MFHPB-20 (Government of Canada, 2009); BAM – Chapter 5: *Salmonella* (FDA, 2011)). Conventional agar media (Hektoen enteric Agar, Xylose Lysine Desoxycholate (XLD) agar) (ISO 6579:2002; MFHPB-20 (Government of Canada, 2009); BAM – Chapter 5: *Salmonella*(FDA, 2011)) and some new chromogenic media (such as Rambach Propylene Glycol (Rambach Agar), CHROMagar) could be used in conjunction with above media as second medium (MFHPB-20 (Government of Canada, 2009)). Typical *Salmonella* colonies are selected based on their morphology, typical biochemical reactions on conventional selective agars or specific colors of colonies based on the incorporation of chromogenic substrates on chromogenic agars, and are then purified by inoculation on nutrient agar in order to obtain isolated colonies prior to confirmatory testing. The last stage is biochemical and serological identification of suspected colonies, including two steps, biochemical screening and serological confirmation. For biochemical screening, well-isolated colonies are screened using determinant biochemical reactions. Key biochemical tests include the fermentation of glucose, negative lactose reaction, hydrogen sulfide production, negative urease reaction, lysine decarboxylase, a negative indole test and fermentation of dulcitol.

Serological confirmation tests are carried out for strains of bacteria which have typical *Salmonella* biochemical profiles in order to detect the presence of somatic O, flagellar H and capsular Vi antigens. Testing with somatic (O) grouping antisera is carried out on isolates to confirm the *Salmonella* species and define the (O) groups when the isolates agglutinate with particular O antisera (Figure 1). *Salmonella* strains are sent to reference laboratories for further serotyping and lysotyping. Lysotyping was carried out to evaluate the susceptibility of strains to selected bacteriophages. Although, there are several phage-typing schemes for typing some *Salmonella* serovars (Typhi, Typhimurium, Enteritidis,…) and *Salmonella* phage-typing does not need expensive equipment, it is not popular to laboratories because it requires well-trained personnel (Wray & Wray, 2000c).

FIGURE 1. Identification of somatic O antigens (from: (Wray & Wray, 2000c))



2.1.4. *Salmonella* infection in human

In terms of pathogenesis, the *Salmonella* genus can be divided into two major groups. The first group consists of a few serovars; characteristically they produce severe systemic disease and are rarely involved in human food poisoning. Serovars in this group initially invade the reticuloendothelial system and are "host species" specific. They include *S.* Typhi, *S.* Paratyphi A in humans, *S*. Typhi, *S*. Paratyphi in mice, *S*. Gallinarum and *S*. Pullorum in poultry, *S*. Dublin in cattle, *S*. Choleraesuis in pigs, *S*. Abortusovis in sheep and a few other serovars. The second group is non-typhoid and typically results in food poisoning; it can also lead to systemic disease under special circumstances. This group comprises more than 2500 serovars and is not restricted to any particular host species (Coburn et al., 2007).

Infection with these bacteria normally occurs via the oral route and all serovars can cause disease in humans (WHO, 2013). All *Salmonella* infections begin with the ingestion of bacteria in contaminated food or water. *Salmonella* then colonize relevant parts of the digestive tract or invade the host. Most are able to colonize the digestive tract or reproductive tract without causing disease and can be excreted in feces. Fecal material is therefore the ultimate source of these bacteria (Doyle et al., 2009). *Salmonella* infections in humans vary from mild to severe and are occasionally fatal. The principal clinical syndromes associated with *Salmonella* infection are enteric (typhoid) fever and gastroenteritis (Pegues & Miller, 2000). Enteric fever is a systemic illness that results from infection with exclusively human pathogens, *Salmonella* Typhi and *Salmonella* Paratyphi A, whose clinical manifestations are fever, abdominal pain, transient diarrhea or

constipation. Other strains usually cause a self-limited enteritis in humans (Ohl & Miller, 2001). Two most important serovars of human salmonellosis caused by animal-origin *Salmonella* are Enteritidis and Typhimurium (WHO, 2013).

2.2. Overview of the broiler production system in Vietnam and supervising governmental agencies

Vietnam is a Southeast Asian country, located on the eastern Indochina Peninsula between the latitudes 8^{o} and 24^{o}N and the longitudes 102^{o} and 110^{o}E. Although it covers a small area of approximately 331,210 km^2 divided into three main parts – northern, central and southern – with different geographical conditions coinciding with differences in latitude. These regional differences are the reason why the Vietnamese climate tends to vary considerably from place to place.

In the economic field, Vietnam is an agricultural country with more than 70% of the population living in rural areas and engaged in agricultural activities. Because of the developing agricultural system, cultivation and animal husbandry activities in Vietnam depend on natural conditions and their development is not synchronous nationwide.

Hanoi, the capital of Vietnam, is 3344 km^{2}in the northern region of the country and has a population of nine million people. Food consumption in Hanoi is very high, about 500 tons per day of which 70% is self-provided and the rest is imported from other provinces (Hanoi Sub-Department of Agriculture and Rural Development, 2012).

2.2.1. Poultry production, processing and marketing

2.2.1.1. Poultry farming systems

In Vietnam, poultry production systems have been in existence for a long time and are well developed in all regions of the country. In 2007, the poultry population was reported to be about 226 millions, and chickens were estimated to make up more than 70% (about 158 million heads) of the total poultry population. Chickens are raised in four main regions that account for about 83.5% of the total chicken population: the Red River Delta (in the North) with 28%, followed by the Northeast region with 22%, the Mekong River Delta (in the South), and the Southeast region with 17.5%,and the Northern Center with 16%. Poultry husbandry provided 358,761 tons of meat which accounted for 11.3% of the total meat produced in 2007 (Department of Livestock, 2007). Poultry meat is the second most important meat of the Vietnamese people, after pork.

In 2005, more than 90% of households (7.9 million households) kept poultry (Department of Livestock, 2006). There are three main poultry production systems in Vietnam: (1) The non-intensive system, which requires limited investment and care, is the most widespread in Vietnam. Ninety-two percent of households raise poultry, contributing to more than 65% of the total products (Duc & Long, 2008). These chickens are kept in small numbers (less than 200heads/year) with chickens scavenging freely for long periods. Products from this system fulfill family needs and some are sold to the market. (2) A semi-intensive system is emerging in Vietnam. It requires more investment than the non-intensive systems, with more chickens raised in larger houses (500–5000 heads/year), better equipment, and better

breed and feed quality. In this system, colored breeds of chicken provided by national or private breeding centers are raised with average performance and better veterinary services than in the non-intensive system. In Vietnam, about 10 – 12% of the households practice this system which produces 10 – 15% of the total products. These products are sold directly to consumers or traders. (3) And finally, there is the intensive system. First established in Vietnam in 1973, this practice has increased in importance since 1995. These integrated industrial farms need large investments with flock sizes ranging from 2000 to 30,000 chicken heads, high quality breeds, improved techniques and good veterinary services. High quality breeds are selected and provided by national breeding centers or foreign companies. These farms produce 20 – 25% of national products. (Department of Livestock, 2006, 2007; Duc & Long, 2008)

There are 11 national breeding farms with 3000 pure breeds and 18,000 grandparent chickens. They provide day-old chicks to private farms or farmers. There are four foreign companies keeping grandparent flocks and producing parent chickens. Chicken breeds in Vietnam are local, imported or crossbred. In contrast, with non-intensive systems, local breeds are kept for laying and eggs are hatched for the next generation (Desvaux, et al., 2008; Duc& Long, 2008).

Vietnamese Good Animal Husbandry Practices (VietGAHP) for poultry was issued along with Decision number 1504/QĐ-BNN-KHCN in May 2008 by the Minister of Agriculture and Rural Development in order to improve conditions in poultry husbandry activities in Vietnam and gradually increase productivity and product quality. This standard is encouraged to be applied nationwide. Approximately 3% of total animal husbandry farms

in Vietnam apply VietGAHP (Ministry of Health, 2011). Under the VietGAHP for poultry, the owners of poultry farms have to set up procedures for good practices during husbandry activities in order to ensure the general hygiene conditions but this guideline does not focus on specific issues, e.g. *Salmonella* on poultry.

2.2.1.2. Poultry processing

Due to the consumption habits and acceptance of consumers, manual poultry slaughtering is still common, raising more difficulties for hygiene control and inspection by competent authorities. There are three types of poultry slaughtering operations under control of governmental agencies but lacking regular inspection. The first type is industrial slaughterhouses with modern infrastructure. They comply to regulations on veterinary hygiene and are certified by provincial inspectors in the animal health sector for the Certificate of meeting requirements on veterinary hygiene of which validation is two years. Daily productivity of these slaughterhouses ranges from 3000 to 64,000 birds per site. The second type is semi-manual abattoirs which partly meet hygiene regulations, with permanent locations and some semi-manual equipment. Daily productivity of these abattoirs is over 2000 birds per abattoir. The last type is small manual slaughtering facilities which are scattered throughout the country at traditional markets, small farms and even at homes. Generally, these small slaughter facilities do not meet requirements of veterinary hygiene for slaughtering, they operate manually on the ground and lack proper practices to ensure food hygiene in slaughtering, transportation and storage (USAID Avian and Pandemic Influenza Initiative, 2012). Governmental agencies face difficulties in controlling

and inspecting these small slaughtering facilities (Hanoi Sub-Department of Agriculture and Rural Development, 2012).

In Hanoi, there are four industrial slaughterhouses, two semi-manual abattoirs and over 3700 small manual slaughtering facilities. The four industrial poultry slaughterhouses were built to comply with hygiene regulations but only one is in use and another is in trial operation. They have a closed slaughtering facility with continuous stages (killing, scalding/defeathering by machine, manual evisceration, washing, and chilling), designed to ensure chicken carcasses do not come in contact with the ground. Due to limitation of input, these slaughterhouses could not be brought to full or moderate operation. Two semi-manual abattoirs, although not in full compliance with regulations on veterinary hygiene and environmental hygiene conditions, are allowed to operate temporarily under regular inspection of veterinary authorities in order to satisfy consumption needs in Hanoi. In contrast, the slaughtering process in manual slaughtering facilities lacks hygiene since all stages are conducted on the ground, carcasses are washed in the same basin without water changes, and there is no chilling. Although carrying and slaughtering live birds at markets is prohibited in Hanoi, at some shops live chickens are killed on site, under limited hygiene conditions, due to buyer requests (Hanoi Sub-Department of Agriculture and Rural Development, 2012).

2.2.1.3. Poultry marketing

The Meat Quality Supervision Program, conducted annually in Vietnam, reported that 71/431 (16.5%) chicken meat and pork samples from retail markets were contaminated by *Salmonella* in 2010 (Ministry of Health, 2011).

In Vietnam, there are two types of markets, traditional markets and supermarkets. Traditional markets are permanent places where there are gatherings of shops and stalls which are allowed by the government to operate food trade. Infrastructural conditions of traditional markets vary, from permanent, semi-permanent to temporary (People's Committee of Hanoi, 2004). At the opposite, supermarkets "constitute a type of modern stores; being general or specialized; having abundant and diversified kinds of goods with assured quality; meeting criteria on business space, technical facilities and management as well as business organization capabilities; adopting civilized and convenient service modes so as to satisfy the customers' "shopping demand" (Ministry of Trade, 2004). Due to origins and storage conditions of chickens, there are three ways to market chicken carcasses in Hanoi; (1) fresh chicken carcasses sold at traditional markets (F/M chicken), (2) fresh ones sold at supermarkets (F/SM chicken) and (3) frozen ones sold at supermarkets (FZ/SM chicken). It is impossible to track the origins of F/M chickens at the markets because there are no records or documents indicating farm or slaughterhouse names, except for a blurry violet stamp on the skin of each carcass to indicate veterinary certification of the slaughterhouses but not all carcasses have this stamp. This stamp is supposed to be given by governmental officers at industrial slaughterhouses and semi-manual abattoirs if the birds are slaughtered in a proper manner. But the veterinary hygiene conditions and slaughtering procedures at the semi-

manual abattoirs generally do not meet the requirements of the legal regulations. In addition, the inability to control the operation at small manual slaughtering facilities leads to difficulties in controlling the meat quality purported by this stamp (Hanoi Sub-Department of Agriculture and Rural Development, 2012).

F/SM chickens are sold in units with labels, are prepackaged individually, pre-weighed and priced, labeled with the producer's name, date packaged and expiry date, and kept chilled on refrigerated shelves and no more information about the origin of the carcasses, e.g. husbandry farm. Raw meat stored at 0ºC – 4ºC must be sold within 72 hours after slaughtering (MARD, 2011a). Concerning where F/SM chickens are packaged, products can be divided into two types: (1) packaged at supermarkets where slaughtered birds are purchased from slaughterhouses and packaged on the premises with styrofoam trays, covered closely by plastic wrap with labels printing the name of supermarket, date packaged and expiry date; (2) prepackaged individually at slaughterhouses in closed plastic bags with labels.

FZ/SM chickens are prepackaged at slaughterhouses and are treated in the same manner as F/SM chickens and stored in freezers at -20ºC for a one year shelf-life after packaging. Supermarkets in Hanoi are graded based on business area and quantity of items; rankings are grade 1, 2 or 3 when they meet the requirements of having more than 5000, 2000 or 500 square meters in area and have more than 20,000, 10,000 or 4000 items, respectively, among other general requirements of infrastructure (Ministry of Trade, 2004). They have all invested in equipment and food safety controls so that food sold at supermarkets generally meets requirements of food safety (Ministry of Health, 2011).

2.2.2. Management of hygiene quality of chicken carcasses

2.2.2.1. Legal and technical documents

In Vietnam, Laws are the highest-level documents in the system of legal documents and encompass general regulations covering all aspects of the Law. Laws rule lower-level documents, including Decrees, Circulars and Decisions.

The Food Safety Law has come into force in Vietnam since July 2011. It regulates the assignment of responsibility in food management systems and must rely on mandatory technical regulations, which are standards from government competent agencies to be promulgated by manufacturers. Raw meat products in particular must meet requirements of food safety without any exceptions, including:

- Meet requirements in correlative technical regulations and comply with regulations on the limit of pathogens, residue of veterinary drugs, heavy metals, contaminated agents and other hazards which can be harmful to human health.

- Be certified in ensuring veterinary hygiene by competent veterinary agencies.

Based on the Food Safety Law, three Ministries involved in food safety management, Ministry of Health, Ministry of Agriculture and Rural Development and Ministry of Industry and Trade, will issue subsidiary circulars to implement the regulations of the Law. Decree number 38/2012/NĐ-CP in April 2012 followed by the issuance of the Food Safety Law, regulates responsibilities of the Ministry of Agriculture and Rural Development (MARD) on management of meat safety, from rearing, slaughtering and processing to

marketing. In order to comply with the above Law and Decree, MARD has issued revised documents. In the veterinary section, MARD has used two sets of documents (legal and technical), including:

- Legal documents: Veterinary Ordinance 2004, Decrees on guidance in implementation of Veterinary Ordinance 2004 and other correlative Circulars and Decisions.

- Technical documents: Technical Regulation on the Veterinary Sections; Technical Regulation on Animal Feeds; Technical Regulation on Veterinary Hygiene Conditions; Regulation on Veterinary Hygiene Conditions at Poultry Slaughterhouses; Circular about the "List of food safety specifications and limits for imported and domestic meat products under the control of Ministry of Agriculture and Rural Development."

Because there are a lot of new regulations in the Food Safety Law regarding to assignment of food safety management which were issued, three Ministries has been step-by-step developing the new subsidiary documents. Recently, some new Circulars, e.g. technical regulations on maximum residue limit of veterinary drugs in meat, are drafted and not available.

In Vietnam, besides some poultry industrial slaughterhouses with modern machines and good hygiene practices, there are many semi-manual abattoirs and manual slaughtering facilities where veterinary hygiene conditions are limited and there are no measures to manage the slaughtering process. A study within the framework of a cooperation project between Vietnam and Thailand has reported the results of a review on hygiene conditions

of small scale poultry slaughterhouses in Vietnam, which are: (1) poultry are slaughtered on the ground with the capacity of 20 to 30 birds per day; (2) 85% of premises are cleaned daily, 80% of premises use underground water, and 75% of premises are inspected by governmental officers 1 to 2 times per year; (3) 100% of premises at wet (traditional) markets are categorized at the lowest level (Poor) of hygienic management (Good/Fair/Poor) (n=16), 90% of family premises are also ranked Poor (n=18) and the rest Fair (n=2); (4) *Salmonella* was isolated from holding pens, live poultry, equipment used in processing, the ground of slaughtering areas, carcasses, soil and waste water. The highest proportion of *Salmonella* contamination was found from the ground, with 70% (n=14) and 56% (n=9) at home and wet market slaughtering facilities, respectively. The proportion of *Salmonella*-positive results in processed meat was 45% (n=90) and 35% (n=56) at home and wet market slaughtering facilities, respectively. *Salmonella* was not detected in water supplies(Rojanasthien & Nguyen, 2011). There have not been any recent studies or assessments on the effects of poultry processing in *Salmonella* contamination of broiler carcasses in Vietnam.

Overall, in Vietnam there are numerous legal and technical documents regarding management of poultry husbandry, slaughtering and marketing that cover food safety issues from the farm to the fork but the level of implementation has not been determined.

2.2.2.2. Management network

Organizational system:

- Governmental level: Department of Livestock, Department of Animal Health, National Agro-Forestry Fisheries Quality Assurance Department (NAFIQAD), all three of which belong to MARD.

- Provincial level: Sub-Department of Animal Health and Sub-National Agro-Forestry Fisheries Quality Assurance Department, which belongs to the Provincial Department of Agriculture and Rural Development.

- District and Commune level: Office of Agriculture, belonging to the People's Committee at the district and commune level.

Inspection and Surveillance system:

- Governmental level: Division of Inspection and Surveillance, which belongs to NAFIQAD.

- Provincial level: Section of Inspection and Surveillance which belongs to Sub-NAFIQAD, established in 63/63 provinces with 1-3 officials/province.

- Testing system: A provincially established network of both national and private veterinary and food safety laboratories that are modernly equipped. These laboratories carry out the testing to find out the hazards in food and factors caused food poisoning or food-borne illnesses, analyze content of ingredients of the food and carry out active national surveillance programs on food safety.

2.2.3. Chicken meat implicated in food poisoning and foodborne illness

In Vietnam, data on food poisoning and foodborne diseases are limited and sporadic cases are reported without detailed information about the implicated agents. In the period of 2002–2010, there were 195 outbreaks of food poisoning annually with 5509 people contaminated and 53 deaths. Microorganisms are suspected to be the cause of food poisoning mainly based on clinical diagnosis and account for 30.7% of total cases, followed by natural toxins (25.2%), chemicals (10.4%) and unidentified agents (33.7%). Food associated with poisoning was a mixture of food (47.4% of total cases), meat and meat products (10.9%), seafood products (10.3%) and others (Vietnam Food Administration, 2011). Unfortunately, baseline information on food poisoning and foodborne illness related to chicken meat in Vietnam is not available.

2.3. Consumption of chicken meat in Vietnam

The poultry meat consumption in Vietnam accounted for 11% of total meat consumption per capita in 2005 (USDA, 2006).

In Hanoi, live chicken are sold widely at the markets because the dominant consumption habit of consumers is to use fresh warm meat (non-frozen meat) (Sub-Department of Agriculture and Rural Development in Hochiminh city, 2012). Even chilled meat is considered less “fresh” by consumers. A predilection for broiler meat sold at markets largely guides consumers, who also believe that products at markets are less expensive and are of better quality; hence the sale of chilled chickens at supermarket is not easy, leading

to difficulties in developing industrial husbandry. Frozen chicken meat is not popular because it is considered less tasty and unfresh.

2.4. Presence of *Salmonella* in broiler carcasses

Chickens and chicken products are widely known to be an important reservoir for *Salmonella* and they play a role as vehicle of *Salmonella* infection in humans (Baumler et al., 2000; Capita et al., 2003; Uyttendaele et al., 1998).

2.4.1. *Salmonella* infection in chicken

2.4.1.1. Relevance of presence of *Salmonella* detected in broiler carcasses to *Salmonella* infection in live broilers at farms

Since *Salmonella* from live chicken flocks still exist in broiler meat after slaughtering, reducing rates of *Salmonella* infection in chicken flocks can be assumed to directly reduce the incidence of human illnesses by *Salmonella*(EFSA, 2012). Some studies also showed that there was probably higher prevalence of *Salmonella* in broiler carcasses from the infected flocks than from the uninfected ones and there was an association between *Salmonella* contamination in carcasses with the *Salmonella* status of the live broilers before slaughtering (Cardinale et al., 2005; McBride et al., 1980).

When the chicken is infected by some serovars of *Salmonella,* Enteritidis and Typhimurium, the microorganisms can be detected from the spleen, liver, heart, gall-bladder, intestinal tissues, ceca or from various sections of the ovary and oviduct (Gast et al., 2004; Gast et al., 2007; Keller et al., 1995). Except for those serovars and some

serovars that can cause fowl diseases, *Salmonella* colonizes the intestinal tract of chicken and is excreted through fecal shedding. Presence of *Salmonella* in day of hatch birds was significantly associated to *Salmonella* contamination of the flock at the end of the rearing period ($P < 0.05$, RR = 1.84) (Rose et al., 1999) and infected flocks that shed *Salmonella* in feces on the first days of rearing keep shedding the bacteria until slaughter represents higher risk batches when slaughtering, which becomes a potential route of processing contamination (Bailey et al., 2001; Marin & Lainez, 2009; Van Immerseel et al., 2004). Another study revealed the presence of *Salmonella* Enteritidis phage type 4 (PT4) in muscle tissues of broiler carcasses (Humphrey et al., 1991), which was imputed to the invasive nature of PT4 in broiler chickens, suggesting that the infection could be septicemic (Lister, 1988; Mickael et al., 2010; Shah et al., 2011). Many factors are also identified as the sources of *Salmonella* contamination in chicken carcasses at the farm level, including: the feed (Corry et al., 2002), hatchery infected broilers (infected breeding and laying flocks) (Corry, et al., 2002; Rigby et al., 1980a; Rigby et al., 1982) and pre-slaughter feed withdrawal (Corrier et al., 1999; Ramirez et al., 1997).

Transmission among live chickens is also a factor that makes chickens become carriers of *Salmonella*. Live birds may have *Salmonella* in their droppings and on their bodies (feathers, feet and beaks) even when they appear clean and healthy, and the bacteria can be distributed widely in the environment where the birds live (CDC, 2012). In Vietnam where backyard chickens are kept, it is very difficult to control and prevent transmission among live chickens at households or between chickens and the environment.

2.4.1.2. Routes of *Salmonella* transmission to live broilers

It is well-known that *Salmonella* can be transmitted vertically or horizontally and horizontal transmission can be direct or indirect. In primary production, control of *Salmonella* within broiler flocks relies on knowledge of the source of infection. Chicken often become infected via horizontal transmission and possible sources include water, feed, litter, farm staff and the environment both inside and outside the broiler house (indirect horizontal transmission) or by contact with other chicks/animals contaminated with *Salmonella* (direct horizontal transmission) (Davies, 2005; FAO/WHO, 2002). Furthermore, commercial poultry production has a pyramid breeding structure with generic selection at the top. Amplification through the system down from breeder flocks to progeny and through the production stage at the baseline can thus lead to a wide scale spread of *Salmonella* if breeding stock is infected. Hatcheries are then another possible source of infection (FAO/WHO, 2002). The presence of *Salmonella* incubated hatching eggs has been clearly identified as a critical control point in the *Salmonella* contamination of broiler chickens (Cox et al., 2000).

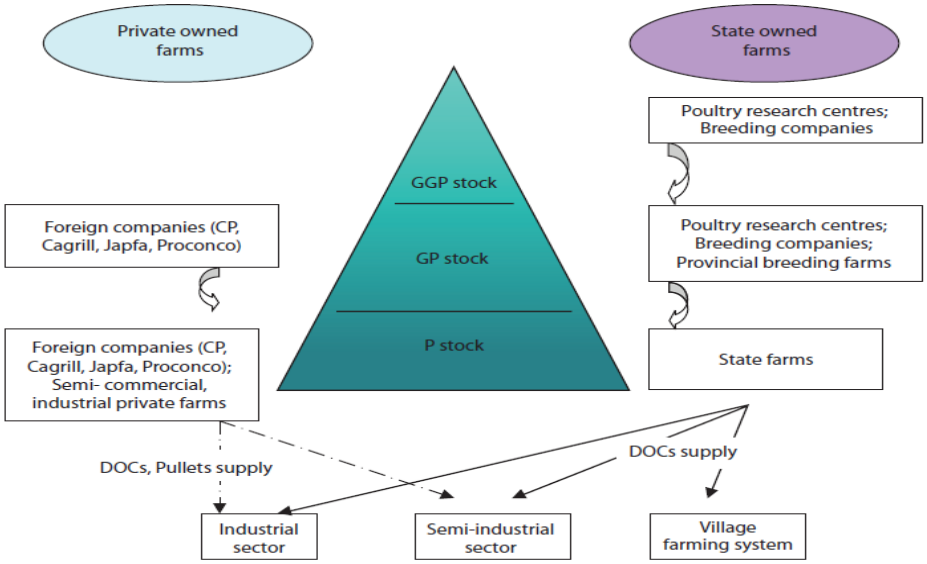
In Vietnam, controlling horizontal transmission among broilers in the non-intensive system is mostly impractical because birds are grazing freely and eat by themselves. There is no available information about measures to control *Salmonella* infection and contamination particularly in semi-intensive and intensive systems, but registered certified broiler farms all meet the requirements of veterinary hygiene conditions regulated in relevant mandatory Technical Regulations. Veterinary hygiene conditions are defined as all conditions regarding to infrastructure conditions, equipments; water supply; feed; breeding management; procedure for caring and raising; procedure for disinfection; control of insect

and harmful animals; control of diseases; hygiene of staffs; management of waste matters; which ensure the animal to be able to grow healthily, in order to produce safe food products, non-harmful to human health and are environmentally friendly (MARD, 2011b).

Theoretically, poultry breeding programs in Vietnam follow the breeding pyramid shaped organization but great grandparent (GGP) breeding stocks of imported breeds are only kept in three of twelve state breeding centers (Delquigny et al., 2004) for selection and for pure breeding multiplication producing grandparent (GP) stocks, whose sizes are small and genetic quality is poor. Only one state farm keeps local breeds – Ri (the name of a local breed) for selection and crossbreeding in small sized flocks. Grandparent and parent flocks of imported breeds are usually kept in 12 state farms and in foreign companies. At a lower level, there are some provincial farms that are supposed to receive GGP and GP stocks from state farms and carry out breeding of parent stocks and production of commercial day old chicks (DOCs), but most public farms, in fact, survive by selling DOCs (Delquigny et al., 2004).

There are foreign companies which are fully integrated. They produce parent stocks privately to supply contracted industrial farms with DOCs from imported GP flocks (Desvaux et al., 2008). Currently there is no detection and control program for *Salmonella* in broiler breeders in Vietnam.

FIGURE 2. Poultry Breeding Pyramid (Desvaux et al., 2008)



In the DOC supply chain, inputs of DOCs to households/farms in each husbandry system are different. In non-intensive systems, most households which keep local breeds produce DOCs by themselves or buy DOCs from suppliers. Local hatcheries collect incubated eggs from breeder households and sell DOCs (Thang et al., 2007). In semi-intensive systems, DOCs are bought from local hatcheries for crossbreeds and from local markets for local breeds (Tung& Rasmussen, 2005). Since sources appear to be quite numerous, the likelihood of vertical transmission is high in both the non- and semi-intensive systems. In intensive systems, however, DOCs are bought from state farms or foreign companies under poultry keeping contracts, no study was found to confirm the better control of vertical transmission in this system than others.

2.4.2. *Salmonella* contamination of chicken carcasses

The routes of *Salmonella* contamination in chicken carcasses are very diverse, with many factors involved from farm to market. *Salmonella* can survive asymptomatically in the gastrointestinal tract of a proportion of chickens and can be present on chickens' feathers via environmental contamination. Consequently, there is a possibility of carcass contamination during processing via fecal contamination at several stages in the slaughter process: transportation, killing, scalding, plucking, evisceration, washing, chilling and cross-contamination from contaminated products or surfaces on the production line. Cross-contamination in the slaughter line has been mentioned and demonstrated in some studies. The slaughter of *Salmonella*-positive birds leads to contamination of the processing line and standard cleaning procedures do not always eliminate this. (Berrang et al., 2009; Byrd et al., 2002; Olsen et al., 2003; Rasschaert et al., 2007; Rasschaert et al., 2008). In Vietnam, because F/M chicken carcasses are sold widely with other meat without separate packaging, chicken carcasses can be cross contaminated through contact with equipment, surfaces or other meat at the market, or by contaminated hand manipulation also.

Transportation from farm to slaughterhouse is considered a source of contamination of chicken carcasses with *Salmonella*. During transportation, uninfected chicken flocks can be contaminated through fecal contamination from another flocks and reusing transport crates is identified as a risk factor. Even when reused crates are washed after use, due to the high frequency of utilization and the presence of fecal materials on crates, they are still often contaminated with *Salmonella* and thus are a potential route of contamination (Heyndrickx et al., 2002). Rigby et al. carried out studies in Canada to investigate the presence of

Salmonella on plastic crates and found that the proportion of crates yielding *Salmonella* before birds were loaded was 86.6% (97/112) and the proportion after washing used cold potable water under pressure, containing 2.5% disinfectant (creolin) was 73.5% (97/132), indicating that washing did not remove *Salmonella* from these crates. Two serovars first isolated from the transport crates were also recovered from six carcasses after slaughtering (Rigby, et al., 1980a; Rigby et al., 1980b) and *Salmonella* was also detected on the feathers of 93.5% (29/31) of sampled chickens. Rigby also reported the *Salmonella* contamination of carcasses (46.4%, 13/28) from *Salmonella*-free flocks which were loaded into contaminated crates. Serovars isolated from carcasses were the same as those isolated from the crates (Rigby, et al., 1982). Inadequate cleaning and disinfection of transport crates performed under normal conditions has also been reported in other studies (Corry, et al., 2002; Mead et al., 1994; Slader et al., 2002), indicating dirty crates as a potential risk factor. In addition, long transportation times and in-plant waiting times were also identified as important risk factors for *Salmonella* contamination of broiler carcasses (Mainali et al., 2009). In fact, these factors are closely associated with increased shedding and spreading of *Salmonella* due to transport stress and movement, leading to the increased possibility of cross-contamination during transportation (FAO, 2005a, 2005b; Mulder, 1995). In Vietnam, chickens are carried to slaughterhouses either in plastic crates, in variably soiled with fecal material, or to the traditional markets or manual abattoirs, where chickens are kept in woven bamboo cages together, sometimes with ducks and geese. At some traditional markets, chickens are kept alive in cages until the consumers purchase them.

At slaughterhouses, chickens go through a process with many stages. These five main stages are identified as potential causes of *Salmonella* contamination and include: scalding, plucking (or defeathering), evisceration, washing and chilling (FAO, 2005a). Each stage can potentially increase the prevalence of *Salmonella* in broilers or the numbers of microorganisms on the surface of chicken carcasses; contamination of the carcasses depends on the installation and the hygiene conditions of processing systems (Mead, 1989; National Advisory Committee on Microbiological Criteria For Foods, 1997). It is reported that the slaughtering process is the main cause of *Salmonella* contamination in chicken carcasses (Goksoy et al., 2004; Rasschaert et al., 2008).

The slaughtering process begins at the scalding stage in order to loosen feathers prior to defeathering. Carcasses with a microbial load on their feathers and skin are immersed in hot water. There are two options for scalding water: "soft" scalding is applied for fresh products using water at 50ºC for 90seconds and "hard" scalding is used for products to be frozen (56ºC for 45seconds). However, even the high temperature in the "hard" scalding process has little effect on the presence of microorganisms in general and *Salmonella* in particular (Buhr et al., 2005; Cason et al., 2004; Slavik et al., 1995). Furthermore, *Salmonella* was found in the scalding water (Cason et al., 2000; Cortez et al., 2006; Reiter et al., 2007) and in particular, some *Salmonella* species may remain viable in the scald tanks for long periods (International Commission for the Microbiological Specifications of Foods, 1996). Therefore, there is a potential cross-contamination among carcasses. The presence of fecal material on the feathers before slaughter and washed off into the water, thereby becoming a source of contamination, was also observed. By this way, most poultry-

associated *Salmonella* found in the intestine of chickens is introduced into the scaldtank water (Wray & Wray, 2000a). In the case of "soft" scalding, because the water temperature is relatively low (about 50^{o}C), death rates of *Salmonella* can be lower and water in the scaldtank is frequently *Salmonella*-positive (Mulder et al., 1978). Scalding has also been shown to facilitate the attachment of *Salmonella* to chicken skin, making them more difficult to remove (Notermans & Kampelmacher, 1975).

The plucking (or defeathering) process can be operated mechanically or manually. Mechanically, the carcasses are in continuous contact with rubber "fingers" which move along the feathers to remove them as the chickens are spun. During the plucking process, a load of fecal material and microorganisms can be extruded. The featherless carcasses can be cross contaminated in this process if the machine is not cleaned and disinfected properly (FAO, 2005a; Rasschaert et al., 2007). Significant increases in the prevalence of *Salmonella*-positive turkeys(Clouser et al., 1995; Nde et al., 2007) and broiler carcasses (Goksoy et al., 2004; Sarlin et al., 1998) after defeathering compared to before defeathering have been reported. In the study of Clouser (1995), the *Salmonella*-positive turkeys before and after defeathering were 3 and 10 out of a total of 14, and in the study of Nde (2007), these numbers are 82 (47%) and 110 (63%) out of a total of 174. In broiler carcasses, the rates of *Salmonella* contaminated carcasses before and after defeathering were 33.3% and 60%, respectively in the study of Goksoy (2004).Another study also observed a higher level of total aerobic bacteria recovered on featherless chicken carcasses than the same feathered birds (Buhr et al., 2003).

During poultry processing, cross-contamination is frequent and can occur at almost any stage of slaughter, but is more likely at the evisceration stage (removal of chest and abdomen contents) when gut contents might be damaged and the exterior of the birds may be contaminated (Wray & Wray, 2000a). Such damage can occur frequently since the machinery used for evisceration is not flexible with respect to the size of the bird (FAO, 2005a). This stage can be carried out manually but the risk is not necessarily due to the mode of evisceration when properly applied; it is the contamination of carcasses from the leakage of the viscera that can significantly contribute to an increase in *Salmonella* prevalence at this stage(Sarlin et al., 1998). When poultry are infected with *Salmonella*, *Salmonella* can colonize and survive in the guts of birds or invade host tissues, so *Salmonella* are found in many organs of the birds, such as: the cecum, cloaca, ileum, crop, spleen, liver/gall bladder, reproductive tracts, etc.(Barrow et al., 1988; Gast, 1994; Gast et al., 2004; Hannah et al., 2011). During commercial processing, the crops have been noticed to be 86 times more likely to rupture than ceca (Hargis et al., 1995) and some studies have also identified the crop as a source of *Salmonella* contamination in broiler carcasses (Byrd, et al., 2002; Corrier, et al., 1999). In Vietnam, this stage is carried out manually. At the industrial slaughterhouses, the workers use knives or scissors to vent an incision while the birds are hung. Leakage of intestines and the spill of eviscerated contents onto the carcasses has been observed (Food Agriculture Production Qualification and Development Control Project, 2010). At semi-manual abattoirs and manual slaughtering facilities, this stage is normally carried out on the ground, the same knife is used throughout the slaughtering process and hygiene conditions are very poor.

Washing is a carcass-cleaning process which is conducted by using water sprays or water immersion for the purpose of removing organic debris and decreasing the numbers of microorganisms on the carcasses. Depending on the method of washing, the prevalence of *Salmonella* may increase or decrease. *Salmonella* can be washed off the exterior of contaminated carcasses however the probability of cross-contamination among positive and *Salmonella*-free carcasses can occur if washing takes place in an immersion tank (FAO, 2005a). The spray washing process can reduce the possibility of cross-contamination but the removal of attached bacteria from carcasses is not enhanced by using chlorine and/or hot water (Northcutt et al., 2005). The reduction of *Salmonella* contamination on carcasses, however, can be enhanced by using acidified electrolyzed water or sodium hypochlorite solutions in spray washing (Northcutt et al., 2007), or using combinations of levulinic acid and sodium dodecyl sulfate in immersion washing (Zhao et al., 2009). In Vietnam, at the industrial slaughterhouses, spray washing is applied but there is no information about using chemicals in washing water(Food Agriculture Production Qualification and Development Control Project, 2010). But at other facilities, one tank of water is used to wash many carcasses after evisceration without proper control of hygiene quality.

Chilling is a process to decrease the inner temperature of broiler carcasses in order to inhibit the multiplication of *Salmonella* and other bacteria and facilitate the storage stage afterward. The methods commonly used are immersion in cold water with/without ice and direct contact with chilled air (FAO, 2005a). In an immersion chiller, although chilled water is replaced and chlorinated in order to control the accumulation of bacteria, large numbers of carcasses share a common water bath, amplifying the opportunity for cross-

contamination. Some recent studies have reported that the water chilling process has raised the potential for cross-contamination or has no effect on the reduction of *Salmonella* incidence in post-chilled carcasses (Goksoy et al., 2004; James et al., 1992a; James et al., 1992b; Sarlin et al., 1998; Smith et al., 2005).Even it has become the largest influence on contaminated carcasses (Bucher et al., 2012). In the air chilling process, although there is less contact between carcasses, the possibility of microbial cross-contamination may also occur, whether or not water sprays are incorporated in the chilling process (Mead et al., 2000). Although there are more studies about the possibility of cross-contamination in the water chilling method, air- and immersion-chilled carcasses without chemical intervention are microbiologically comparable (Huezo et al., 2007).

2.4.3. Growth of *Salmonella* in raw chicken meat

As mentioned above, *Salmonella* detected from raw chicken meat derives mostly from contamination during rearing, transportation, processing and marketing, so that *Salmonella* on chicken carcasses survives and grows on the skin or the meat surface. Chicken skin is one of the best surfaces for attachment of bacteria (Firstenberg-Eden et al., 1978).

When broiler carcasses are contaminated with *Salmonella*, *Salmonella* can still survive under refrigerated storage conditions, although some studies have indicated the reduction of proportion of *Salmonella* recovery after refrigerated storage. In the study of Bourassa (2004), there was a significant decrease in the percentages of *Salmonella* recovery on the day of processing and after 7 days of storage at 2ºC, 46% and 20% (n=50), respectively (*P=0.0002*) (Bourassa et el., 2004). Another study by Cason (2006) reported the same

statistical incidences of *Salmonella* detected from whole chicken carcasses between 0 and 24-hour storage in 4ºC (Cason et al., 2006). However, the storage period in this study was shorter than others and it could not be concluded that there was the microbiological equivalence of *Salmonella* recovery from whole-carcass rinse samples before and after refrigerated storage. At the ideal refrigerated temperature 4ºC, some studies have showed that the changes in *Salmonella* numbers were not significant during a varying storage period. There was no significant difference on counting observed between day 0 and day 5, day 8at 4ºC for *Salmonella* Typhimurium (Pintar et al., 2007). The same result was also noted by Oscar (2011) that *Salmonella* Typhimurium DT104 with a low initial dose (0.9 log) on chicken skin survived and death was not observed at 4ºC after 1, 3, 6 and 10 days (Oscar, 2011). Another study of Pradhan et al. (2012) proved the inability of proliferation of *Salmonella* Typhimurium (started at 4.7 log cfu/g) at 4ºC after 8 days and 15 days ($P > 0.05$) (Pradhan et al., 2012).

On the other hand, when storage temperature cannot be maintained below 4ºC, *Salmonella* can reproduce; in many studies growth has been observed at 8ºC after 3 to 9 days of storage. In the study of Jimenez at al. (2009), the number of inoculated *Salmonella* on chicken carcasses decreased about 1 log at 2ºC but increased 1.5 log at 8ºC after 9 days (Jimenez et al., 2009).Pradhan (2012) also proved the growth of *Salmonella* Typhimurium at 8ºC by the increase from 4.7 log cfu/g to 5.1 and 5.9 log cfu/g after 3 and 7 days of experiment (Pradhan et al., 2012). At a temperature range from 8ºC to 12ºC, the growth of *Salmonella* Typhimurium DT4 on chicken skin was observed at 9ºC (0.7 log cfu/ml), 10ºC (1.1 log), 11ºC (1.8 log), and 12ºC (2.9 log) within 10 days (Oscar, 2011). *Salmonella*

Typhimurium and *Salmonella* Enteritidis growth on aerobically packed chicken thighs were observed at 8ºC, 10ºC and 12ºC (2-3 log) within 9 days (Betts et al., 2003). At higher temperature, the growth of *Salmonella* which was inoculated into raw chicken was observed at temperature ranges of 10ºC – 28ºC, of 30ºC – 37ºC (Dominguez & Schaffner, 2008), and of 25ºC – 45ºC; the growth rate increased gradually along with the increase of temperature and reached the peak at 40ºC (1.1 log/h in 8-hour experiment) (Oscar, 2009).

Frozen storage at a range of temperature from -20ºC to 0ºC did not produce changes in population of *Salmonella* in raw chicken meat. At -18, -12, -14 and 0ºC, *Salmonella* did not grow but survived on processed chickens after 14 days (Bailey et al., 2000). The same results were also reported for the survival and absence of growth of *Salmonella* Typhimurium on raw chicken breast samples at -20, -12 and 0ºC after 21 days (Pradhan et al., 2012), and *Salmonella* in ready-to-cook chicken nuggets and strips at -20ºC after 16 weeks (Dominguez & Schaffner, 2009).

2.4.4. Prevalence of *Salmonella* contamination in chicken meat

Consumption of *Salmonella* contaminated chicken meat is associated to human salmonellosis of foodborne illnesses (Fearnley et al., 2011; Humphrey et al., 1988; Reilly et al., 1988; Wegener et al., 2003). There are many studies on the prevalence of *Salmonella* in raw chicken meat and the results vary geographically and change over time. However, it is impossible to compare on paper the prevalence among countries or regions because of differences between sampling plans, laboratory isolation methods and capacities.

In the United States, the United States Department of Agriculture (USDA) has conducted nationwide microbiological baseline studies on *Salmonella* isolates from meat and poultry products, along with complementary data from molecular and phenotypic analyses, to examine the association among serotypes from meat and poultry products and from human cases of salmonellosis. With the aim to reduce the risk of foodborne illness associated with the consumption of meat and poultry products, the Food Safety and Inspection Service (FSIS) issued the Pathogen Reduction/Hazard Analysis and Critical Control Point (PR/HACCP) Systems, Final Rule, also known as the Mega-Reg, and *Salmonella* was selected as the target organism. *Salmonella* performance standards for slaughtering and producing establishment of food animals or raw ground products were set to achieve the targets for pathogen reduction, which requires the prevalence of *Salmonella* contamination, as a percentage of positive samples from carcass, lower than the findings of the current national microbiological baseline surveys. At first, the *Salmonella* Reduction Performance Standard were set at 20% positive for *Salmonella* in broilers but it will be revised periodically based on the new baseline prevalence data to achieve the goal of reducing the risk of foodborne illness (Federal Register, 1996).

The prevalence of *Salmonella* contamination from broiler carcasses had a decreasing tendency during the period of 2005 to 2012, with incidences of 16.3%, 11.4%, 8.5%, 7.4%, 7.2%, 6.7%, 6.5% and 4.3%, respectively. Since 2011, the *Salmonella* Reduction Performance Standard has been set at 7.5% positive for *Salmonella* on young chicken carcasses (USDA, 2013). Serovars Kentucky and Heidelberg have been predominant in non-targeted broiler samples from 1998 to 2005. Enteritidis has become one of the ten most

popular serovars isolated from poultry meat since 2000, and from 2006 to 2010, Kentucky, Heidelberg, Enteritidis and Typhimurium were the most dominant serovars isolated from poultry meat (USDA, 2011b).

In the European Union (EU), the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) have confirmed the clear decrease of *Salmonella* prevalence in poultry at the EU level (EFSA, 2012). That result is the success of EU *Salmonella* control programs for reducing the prevalence of the bacteria in poultry populations which aim at reaching the *Salmonella* reduction target of the maximum *Salmonella* positive percentage to 1% or less by 31 December 2011. These programs were set by Regulations (EC) No 1003/2005, No 1168/2006, No 646/2007 covering the following *Salmonella* serovars: Enteritidis, Typhimurium, Infantis, Virchow and Hadar in breeding flocks, and Enteritidis and Typhimurium in laying hen flocks, chickens and turkeys, having regard to Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November2003 on the control of *Salmonella* and other specified foodborne zoonotic agents(EU, 2003; 2005; 2006; 2007). In 2008, an EU-wide baseline survey was carried out at slaughterhouses to determine the prevalence of *Salmonella* on broiler carcasses. This survey was conducted in 26 EU countries, sampling 10,132 broiler batches (one carcass per batch) from 561 slaughterhouses. All members followed a shared protocol in order to ensure the equivalence in results among countries. The prevalence of *Salmonella*-contaminated broiler carcasses varied widely, from 0.0% to 26.6%, with a Community prevalence of 15.6%. Hungary was a notable exception, witha prevalence of 85.6% and the majority of isolates being Infantis. In the EU, the four most

common serovars were Infantis (29.2%), Enteritidis (13.6%), Kentucky (6.2%) and Typhimurium (4.4%) (EFSA, 2011).

In Asian countries, including Vietnam, there is no nation-wide study on the prevalence of *Salmonella* in retail broilers, and there is a lack of data on predominant *Salmonella* serovars. In Vietnam, there are some recent studies on the prevalence of *Salmonella* in chicken meat, all of which have focused on traditional type markets only. The samples used in these studies were parts of chicken and the results obtained varied from 21% to 53.3% (see Table 2).

TABLE 2. Prevalence of *Salmonella* in raw chicken meat

Country	Type of samples	% positive	Predominant identified serovars	Reference
USA	1999-2000: retail raw chicken meat	4.2% (9/212)	N/A	(Zhao et al., 2001)
	2010: raw poultry meat (baseline studies)	6.7%	Kentucky, Heidelberg, Enteritidis and Typhimurium	(USDA, 2013)
Canada	2008: retail raw chicken	40% (382/960)	Kentucky, Heidelberg, Enteritidis and Hadar	(Government of Canada, 2008)
The EU	2008: raw carcasses at slaughterhouses	0-85.7%	Infantis, Enteritidis, Kentucky, Typhimurium	(EFSA, 2011)
Spain	1993: retail raw chicken meat	55% (40/73)	Enteritidis, Poona, Infantis, Newport, Typhimurium	(Alvarez-Fernandez et al., 2012)
	2006: retail raw chicken meat	12.4% (19/153)		
	1999: retail raw chicken meat	35.83% (71/198)	Enteritidis (47.88%), Hadar (25.35%) and serotype 4,12:b:-(II) (19.71%)	(Dominguez et al., 2002)
China	2010: whole carcasses	52.2% (1152)	N/A	(Yang, et al., 2011)
	2010	54% (276/515)	Enteritidis, Typhimurium, Shubra, Indiana	(Yang et al., 2010)
Japan	2006-2008: retail raw chicken meat	20% (164/821)	Infantis, Kalamu, Schwarzengrund	(Iwabuchi et al., 2011)
Thailand	2003: retail raw chicken meat	62% (31/50)	Corvallis, Hadar,Give, Schwarzengrund,	(Vindigni et al., 2007)

			Vichow, Amsterdam, Mbandaka, Paratyphi B *var.* Java	
Vietnam	2000-2001: retail raw chicken meat	21% (202)	Typhimurium, Dessau	(Phan et al., 2005)
	2004-2005: retail raw chicken meat	48.9% (128/262)	Agona, Emek, London	(Luu et al., 2006)
	2004: retail raw chicken meat	53.3% (16/30)	N/A	(Van et al., 2007)
	2007-2009	42.9% (115/268)	Emek, Infantis, Blockey, Anatum	(Thai et al., 2012)

N/A: not available in the study

Conclusion

The *Salmonella* genus can be divided into two major groups: typhoidal and non-typhoidal *Salmonella*, which can cause salmonellosis in humans. Chicken and chicken products are widely known to be an important reservoir for *Salmonella*, and they have been pinpointed as vehicles of *Salmonella* infections in humans. Infection with these bacteria is normally oral and causes enteric fever or self-limited enteritis in humans.

Presence of *Salmonella* in broiler carcasses at slaughter has been associated with *Salmonella* infection in live broilers at the farm. *Salmonella* can be transmitted vertically or horizontally and horizontal transmission can be direct or indirect. In Vietnam, the sources for infection appear to be quite numerous. The likelihood of vertical transmission is high in both the non- and semi-intensive systems. During processing, potential *Salmonella* contamination in broiler carcasses occurs at almost every stage, especially in manual slaughterhouses with poor hygienic conditions in Vietnam and even in larger slaughterhouses where evisceration techniques have not been standardized. The possibility of contamination at traditional markets in Vietnam is high since chickens are not wrapped and are in contact with other meat and the environment.

In order to evaluate the risk for human infection, it is important to evaluate the level of exposure. Chicken meat is the most important vector of infection so the evaluation of prevalence or incidence of carcass contamination by *Salmonella* will indicate the level of human exposure and the level of the risk.

The objectives of this study are:

- To establish the prevalence of *Salmonella* contamination in retail raw whole chicken carcasses.

  + To distinguish proportions of *Salmonella*-positive samples among three types of chickens: F/M, F/SM and FZ/SM.

  + To examine the diversity and distribution of *Salmonella* serovars.

  + To determine if other factors have an impact on *Salmonella* proportions.

- To measure and compare inner carcass temperature at various purchasing sites during a six week period in the summertime.

CHAPTER 3. ARTICLE

Prevalence of *Salmonella* in retail whole chicken carcasses in Hanoi, Vietnam

Prevalence of *Salmonella* in retail whole chicken carcasses in Hanoi, Vietnam

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ABSTRACT

In Vietnam, the data on the prevalence of *Salmonella* contamination in retail chicken meat is limited. We wanted to establish and compare that prevalence at traditional and modern supermarkets, as well as in fresh versus frozen carcasses, and at the same time to measure the inner carcass temperatures at time of purchase. A collection of 245 whole chicken carcasses were purchased from traditional markets and supermarkets, in seven urban district areas of Hanoi in June and July, 2011. Sampling plan included 110 fresh chickens from traditional markets (F/M), 109 fresh chickens from supermarkets (F/SM) and 26 frozen chickens from supermarkets (FZ/SM). The inner carcass temperature was collected at the time of purchase. *Salmonella* was isolated from carcass rinses and isolates were serotyped. The overall prevalence of *Salmonella*-positive carcasses was 66.5% (163/245). The *Salmonella* prevalence in the three types of chickens varied significantly, 84.55% (93/110) from F/M, 59.63% (65/109) from F/SM and 19.23% (5/26) from FZ/SM ($P<$ 0.05). A total of 25 serovars were recovered. The predominant serovars were Agona (24.78%), Albany (20.43%) and Corvallis (10%). Two different serovars were isolated and coexisted on the same carcass in 66 samples (26.9%). The inner carcass temperatures of fresh samples from traditional markets and supermarkets were significantly different (P <0.05) with a mean inner carcass temperature of 27.3°C and 15.8°C respectively. This study revealed a high prevalence of *Salmonella* spp. from retail chickens in Hanoi and uncovered the difficulty encountered by all market types to store broiler chicken carcasses at a safe temperature.

Key words: *Salmonella*, chicken carcass, prevalence, serovar, inner carcass temperature, Vietnam.

INTRODUCTION

Salmonellosis has become one of the most prevalent foodborne diseases worldwide (Altekruse, et al., 2006; CDC, 2009, 2010, 2011; Cogan & Humphrey, 2003; Kennedy, et al., 2004; WHO, 2005). Most human *Salmonella* outbreaks are associated with the consumption of contaminated products from animal origin (Wray &Wray, 2000a; Domingues, et al., 2011; Pires, et al., 2010), especially chicken meat and egg products (Altekruse, et al., 2006; Baumler, et al., 2000; Capita, et al., 2003; FAO & WHO, 2009; Kim, et al., 2007; Madden, et al., 2011; Murchie, et al., 2007; Pires, et al., 2010; Uyttendaele, et al., 1998; Yang, et al., 2011). Baseline surveys conducted in the United States and the European Union have shown a wide range of *Salmonella* isolation rates in chicken carcasses, varying from 0%in some Nordic countries (2008) to 85.6% in Hungary (2008)(EFSA, 2011; USDA, 2013).

In Vietnam, the data on prevalence of *Salmonella* contamination in retail chicken meat is limited. A few studies have been conducted on chickens parts only, with results ranging from 21% to 53.3% of the samples being positive for *Salmonella* (Ha & Pham, 2006; Luu, et al., 2006; Tran, et al., 2006; Tran, et al., 2004; Van, et al., 2007). All of these studies have focused on traditional type markets. These markets are designated places where shops and stalls are grouped and whose infrastructures are varied, from permanent and semi-permanent to temporary (People's Committee of Hanoi, 2004). Since there is an increasing number of modern supermarkets to meet the demands of a rapidly growing Vietnamese middle class, we wanted to compare the prevalence of *Salmonella* on chicken carcasses between these markets types.

Purchase of fresh warm meat sold at traditional markets is still very common in Vietnam. It is known that the growth rate of *Salmonella* is positively correlated with the increase of temperature (Chavez et al., 2004; Dominguez & Schaffner, 2008; Jimenez, et al., 2009; Oscar, 2009, 2011; Pradhan, et al., 2012). Controlling chicken meat temperature is thus very important because it directly affects the microbial quality of products. Since appropriate storage temperature is difficult to achieve in traditional type markets, we also wanted to verify what the inner carcass temperature for both facility types was and if frozen carcasses had a lower *Salmonella* prevalence.

The objectives of our study were (1) to measure the prevalence of *Salmonella* contamination in retail raw whole chicken carcasses and to compare the prevalence of *Salmonella* contamination of three groups of chicken carcasses: fresh from traditional markets (F/M chicken), fresh from supermarkets (F/SM chicken) and frozen from supermarkets (FZ/SM chicken); (2) to examine the diversity and distribution of *Salmonella* serovars in retail raw whole chicken carcasses; and (3) to measure and compare inner carcass temperature in fresh carcasses at the time of purchase at the markets and supermarkets.

MATERIALS AND METHODS

Sampling

Whole chicken carcasses were purchased from seven urban district areas of Hanoi in a six-week period in June and July 2011. Each area was visited three times; supermarkets and markets were chosen based on the availability of whole carcass chickens and if the distance between paired supermarkets and markets was less than five kilometers. Each time, the same sampling scheme was applied for collection of F/M, F/SM and FZ/SM chicken carcasses. Four carcasses of each type, when available, were collected every day, or six F/M chickens and six F/SM chickens were purchased if no FZ/SM chickens were available.

All samples were collected in the morning. The temperature of fresh carcasses was measured at the time of purchase, using a needle electronic thermometer (ERTCO Digital Thermometer (ERT 300) Barnstead International, USA) by piercing a hole in the side of the breast of the carcass to reach the deep pectoral muscle. Samples were kept separately in closed individual plastic bags and carried to the lab in insulated boxes with ice packs within three hours after purchasing. At the lab, samples were kept in a refrigerator and tested for *Salmonella* prevalence within 24 hours after collecting. Further information regarding provider name, packaging place, date of packaging and expiry date was also gathered at the time of purchase either from the seller or the label.

Microbiological analysis

Whole carcass chicken rinse: A whole carcass chicken rinse was performed as described by the Laboratory Guidebook issued by the United States Department of Agriculture, using 400 ml of buffered peptone water (BPW, Difco) (USDA, 2011a). The Whirl-pak® bags with 30 ml of chicken rinse mixed with 30 ml of sterile BPW were incubated at 37ºC±1ºC for 18h±2h.

Isolation and identification of Salmonella: Detection of *Salmonella* in this study followed a four-stage culture-based method as per protocol ISO 6579:2002. The three first stages of testing were carried out at the microbiological lab of the Department of Animal Health in Vietnam. Briefly, the first stage used a non-selective pre-enrichment broth (BPW) after incubation of the rinse carcass fluid. Second, the selective enrichment stage was performed in Rappaport Vassiliadis *Salmonella* Soy Broth (RVS, Difco) and tetrathionate broth (Tetrathionate Broth Base, Difco) with brilliant green (10 mg/l). A portion (0.1 ml) of the pre-enrichment culture was added to 10 ml of RVS and 1 ml of the same culture was transferred to 9ml of a tetrathionate broth with 0.2 ml of iodine solution, then was vortexed and incubated at 41.5ºC±1ºC for 24h±3h. Third, each culture obtained after incubation was inoculated on two petri dishes of xylose lysine deoxycholate agar (XLD agar, Difco) and brilliant green sulfa agar (BG Sulfa Agar (BGS), Difco) with novobiocin (20 mg/l). The dishes with presumptive *Salmonella* colonies were wrapped closely by parafilm and shipped to Canada by FedEx service. The transportation took about one week without chilling preservation.

The last stage of biochemical and serological identification was carried out in Canada. When they arrived in Canada, the petri dishes were stored at 4ºC for one month before the next stage was completed. Because of possible injury to the bacteria due to transportation, two techniques were used before biochemical screening: selection of colonies for confirmation and creation of backup samples for another analysis at the lab of the Research Chair of Meat Safety in the Faculty of Veterinary Medicine, University of Montreal. For colony selection, one typical colony was selected and streaked onto the surface of a blood agar and incubated at 37ºC±1ºC for 18h±2h; for the backup samples, all colonies from one dish that did not contain the typical colony for the first technique were transferred to a brain heart infusion (BHI, Difco), incubated at 37ºC±1ºC for 18h±2h for a pre-enrichment stage. The culture obtained with BHI was inoculated with a total volume of 0.1ml culture divided into three drops (about 33 µl per drop) on Rappaport-Vassiliadis Medium Semisolid Modification (MSRV, Difco) and incubated at 42ºC±1ºC for 24h±2h and 48h (MFLP-75 (Government of Canada, 2004)). Bacteria were picked at the outer most edge of the migration area and were streaked onto the blood agar surface for purification. All the colonies on the blood agar were inoculated in triple sugar iron agar (TSI Agar, Difco), urea agar (Urea Agar, Difco) and lysine iron agar (LIA, Difco) for biochemical screening. A collection of isolates identified as *Salmonella* from two techniques were serogrouped using *Salmonella* antisera and sent to the reference lab of Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec for serotyping according to the Kauffmann-White-Le Minor serotyping scheme.

Statistical analysis

Data involving *Salmonella* prevalence, detected proportions and serotype levels (with chicken type, producer, place of packaging, and frequency of serovars as variables),and distribution figures of inner carcass temperatures at purchase were obtained using SAS® version 9.2 (SAS Institute Inc.). The relationship between paired variables with *P* value was calculated with a non-parametric Mann-Whitney U test for independent samples, using SPSS Statistics Software version 16.0 (IBM Corp.).

RESULTS

Sampling

A total of 245 whole chicken carcasses were collected from seven urban district areas. The F/M chickens sampled were all killed on the same day and sold in small butcher shops with or without other kinds of meat. None of the chicken carcasses were frozen, prepackaged or pre-weighed, and they were exposed to an external environment when purchased. However, in three shops, chicken carcasses were kept in a refrigerator (temperature was not measured) after killing, and in one shop, carcasses were chilled in large styrofoam boxes with ice, before being exposed to environmental temperatures when placed on table surfaces for sale in all cases. All F/SM samples were collected before the expiry date i.e., the official three days of shelf-life after packaging and storage at 4ºC. F/SM chickens carcasses were sold fresh, prepackaged in individual sealed plastic bags, pre-weighed with price, name of provider and expiry date and kept chilled on refrigerated shelves. FZ/SM

chickens were packaged the same way as F/SM chickens, but stored in freezers with a one-year shelf-life post-packaging expiry date.

Since FZ/SM chickens appeared not to be popular in Hanoi, they could only be found in two supermarkets. The sample collection included 110 F/M chickens, 109 F/SM chickens and 26 FZ/SM chickens.

Detection level: Prevalence of *Salmonella* in raw chicken carcasses

The overall prevalence of *Salmonella* contamination in retail raw whole carcass chicken samples in Hanoi during the summer time in the period of June to July 2011 was 66.5% (163/245). The percentages varied significantly among types of chicken: (1) fresh chicken from markets (84.55%, 93/110), (2) fresh chicken from supermarkets (59.63%, 65/109), (3) frozen chicken from supermarkets (19.23%, 5/26). Each paired proportion was significantly different from the other ($P< 0.05$) (Table 1).

Information regarding providers (name of company) who packed the chicken carcasses was collected from the F/SM packaged chicken label, but the information regarding the source (grower or slaughterhouse) was scarce or absent. In our study, providers A and B are the main fresh chicken providers for almost all supermarkets in Hanoi and a group of certain supermarkets that provide fresh chickens packaged on-site was designated as provider C. Regarding packaging location of F/SM chickens, products can be divided into two types: (1) packaged at supermarkets where slaughtered birds are transported from slaughterhouses and packaged on the premises in styrofoam trays and closely covered with a plastic wrap; (2) individually prepackaged at slaughterhouses in closed plastic bags with labels. FZ/SM

chickens are prepackaged at slaughterhouses and kept in freezers at -20^{o}C. In this study, the producer and location of packaging did not significantly (P>0.05) affect *Salmonella* prevalence.

Detection and distribution of *Salmonella* serovars

A total of 25 different serovars were recovered from 230 isolates. Two different serovars were isolated and coexisted on the same carcass in 66 samples (26.9%) (Table 1).

As shown in Table 3, the two most dominant serovars were Agona and Albany (24.78%, 57/230 isolates and 20.43%, 47/230 respectively), followed by the serovars Corvallis, Derby and Aarhus (10%; 8.7% and 7.3%, respectively). The diversity of *Salmonella* serovars recovered from F/M and F/SM samples are similar, with 18 serovars and 20 serovars, respectively in total. They also had 13 serovars in common. *S.* Enteritidis was recovered solely from F/SM samples (3.26%) (Table 2).

From F/SM samples, there was no significant difference in diversity and distribution of serovars among the three grades of supermarket or among the three providers(P>0.05). From F/M samples, there was no significant difference in diversity and distribution of serovars among the seven geographical areas (P>0.05).

Inner carcass temperature at time of purchase (ICT)

ICT between two types of chickens

There was a significant difference between the ICT of F/M and F/SM chickens. The ICT of F/M chickens (*n*=107) ranged from 5.4ºC to 39.7ºC with a mean of 27.3ºC (median=28.4ºC), while the ICT of F/SM chickens (*n*=109) ranged from 0.5ºC to 25.8ºC with a mean of 15.8ºC (median=15.5ºC) (Figure 1).

ICT between positive and negative samples

The ICT of F/M chickens positive for *Salmonella* (*n*=92) ranged from 5.4ºC to 39.7ºC with a mean of 27.23ºC, while the ICT of F/M chickens negative for *Salmonella* (*n*=15) ranged from 8.5ºC to 38.9ºC with a mean of 27.6ºC. The ICT of F/SM chickens positive for *Salmonella* (*n*=65) ranged from 4.3ºC to 25.6ºC with a mean of 15.2ºC, while the ICT of F/SM chickens negative for *Salmonella* (*n*=44) ranged from 0.5ºC to 25.8ºC with the mean of 16.8ºC. There was no significant difference between the ICT of F/M and F/SM chickens in terms of testing negative or positive for *Salmonella* (*P*>0.05).

DISCUSSION

Salmonella detection

There have been a few studies on prevalence of *Salmonella* in retail chicken meat in Vietnam at traditional markets (Luu, et al., 2006; Van, et al., 2007; Vo et al., 2006), but all studied chicken parts not whole carcasses. In comparison with these studies, *Salmonella* prevalence from our sampled retail chicken meat was higher (Luu, et al., 2006; Thai, et al., 2012; Van, et al., 2007). Indeed, while these authors found *Salmonella* prevalence ranging from 42.9% to 53.3% in chicken part samples, 66.5% of our whole carcass chickens were

positive for *Salmonella*. Our results are however similar to the findings of smaller scale studies in Thailand where a prevalence of 62% (31/50) from chicken samples from fresh markets and supermarkets was reported (Vindigni, et al., 2007). Our results are higher than the results in studies from Algeria, Japan, Mexico and China (17.97%, 20%, 39.7% and 52.2% respectively) (Iwabuchi, et al., 2011; Mezali & Hamdi, 2012; Yang, et al., 2011; Zaidi et al., 2006). However, market and sample types should be taken into account when making such comparisons; only supermarkets were sampled in the Japanese study, while the samples from other studies were a mix collected from supermarkets, retail outlets and fresh/wet markets.

Interestingly, the significantly higher prevalence of *Salmonella* from F/M chickens than F/SM chickens in our study (84.55% and 59.63%, respectively) supports the results of Vindigni (2007) in Thailand (85% and 35%, respectively) (Vindigni, et al., 2007). Because of the similar conditions of tradition markets in Vietnam and fresh markets in Thailand, along with the similarity of sample preservation, the possible explanation for this is meat exposure to environmental contamination. It is readily apparent that the conditions at traditional markets in Vietnam are ideal for cross-contamination among carcasses because they are exposed to the environment outdoors and are in direct contact with other meats, table surfaces and insects. And during night, open table surface may be visited by pets or rodents that also contribute to contamination. Given the warm climate, exposure to high temperatures allows for rapid bacterial growth, contributing to easier cross-contamination and high *Salmonella* prevalence. Finally, the hygienic status of chicken carcasses can also be affected by various factors during rearing, transportation and slaughtering operations.

The significantly lower *Salmonella* prevalence of frozen versus fresh carcasses observed in our study (19.23% versus 84.55% (F/M) and 59.63% (F/SM)) is consistent with the results of Yang (2011) in China (Yang, et al., 2011) and Donado-Godoy (2012) in Columbia (Donado-Godoy., et al., 2012). One could assume that carcasses sold frozen to supermarkets are rapidly and completely chilled after slaughter, hence decreasing the chance of *Salmonella* proliferation. In contrast, this result is not in agreement with other studies that noticed the higher prevalence of *Salmonella* in frozen chickens than in freshly slaughtered and chilled chickens (Alali., et al., 2012; Wang., et al., 2013). It could be explained by the differences in manipulation and hygiene practices at the place of chicken providers (company) causing *Salmonella* contamination which may impact the *Salmonella* prevalence in frozen chickens and other types, especially in this study F/M samples came from many local butchers, F/SM ones from three companies and a small quantity of FZ/SM ones (n = 26) came from one company.

***Salmonella* serovars**

The higher frequency of serovar Agona in this study is similar to the results of a 2005 study on chicken meat in Hanoi (in the northern part of Vietnam)(31%, 40/129) (Luu, et al., 2006). Interestingly, this serovar was not found at retail markets in Hanoi and in two surrounding provinces in another study in 2007-2009 (Thai, et al., 2012) nor was it the dominant serovar in southern Vietnam in 2001 (Phan, et al., 2005). It supports conclusions of recent surveys that *Salmonella* serovars do vary greatly geographically and in time (EFSA, 2011; USDA, 2011b).

The high number of serovars identified might be explained by the variety of chicken sources in our study and the multiple sources of cross-contamination. The cross-contamination can occur at all stages, from rearing farm (Mainali, et al., 2009), to slaughterhouse (Goksoy, et al., 2004; Rasschaert, et al., 2008) and market (Scheinberg, et al., 2013) which assumes the association between distribution of *Salmonella* serovars and hygiene practices from farm to market.

In our study, about 40.5% of single carcasses testing positive for *Salmonella* (66/163) contained 2 or 3 serovars. The presence of more than one serovar in a single sample shows that there is a lot of cross-contamination from farm to market stressing the importance of serotyping several isolates from a single sample in epidemiological studies and underlining the consequences of cross-contamination.

Inner carcass temperature at the time of purchase

In our study, there was only one F/M sample with a compliant inner temperature between 4ºC – 7ºC (MARD, 2012) i.e., 5.4ºC. All other samples showed an inner temperature between 8 and 39.7ºC. Some (7) F/M samples had low temperatures (5.4ºC – 11.1ºC) because they were stored either in styrofoam boxes with ice or in a refrigerator prior to sale. Because other carcasses had extremely high temperatures, it is possible that some were slaughtered right before selling because the average temperature in June-July in Hanoi is about 30ºC – 35ºC.

According to the supermarket package labels, all F/SM chickens have a 3-day shelf-life and a packaging date rather than a slaughter date provided on the label, which makes inference

to the latter rather difficult. It was worrisome in our study to observe that only three samples complied with the regulations (0.5ºC and 4.3ºC) while other samples had unacceptable inner temperatures varying between 6.8ºC to 25.8ºC (mean=16.2ºC). We could not obtain any information about the standardized operating procedures to ensure proper temperature of the supermarket refrigerators. It is also possible that there was an inappropriate chilling process at slaughter or inappropriate storage temperature during transportation (without chilling) to supermarkets. Care should be taken to verify not only if carcasses have adequately been cooled before leaving the slaughterhouse premises, but also that the cold chain protocol is fully respected throughout the various steps leading to the consumer. Not respecting the cold chain not only jeopardizes the bacteriological quality of the meat, but also its sensory and nutritional qualities.

CONCLUSION

This was the first study on prevalence of *Salmonella* contamination in retail chicken carcasses from different types of markets and types of chicken (F/M, F/SM and FZ/SM) in Vietnam. Although there are some regulations on controlling food safety during storage and marketing, they are apparently not fully complied with by retailers. These results should be useful for governmental agencies to establish effective strategies for improving food safety in Vietnam. In the future, extended studies should be conducted nationwide in order to establish a baseline prevalence of *Salmonella* in retail chickens in Vietnam and further studies on genotyping occurrences to track the factors bearing on contamination should be considered.

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TABLE 1. Prevalence of *Salmonella* contamination in retailed raw whole carcass chicken in Hanoi

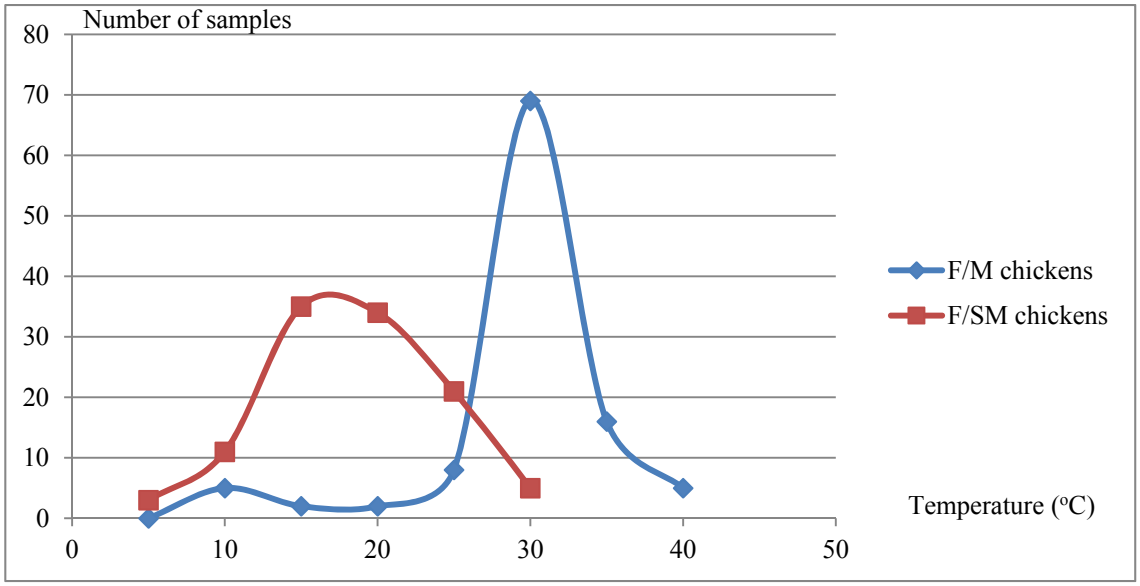
Types of chickens	***No. of samples tested***	***No. (%) of samples positive for Salmonella***			
		Total	***Percent (95% CI)***	***No. of serovars isolated***	
				1	***2***
Fresh/Market	110	93	84.55[a] (77.80 - 91.30)	55	38
Fresh/Supermarket	109	65	59.63[b] (50.42 - 68.84)	38	27
Frozen/Supermarket	26	5	19.23[c] (9 - 38)	4	1
Total	*245*	*163*	*66.5 (60.61 - 72.39)*	*97*	*66*

[a, b, c] Denote the significant differences among proportions ($P < 0.05$)

TABLE 2. Distribution of the most frequent *Salmonella* serovars recovered from retail raw whole chicken carcasses in Vietnam

Serovars	***Frequency (%)***	***No. (%) of isolates per type of chickens***		
		F/M	***F/SM***	***FZ/SM***
Agona	57 (24.78)	33 (2500)	22 (23.91)	2 (33.33)
Albany	47 (20.43)	26 (19.70)	20 (21.74)	1 (16.67)
Corvallis	23 (10.00)	11 (8.33)	11 (11.96)	1 (16.67)
Derby	20 (8.70)	13 (9.85)	7 (7.61)	
Aarhus	18 (7.83)	13 (9.85)	4 (4.35)	1 (16.67)
Infantis	11 (4.78)	8 (6.06)	3 (3.26)	
Typhimurium	6 (2.61)	3 (2.27)	3 (3.26)	
Cerro	6 (2.61)	3 (2.27)	2 (2.17)	1 (16.67)
Hadar	6 (2.61)	3 (2.27)	3 (3.26)	
Kentucky	6 (2.61)	4 (3.03)	2 (2.17)	
Indiana	5 (2.17)	5 (3.79)		
Meleagridis	4 (1.74)	3 (2.27)	1 (1.09)	
Enteritidis	3 (1.30)		3 (3.26)	
London	3 (1.30)	2 (1.52)	1 (1.09)	

FIGURE 1. Distribution of inner carcass temperatures of F/M and F/SM chicken carcasses



CHAPTER 4. DISCUSSION

National data on *Salmonella* contamination in raw chicken are not available in Vietnam. To our knowledge, this is the first study of prevalence of *Salmonella* contamination in retail whole chicken carcasses in Hanoi. There have been a few studies on prevalence of *Salmonella* in retail chicken meat (Luu, et al., 2006; Van, et al., 2007; Vo, et al., 2006) at traditional markets, however all of these studies focused on chicken parts. Furthermore none of them compared the *Salmonella* proportions between F/M and F/SM chickens and between fresh and frozen chickens, and none measured inner carcass temperature. The objectives of our study were (1) to measure the prevalence of *Salmonella* contamination in retail raw whole chicken carcasses and to compare the prevalence of *Salmonella* contamination of three groups of chicken carcasses: fresh from traditional markets (F/M chicken), fresh from supermarkets (F/SM chicken) and frozen from supermarkets (FZ/SM chicken); (2) to examine the diversity and distribution of *Salmonella* serovars; and (3) to measure and compare inner carcass temperature of fresh carcasses at the time of purchase at markets and supermarkets.

Salmonella **detection**

In comparison with the results of other studies conducted in Vietnam, *Salmonella* prevalence from retail chicken meat in our study was higher (Luu et al., 2006; Thai et al., 2012; Van et al., 2007). Indeed, while Luu, Van and Thai found a *Salmonella* prevalence of 48.9%, 53.3% and 42.9% respectively, in their chicken part samples, 66.5% of our whole carcass chickens were *Salmonella* positive. In comparison with the results from other Asian countries and other countries, this result is similar to the findings of studies in Thailand (66%, 467/705) (Jerngklinchani, et al., 1994), (62%, 31/50) (Vindigni, et al., 2007) and in

Portugal (60%, 36/60) (Antunes et al., 2003); but higher than the results in China (52.2%, 601/1152) (Yang et al., 2011), (54%, 276/515) (Yang et al., 2010), in Japan (20%, 164/821) (Iwabuchi et al., 2011), in Algeria (17.97%, 23/128) (Mezali & Hamdi, 2012) and in Mexico (39.7%, 121/295) (Zaidi et al., 2006). However, market and sample type should be taken into account when making comparisons. Only supermarkets were sampled in the Japanese study, while the samples from other countries were collected from supermarkets, retail outlets and fresh/wet markets. Since the conditions of fresh/wet markets in Thailand and China are quite similar to the conditions of the traditional markets in Vietnam, the results from these studies should be more appropriate for making comparisons. Apart from the study of Yang et al. (2011), the other studies did not describe their sample types as whole chicken carcasses, hence the difference of sample types could lead to inequivalence. The *Salmonella* incidence from F/M chickens in our study is similar to the results obtained in Cambodia (88.2%, 134/152) where neck skin from carcasses slaughtered directly at market sites (Lay, et al,, 2011) were sampled, as well as in Thailand where chicken meat was sampled at fresh markets (85%, 23/27) (Vindigni, et al., 2007). None of these used whole chicken carcasses.

Interestingly, the significantly higher prevalence of *Salmonella* from F/M chickens than F/SM chickens in our study (84.55% and 59.63%, respectively) supports the results of Vindigni (2007) in Thailand (85% and 35%, respectively) (Vindigni, et al., 2007). Because of the similar conditions of tradition markets in Vietnam and fresh markets in Thailand, along with the similarity of sample preservation, a possible explanation for this is the exposure of the meat to environmental contamination. It is readily apparent that the

conditions at the traditional markets in Vietnam are ideal for cross-contamination among carcasses because the carcasses are exposed to the environment outdoors, and are in direct contact with other meats, table surfaces and insects. And during night, open table surface may be visited by pets or rodents that also contribute to contamination. Given the warm climate, exposure to environmental high temperature allows for a rapid bacterial growth, contributing to an easier cross-contamination and a high *Salmonella* prevalence. Finally, the hygienic status of chicken carcasses can also be affected by various factors during rearing, transportation and slaughtering operations.

The significantly lower *Salmonella* proportion of frozen chickens over fresh chickens in our study (19.23% versus 84.55% and 59.63%) is in agreement with the results of Yang (2011) in China (45.7% versus 52.4% and 56%) (Yang, et al., 2011) and Donado-Godoy (2012) in Columbia(42% (n = 191) in chilled chickens versus 14% (n = 79) in frozen ones) (Donado-Godoy, et al., 2012). Injury and decrease of viability of *Salmonella* in meat after frozen storage has been shown in previous studies (Barrell, 1988; Foster & Mead, 1976) and this could be the reason for the lower prevalence in frozen chickens. However, recent studies have shown the contrary results which proved that frozen storage has no effect in viability of *Salmonella*. Dominguez and Schaffner (2009) examined the number of *Salmonella* in chicken at -20^{o}C and found that the number remained after 16 weeks (Dominguez & Schaffner, 2009). A study of Pradhan (2012) also found that *Salmonella* Typhimurium can survive at -20, -12 and 0^{o}C without producing significant changed in population (Pradhan et al., 2012). Although there is no data on the period after slaughter until becoming frozen thoroughly, one could also assume that carcasses sold frozen to supermarkets are rapidly

and completely chilled after slaughter, hence decreasing the chance of *Salmonella* proliferation. In contrast, this result is not in agreement with other studies that noticed the higher prevalence of *Salmonella* in frozen chickens than in freshly slaughtered and chilled chickens (53.3% (n = 60) versus 37.5% (n = 120) and 45% (n = 60), respectively) (Wang et al., 2013) or no significant difference between chilled and frozen chickens (34.35% (n = 482) versus 26.48% (n = 216)) (Alali et al., 2012). It could be explained by the differences in manipulation and hygiene practices at the place of chicken providers (company) causing *Salmonella* contamination which may impact the *Salmonella* prevalence in frozen chickens and other types, especially in this study F/M samples came from many local butchers, F/SM ones from three companies and a small quantity of FZ/SM ones (n = 26) came from one company. Interestingly, in the study of Wang (2013), although the level of *Salmonella* contamination of frozen chickens was the highest, the highest average MPN value of *Salmonella* observed in freshly slaughtered samples collected at wet markets (avg. MPN value = 0.1912 MPN/g) (Wang et al., 2013).It proposes a further study of determination of the MPN value of *Salmonella* in our three types of samples to confirm the level of contamination and identify risk factors associated with the *Salmonella* contamination in chicken carcasses in Vietnam.

Type of samples appears to be associated to prevalence of *Salmonella* from chicken meat. Some studies reported a higher *Salmonella* incidence from carcasses versus other parts, 57.1%(n = 70) versus 28.6% from left front breast parts and 25.7% from right front breast (n = 70) (Oscar et al,, 2010), or 55% versus 40% from chicken parts (Capita et al., 2003). In a 2005 study, the breast portions studied showed a lower prevalence of *Salmonella* of

48.9% (*n* = 262) (Luu et al., 2006) than the 66.5% found in whole chicken carcasses (*n* = 245) in this study, which is consistent with the studies above. This is surprising since increased meat handling due to carcass cutting should increase chances of cross-contamination and lead to higher *Salmonella* prevalence. This difference in prevalence between sample types underlines the necessity of considering the pros and cons of different sampling plans in diagnostics.

Another reason for the difference in reported *Salmonella* prevalence might be related to the isolation techniques used in the different studies. The four-stage method used in this study is the "gold standard" for *Salmonella* identification from food and food ingredients, which includes a pre-enrichment stage reported to be more sensitive than the direct culture method (Barrell, 1988; Gast, 1993) and direct selective agar plating (Valentin-Bon, et al., 2003). In addition, RVS-XLD combination is found to have the highest sensitivity (0.99) for *Salmonella* detection in chicken carcasses and RVS broths are recommended for selective enrichment (Hyeon et al., 2012). Another study also revealed that RVS broths and tetrathionate broths with novobiocin yielded a higher recovery of *Salmonella* (97.4% and 94.9%, respectively) than a Selenite Cystine broth (38.5%) (Schonenbrucher et al., 2008). Our study used the combination of an RVS/TT broth and XLD/BGS agar, compared with an RVS broth and XLT4 agar (Thai et al., 2012) and an RVS/TT broth and XLT4/Rambach agar (Luu et al., 2006) used in the other studies in Vietnam. The combination of a more selective broth and agar with higher sensitivities complied with the most recent protocol on the most acceptable method for detection (ISO 6579:2002). In brief, our combination of

selective broths coupled with pre-enrichment (BPW) could have provided a higher sensitivity in *Salmonella* detection, which would explain our higher prevalence.

To avoid a sampling bias related to location, the geographical area factor was considered in our study when designing a sampling plan. Our statistical analysis revealed that geographical area had a significant effect on the proportions of *Salmonella* in F/M chickens ($P< 0.05$). Among seven areas, there are three areas in which *Salmonella* was detected from all samples (districts number 2, 3 and 4, n = 12, 12 and 17, respectively). In other areas, the proportions fluctuated between 61.11% and 88.89% (n = 15 or 18) (Table 3). However, our study was not designed to compare geographical areas; sampling was done in various areas to optimize sampling size and give an overview of the Hanoi region and avoid bias. Since there were no statistical difference between the grades of supermarket and providers it is difficult to explain this geographical difference.

The supermarket grade factor was also considered to measure the effect of infrastructure. Supermarkets in Hanoi are graded based on business area and quantity of items; ranking grade 1, 2 or 3 when they meet the requirements of having more than 5000, 2000 or 500 square meters in area and have more than 20,000, 10,000 or 4000 items, along with other general requirements of infrastructure (Ministry of Trade of Vietnam, 2004). Supermarkets have invested in equipment and food safety controls (Ministry of Health of Vietnam, 2011). In our study, although supermarket grade did not significantly ($P> 0.05$) affect *Salmonella* prevalence (Table 3), the investment in refrigerators and freezers appeared not to be uniform, with more modern equipment observed at the higher grade supermarkets during the course of

our study. Producer and location of packaging also did not significantly affect *Salmonella* prevalence in F/SM chicken carcasses (Table 5).

TABLE 3. Proportion of *Salmonella* contamination in retailed raw whole chicken carcasses in Hanoi

Type of chicks	***n*** [*]	***n+*** [¶]	***%***
Fresh/Market			
Location of markets/District (P = 0.004)			
No. 1	15	10	66.67
No. 2	12	12	100.0
No. 3	12	12	100.0
No. 4	17	17	100.0
No. 5	18	15	83.33
No. 6	18	11	61.11
No. 7	18	16	88.89
Fresh/Supermarket			
Grade of supermarket (n_1) (P = 0.693)			
Grade 1(2)	25	16	64.00
Grade 2(3)	30	16	53.33
Grade 3(4)	54	33	61.11

[*] Number of observations
[¶] Number of *Salmonella*-positive observations
Grade1 supermarkets are more 5000 square meters in area and have more than 20,000 items
Grade2 supermarkets are more 2000 square meters in area and have more than 10,000 items
Grade3 supermarkets are more 500 square meters in area and have more than 4,000 items
The n_1 values are the number of supermarkets at each level

Salmonella serovars

A total of 25 serovars were recovered from 230 isolates and Agona, Albany and Corvallis were the most common serovars isolated from chicken carcasses in this study. Notably, the highest frequency of serovar was Agona, which is similar to the results of a 2005 study on chicken meat in Hanoi (in northern part of Vietnam)(31%, 40/129) (Luu et al., 2006); however, this serovar was not found on chicken meat at retail markets in Hanoi and the two surrounding provinces in another study in 2007-2009 (Thai et al., 2012) and was not the dominant serovar from chicken meat in the south in 2001 (Phan et al., 2005). The

differences between our study, Luu's (2006) and Phan's (2005) are easily understandable since the southern region was the geographical area of focus for the latter and the other two were based in the north. But in the case of the study of Thai et al. (2012), the samples were collected from July 2007 to June 2009 and the difference could be explained by the larger geographical area of his study, which focused not only on Hanoi city, but also on two other surrounding provinces where the distribution of *Salmonella* in chicken meat was possibly different.

Interestingly, Agona is one of twenty of the most common serovars recovered from humans in Asian countries (Hendriksen et al., 2011). Since S. Agona was one of the most prevalent serovar observed in our study, one can question the relationship between contaminated chicken meat consumption and human infection with this serovar in Vietnam. Conversely, *S.* Emek was one of the predominant serovar in previous studies in Vietnam (Luu et al., 2006; Phan et al., 2005; Thai et al., 2012) but it was not isolated in this study. This is very surprising since two of these studies were conducted in the same geographical areas as ours. However, there is a difference between our study and two of these studies, which is the time of sampling. Our study was conducted during summer time, while the study of Luu et al. (2006) was conducted during winter and spring time and the samples of the study of Thai et al. (2012) were collected randomly for two years. However, at the point of *Salmonella* serovar distribution, a further study should be designed with longer sampling time to determine any temporal pattern.

Regarding the relationship between *Salmonella* isolates from humans and chicken meat consumption, among the 20most frequently serotyped human *Salmonella* isolates from

Asian countries (Hendriksen et al., 2011), 14 serovars were recovered in this study. Although *S.* Typhimurium and *S.* Enteritidis have been the most frequent serovars recovered from humans (Hendriksen et al., 2011; Vo et al., 2006), according to our results chicken meat may not be such an important source of transmission of these serotypes to humans in Vietnam, this because of the low frequency of these serovars from chicken meats in some recent studies (Luu et al., 2006; Thai et al., 2012). The most common serovars in North America and the European Union (e.g., *S.* Kentucky, *S.* Enteritidis, *S.* Typhimurium) were found at low frequencies and *S.* Heidelberg was not recovered in this study at all.

S. Derby is commonly associated with pork and swine (Kich et al., 2011; Piras, et al., 2011; Schmidt et al., 2012) but it is also the fourth predominant serovar (8.7%) in this study. From other studies in Vietnam, *S.* Derby was also recovered at low rates from F/M chickens (Luu et al., 2006; Thai et al., 2012). This result might be explained by the possible cross-contamination between pork and chicken meat occurring at market since they are sold on the same table. This serovar has not been reported dominantly from chicken meat in the EU, the US, Thailand and Japan (EFSA, 2011; Iwabuchi et al., 2011; USDA, 2011b; Vindigni et al., 2007). Interestingly, this serovar was also recovered at high frequency from F/SM chickens (7.61%) in this study and all of them were packaged at slaughterhouses. In this case, further study should be conducted to trace the origin of this serovar from the farm to the slaughterhouse where poultry and pork are slaughtered at the same place.

In our study, about 40.5% of single carcasses positive for *Salmonella* (66/163) contained two serovars. The presence of more than one serovar in a single sample stresses the importance of serotyping several isolates from a single sample in epidemiological studies.

Inner carcass temperature at the time of purchase

The storage temperature is one of important factors to define the shelf-life of fresh raw meat products. Obviously, raw meat has a diversified bacterial flora. Under proper temperature for growth, the quantity of bacteria can reach the maximum acceptable level after which the meat spoils, producing unacceptable odours and an off-flavor or appearance (Borch, et al., 1996). The predominant bacteria associated with broiler spoilage are *Staphylococcus* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Aeromonas* spp., *Enterobacteriaceae* and *Weissella* spp. (Chouliara et al., 2008; Patsias et al., 2008; Zhang et al., 2012). In order to extend the shelf-life and inhibit the growth of spoilage bacteria, proper chilled storage is required for fresh raw chicken meat (Likar & Jevšnik, 2006; Mielnik et al., 1999). The study of Zhang indicated a significantly higher count of *Pseudomonas* from samples kept at 4^{o}C to 10^{o}C for 1 day oversamples stored below 4^{o}C. And culture-dependent analysis reported the slower growth of microflora at the range of 0^{o}C – 4^{o}C (Zhang et al., 2012).

Recently in Vietnam, the Ministry of Agriculture and Rural Development issued the Legal Circular No. 33/2012/TT/BNNPTNT dated 20 July 2012regulating the veterinary hygiene conditions in order to ensure food safety in marketing of raw meat and foodstuffs which was going to come into force on 3 September 2012. In this Circular, it is stated that raw

meat and foodstuffs stored at ambient temperature must be sold within 8h after slaughtering, and those stored between 0 and 5ºC must be sold within 72h after slaughtering (MARD, 2012). However, this document has been postponed due to the inability of inspection in Vietnam and the impracticability of its regulations. Consumers and inspectors do not have equipments to check how long the meat is kept and the sellers could not show the evidence to prove their raw meat products have been stored less than 8 hours. Thus, Vietnam still lacks of regulation on controlling raw meat at markets.

In our study, there was only one F/M sample with a compliant inner temperature between 4ºC –7ºC i.e., of 5.4ºC. All other samples showed an inner temperature between 8ºC and 39.7ºC (Table 6). Some (7) F/M samples had low temperatures (5.4ºC–11.1ºC) because they were stored either in styrofoam boxes with ice or in a refrigerator prior to sale. It is possible that some were slaughtered right before selling because the inner temperature was higher than the average temperature in June/July in Hanoi, which is about 30ºC– 35ºC. Care should therefore be taken to educate customers regarding the importance of quickly refrigerating freshly killed chicken carcasses they purchase.

At supermarkets, according to the package label, all F/SM chickens have a 3-day shelf-life. Packaging date rather than slaughter date is provided on the label which makes inference to the latter rather difficult. Only three samples had a temperature in compliance with the regulations (0.5ºC and 4.3ºC) while other samples had inner temperatures varying between 6.8ºC to 25.8ºC (mean = 16.2ºC) (Table 7). At grade1 supermarkets (2 supermarkets), there are thermal sensors inside the refrigerators connected to computers for automatic control, and at other supermarkets, temperature is controlled with refrigerator thermometers. We

could not obtain any information about the operational control of the equipment that maintains proper temperatures. In general, the ICT of F/SM chicken carcasses in supermarket refrigerators was unacceptable, with a median of 15.5ºC. The reason for this finding, apart from an interruption of operation, could be related to an inappropriate chilling process at slaughter or an inappropriate storage temperature during transportation (without chilling) from the slaughterhouses to the supermarkets. Care should be taken to verify not only if carcasses have adequately been cooled before leaving the slaughterhouse premises, but also that the cold chain protocol is fully respected in the various steps leading to the consumer.

In the case of F/SM chicken carcasses of our study, if the ICT could not be maintained at 4ºC, the safety of the products for consumers and the 3-day shelf-life of products should be questionable. For corrective action at the supermarket, retailers must not only control the temperature at the refrigeration thermometers, but also check the surface temperature of chicken carcasses regularly.

Even if some F/M chickens were not contaminated with *Salmonella*, keeping the meat at room temperature for 24hours is not adequate in ensuring the safety of fresh meat, since other processes, such as rancidity, might take place, affecting both the sensory and microbiological quality of products.

The point of maximum temperature of fresh/supermarket chickens (25.8ºC) is used to calculate the proportion of *Salmonella* in fresh/market chickens at the same temperature range of F/SM chickens. At this range (< 25.8ºC), the proportions of *Salmonella* in F/M

chickens (n = 18) and in F/SM chickens (n = 109) are significantly different ($P< 0.05$) (Table 4). The medians of two temperature ranges of two types of chickens are used as the points to compare the difference between proportions in each group. There is no significant difference between proportions of *Salmonella* contamination below and over the median point of range of temperature at purchase in the two types of chickens (Table 4). In this study, since the chicken carcasses could be contaminated with *Salmonella* during preservation, *Salmonella* could survive and grow, depending on storage temperature.

TABLE 4. Proportion of *Salmonella* contamination in retailed raw whole chicken carcasses in Hanoi, depending on temperature at purchase

ICT	***Fresh/Market chickens***			***Fresh/Supermarket chickens***			***p-value***
	n	***n+***	***%***	***n***	***n+***	***%***	
<25.8°C (a)	18	16	88.89	109	65	59.63	0.017
28.4°C (b)							
< 28.4°C	52	45	86.54				0.872
≥ 28.4°C	55	47	85.45				
15.5°C (c)							
< 15.5°C				53	35	66.04	0.187
≥ 15.5°C				56	30	53.57	

(a) Maximum temperature at purchase of fresh/supermarket chickens
(b) Median of range of temperature at purchase of fresh/market chickens
(c) Median of range of temperature at purchase of fresh/supermarket chickens
n values are the number of total samples
n+ values are the number of positive samples

In our study, the ICT were obtained by piercing a hole in the side of the breast of the carcass, so we could not obtain the ICT of FZ/SM carcasses given their frozen state.

Since the bacteria can grow at a wide range of temperatures on chicken meat (Betts et al., 2003; Cason et al., 1997; Dominguez & Schaffner, 2008; Jimenez, et al., 2009; Oscar,

2009, 2011; Pradhan et al., 2012), the high prevalence of *Salmonella* detected on chicken and the high inner carcass temperatures found in this study have raised an important issue about ensuring the quality of fresh chicken meat.

On the other hand, there are some limitations in this study which should be improved in further studies. First, this study was conducted in a short period (6 weeks) during summertime in Hanoi, so the results do not reflect any temporal trend of prevalence. The weather conditions can greatly affect the quality of the F/M meat when the meat is sold without refrigeration. This is particularly true in Hanoi, where there is a substantial difference of temperature between summer (30^{o}C– 35^{o}C) and winter (10^{o}C– 18^{o}C). However, some sellers were able to circumvent this challenge and the use of a refrigerator or a simple styrofoam box with ice was enough to cool carcasses to almost adequate ICT. Second, there are many factors which can affect the *Salmonella* contamination in chickens while there are few studies determining the risk factors associated with carcass contamination at the markets in Vietnam. If we could find out the risk factors, it would be easier for governmental officials to control and inspect conditions. To make a comprehensive analysis of risk factors in the broiler production system in Vietnam, the whole process from breeding farms to markets should be examined. Our study was only conducted at markets, but the result of high ICT of F/SM chickens might also indicate the failure of properly chilling carcasses at slaughterhouses and respecting the cold chain. At traditional markets, the risk factors are apparent, i.e. poor hygiene conditions of butcher shops, unhygienic slaughtering practices of live chickens at market, storage of many live chickens together in one place, improper storage temperature of fresh carcasses, etc.

However, further studies should be conducted to determine the specific risk factors from farm to market. Third, inner carcass temperature is very important across the food chain because it dictates the post-slaughter microbial quality of the meat. In order to identify the impact of ICT on proportions of *Salmonella* contamination, a quantitative analysis should have been conducted to measure the quantity and growth of *Salmonella* at specific temperatures. Unfortunately, we did not have the necessary laboratory means to achieve this at the time of sampling.

CHAPTER 5. CONCLUSION

Our study reported an overall prevalence of 66.5% of *Salmonella* from raw whole chicken carcasses in Hanoi, Vietnam. There were significant differences among *Salmonella* proportions from F/M, F/SM and FZ/SM chickens (84.55%, 59.63% and 19.23%, respectively). Risk factors such as chicken producer and place of packaging did not significantly affect the proportions from F/SM chickens which were produced by three different producers and may be packed at supermarket or at the slaughtering place. From the results of our study, frozen chickens seem to be the safest for consumers.

There were 25 serovars from 230 isolates recovered from whole chicken carcasses in this study. The most dominant serovars were Agona (24.78%), Albany (20.43%), Corvallis (10%) and Derby (8.7%).

This result indicates that further studies should be conducted to identify the relationship between hygiene conditions of butcher shops at traditional markets and *Salmonella* contamination in chicken carcasses. Furthermore, another study should be conducted to determine the specific risk factors from the farm to the market because chicken carcasses can be contaminated not only at markets but along any stage of the food delivery chain. In the meantime, a national surveillance program of human illnesses should be put in place to collect data of foodborne diseases supporting other studies on food safety risk assessment.

The inner carcass temperature of F/M chickens ranged from 5.4^{o}C to 39.7^{o}C with the mean of 27.3^{o}C, and for F/SM chickens, the range was from 0.5^{o}C to 25.8^{o}C with the mean of 15.8^{o}C. The two temperature ranges are significantly different. Generally, the ICT of fresh chickens in Hanoi was not in compliance with the current governmental regulations. This is

a big issue when trying to ensure the quality of fresh chicken meat. The Vietnamese Government should take effective action to control food safety and verify the implementation of the regulation. At the same time, the Government should organize campaigns to raise consumer awareness of food safety not only about implementing good practices in food handling and preparation but also about storage temperature and the importance of the cold chain protocol at all steps post slaughter.

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ANNEXES

TABLE 5. Proportion of *Salmonella* contamination in F/SM chicken carcasses in Hanoi

Variable	*n*[*]	*n*+[¶]	*%*
Producer (P = 0.209)			
A	38	27	71.05
B	42	24	57.14
C	26	13	50.00
Packaged at supermarkets (P = 0.25)			
Yes	26	13	50.00
No	83	52	62.65

[*] Number of observations
[¶] Number of *Salmonella*-positive observations

TABLE 6. Temperature at purchase of all fresh chickens, categorized by types of chickens

Group 1. Chickens from markets						*Group 2. Chickens from supermarkets*					
5.4	26.3	28	28.7	29.5	32.1	**0.5**	11	13.5	16.3	19.5	24.5
8	26.3	28	28.7	29.7	33.3	4.3	11.1	13.7	16.4	19.6[**]	24.8
8.1	26.5	28	28.7	29.7	35.7	4.3	11.2	13.7	16.4	19.8	24.9
8.5	26.5	28	28.7	29.7	38.1	6.8	11.5	13.8	16.5	20.5	25
8.9	26.6	28.1	28.7	29.7	38.9	7.0	11.5	14	16.6	20.7	25.4
11.1	26.9[**]	28.1	28.8	29.9	39.2	7.1	11.6	14.2	16.8	21	25.4
14	26.9	28.2	28.9	30.1	**39.7**	7.2	11.7	14.8	17.3	21.6	25.6
17.5	27.1	28.2	28.9	30.1		7.2	11.8[**]	15	17.6	21.7	25.8
19.2	27.1	28.2	28.9	30.1		8.2	12	15	17.8	22	**25.8**
22.2	27.1	28.2	29	30.2		8.7	12	15.1	17.8	22	
22.6	27.2	28.3	29	30.3		9.1	12.2	15.1	18.1	22	
22.7	27.3	28.3	29	30.3		9.2	12.3	15.1	18.6	22	
23.5	27.3	28.4[*]	29.1	30.5		9.5	12.4	15.3	18.9	22.2	
23.8	27.5	28.4	29.2	30.6		9.5	12.5	15.5	19	22.4	
24	27.7	28.5	29.2	30.6		10.1	12.5	15.5	19.1	22.7	
24.4	27.7	28.5	29.3	30.7		10.2	12.6	15.5[*]	19.3	22.9	
24.5	27.7	28.5	29.3	30.9		10.7	12.7	15.5	19.3	23.4	
25.2	27.8	28.6	29.3	30.9		10.9	12.7	15.5	19.3	23.9	
26.2	27.9	28.6	29.5[**]	31.7		10.9	12.9	15.7	19.4	24	
26.3	27.9	28.7	29.5	32		10.9	12.9	16.1	19.4	24.2	
n = 107; mean = 27.29; SD = 5.83; SIR = 1.3						n = 109; mean = 15.84; SD = 5.57; SIR = 4.0					

[*] Median
[**] Quartiles

TABLE 7. Temperature at purchase of all fresh chickens, categorized by types of chickens and results of *Salmonella* contamination

Group 1. Chickens from markets						*Group 2. Chickens from supermarkets*						
G 1.1. Positive samples					*G 1.2. Negative samples*	*G 2.1. Positive samples*				*G 2.2. Negative samples*		
5.4	26.9	28.2	28.9	30.3	**8.5**	**4.3**	12.0	16.6	24.0	**0.5**	15.5	25.0
8.0	26.9	28.2	29.0	30.5	11.1	4.3	12.2	17.6	24.2	6.8	15.7	25.4
8.1	27.1**	28.2	29.0	30.6	26.3	7.0	12.3	17.8	24.5	8.2	16.3	25.8
8.9	27.1	28.2	29.1	30.6	26.3**	7.1	12.4	18.1	25.4	9.5	16.5	**25.8**
14.0	27.2	28.3	29.2	30.7	26.6	7.2	12.5	18.9	**25.6**	9.5	16.8	
17.5	27.3	28.4*	29.2	30.9	27.1	7.2	12.6	19.0		10.9	17.3	
19.2	27.3	28.4	29.3	30.9	28.3	8.7	12.7	19.1		11.0	17.8	
22.2	27.5	28.5	29.3	31.7	28.5*	9.1	12.9	19.3		11.5	18.6	
22.6	27.7	28.5	29.3	32.0	29.0	9.2	12.9	19.4**		11.6	19.3	
22.7	27.7	28.6	29.5	35.7	30.1	10.1	13.7	19.5		12.5	19.3	
23.5	27.7	28.6	29.5	39.2	30.3	10.2	14.0	19.8		12.7	19.4	
23.8	27.8	28.7	29.5	**39.7**	32.1**	10.7	14.2	20.5		13.5	19.6	
24.0	27.9	28.7	29.7		33.3	10.9	14.8*	20.7		13.7	22.0	
24.4	27.9	28.7	29.7		38.1	10.9	15.0	21.0		13.8	22.0**	
24.5	28.0	28.7	29.7		**38.9**	11.1	15.1	21.6		15.0	22.2	
25.2	28.0	28.7	29.7			11.2	15.5	21.7		15.1	22.9	
26.2	28.0	28.7	29.9			11.5**	15.5	22.0		15.1	23.4	
26.3	28.0	28.8	30.1			11.7	16.1	22.0		15.3	23.9	
26.5	28.1	28.9	30.1			11.8	16.4	22.4		15.5	24.8	
26.5	28.1	28.9	30.2			12.0	16.4	22.7		15.5	24.9	

G 1.1. n = 92; mean = 27.23; SD = 5.39; SIR = 1.15; Q_3=29.4
G 1.2. n = 15; mean = 27.63; SD = 8.23; SIR = 1.3

G 2.1. n = 65; mean = 15.21; SD = 5.38; SIR = 3.95
G 2.2. n = 44; mean = 16.76; SD = 5.77; SIR = 4.45;
Q_1=13.1; Q_2=16; Q_3=22

* Median
** Quartiles