ISOLATION AND IDENTIFICATION OF *SALMONELLA* SPP. FROM CHICKEN MEAT IN HANOI, VIETNAM

LUU QUYNH HUONG

MASTER OF SCIENCE
IN VETERINARY PUBLIC HEALTH

CHIANG MAI UNIVERSITY AND FREIE UNIVERSITÄT BERLIN
SEPTEMBER 2005
ISBN 974-9887-83-2

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A THESIS SUBMITTED TO CHIANG MAI UNIVERSITY AND FREIE UNIVERSITÄT BERLIN IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN VETERINARY PUBLIC HEALTH

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21 September 2005

Thesis title Isolation and Identification of Salmonella spp. from Chicken Meat in Hanoi, Vietnam

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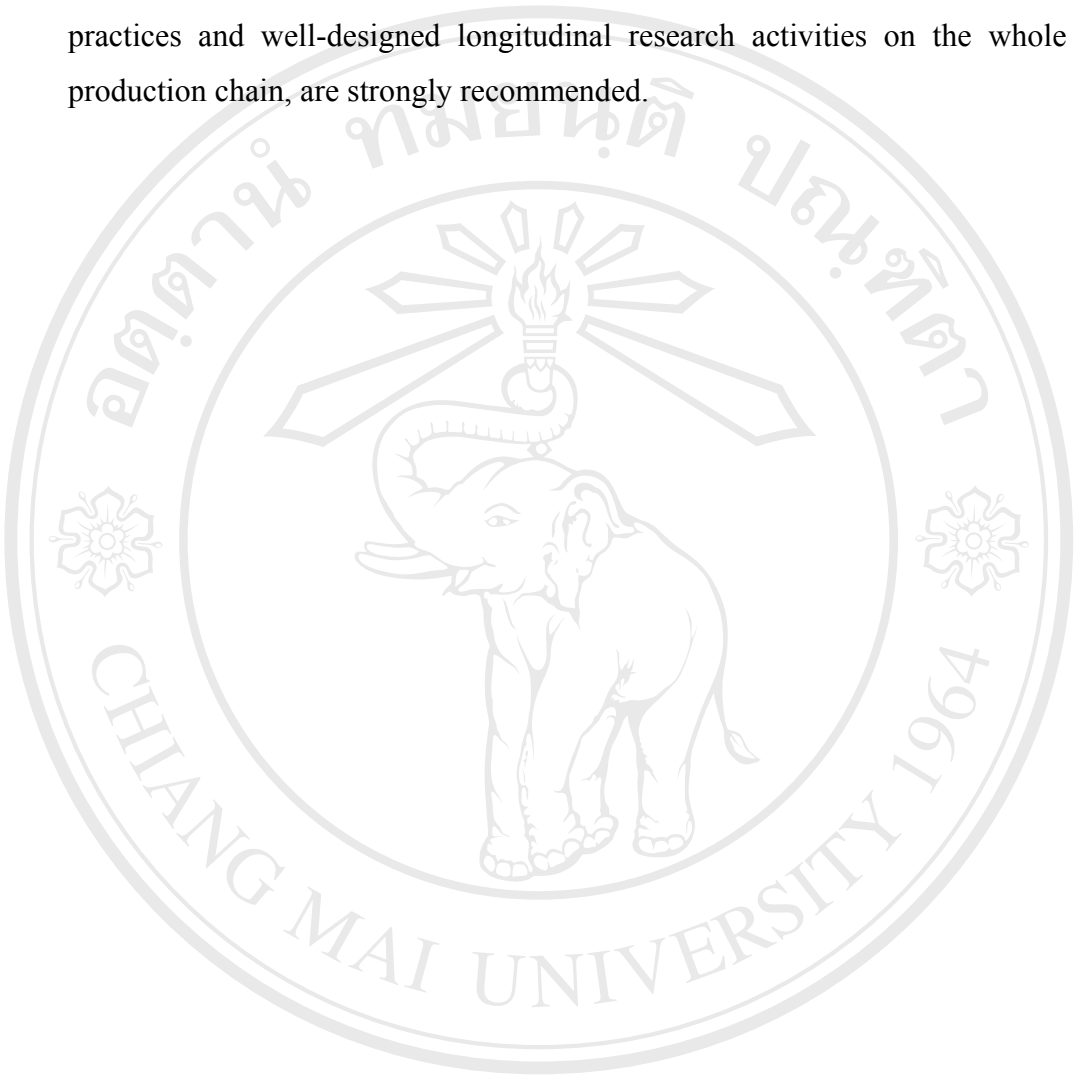
ABSTRACT

Salmonella is the most frequently reported cause of foodborne bacterial illness worldwide. The ultimate objective of controlling this foodborne hazard is to reduce or eliminate its potential risk to consumers, in addition to the economic burden associated with adverse impacts on human health. In recent years, much attention has been focused in determining the prevalence of *Salmonella* at different stages in poultry production chain.

Therefore, this study was designed to investigate the prevalence of *Salmonella* serovars in the retail chicken meat in Hanoi. A total of 262 random samples were collected from retail markets. They were examined for the presence of *Salmonella* using conventional (culturing and serotyping) methods. Of these samples, 48.9% were contaminated with *Salmonella.* The most prevalent serotype was *S*. Agona, followed by *S*. Emek and *S*. London. The proportions of *Salmonella* Enteritidis and *Salmonella* Typhimurium, were 1.55% and 7.75%, respectively. Among the risk factors examined, "Number of knives were used", "Number of choppers were used", "Hygiene status of shop" and "Type of table surface" were significantly ($p \leq 0.001$) associated with *Salmonella* contamination in chicken meat. These findings have highlighted the magnitude of the *Salmonella* contamination in retail chicken meat in Hanoi.

Therefore, based on these results, setting up of cost-effective *Salmonella* monitoring and surveillance systems, augmented by good agricultural and hygienic practices and well-designed longitudinal research activities on the whole poultry production chain, are strongly recommended.



ชื่อเรื่องวิทยานิพนธ์	การแยกและจำแนกเชื้อซัลโมเนลลาในเนื้อไก่ในเมืองฮานอย ประเทศเวียดนาม
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บทคัดย่อ

เชื้อซัลโมเนลลา เป็นเชื้อแบคทีเรียก่อโรคในอาหารที่มีอุบัติการณ์สูงทั่วโลก ซึ่งนอกจากจะเป็นอันตรายต่อสุขภาพแล้ว ยังสร้างความสูญเสียทางเศรษฐกิจด้วย การควบคุมอันตรายจากเชื้อซัลโมเนลลา อาจทำได้โดยการกำจัดแหล่งของเชื้อ จึงมีผู้สนใจศึกษาความชุกของเชื้อซัลโมเนลลาในขบวนการผลิตไก่เป็นจำนวนมาก

การศึกษานี้มีวัตถุประสงค์เพื่อหาความชุกของเชื้อซัลโมเนลลาในเนื้อไก่ที่จำหน่าย ณ.กรุงฮานอยประเทศเวียดนาม ทำการเก็ยตัวอย่างเนื้อไก่จำนวน 262 ตัวอย่าง โดยการสุ่มอย่างเป็นระบบ ตรวจหาเชื้อซัลโมเนลลาโดยวิธีมาตรฐาน ผลปรากฏว่าตัวอย่างร้อยละ 48.9 มีการปนเปื้อนเชื้อซัลโมเนลลา ชนิดของเชื้อซัลโมเนลลา ที่พบสูงสุดคือ S. Agona รองลงมาได้แก่ S. Emek และ S. London ตัวอย่างร้อยละ 1.55 ปนเปื้อนเชื้อ S. Enteritidis ตัวอย่างร้อยละ 7.75 ปนเปื้อนเชื้อ S.Thyphimurium ข้อมูลจากแบบสอบถามแสดงให้เห็นว่าจำนวนมีด จำนวนเขียง ความสะอาดของร้าน และชนิดของพื้นผิวต๊ะวางไก่มีความสัมพันธ์กับการปนเปื้อนเชื้อซัลโมเนลลอย่างมีนัยสำคัญ ($p<0.01$)

จากผลการศึกษาดังกล่าวแสดงให้เห็นว่าควรทำการศึกษาแบบติดตามเชื้อซัลโมเนลลาในกระบวนการผลิตไก่ และควรสร้างระบบการเฝ้าระวังเชื้อซัลโมเนลลาในเนื้อไก่ตลอดจนใช้ระบบการผลิตและการเกษตรที่ได้มาตรฐานเพื่อควบคุมการปนเปื้อนช้อซัลโมเนลลาในเนื้อไก่ต่อไป

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ABBREVIATIONS AND SYMBOLS

-	Negative
+	Positive
^{0}C	Degree Celsius
ACMSF	Advisory Committee on the Microbiological Safety of Foods
ACT	Australian Capital Territory Government Health
CDC	Center for Disease Control and Prevention
D	District
DIN	Deutsches Institut für Normung e. V.
DNA	Deoxyribonucleic Acid
EC	European Commission
et al	et alii
EU	European Union
FDA	Food and Drug Administration
FSRIO	Food Safety Research Information Office
G	Group
H antigen	Flagella antigen
ICMSF	International Commission on Microbiological Specifications for Foods
IFST	Institute of Food Science & Technology
ISO	International Standardization Organization

KIA	Kligler iron agar
M	Market
MLEE	Multi locus enzyme electrophoresis
MOH	Ministry of Health
NIAID	National Institute of Allergy and Infectious Diseases
O antigen	Somatic antigen
OR	Odds ratio
P	P-value
PFGE	Pulsed field gel electrophoresis
RAPD	Random amplified polymorphic DNA
S	Shop
S.	*Salmonella*
SE	*Salmonella* Enteritidis
spp.	Species
ST	*Salmonella* Typhimurium
UK	United Kingdom
US	United States
USDA	United States Department of Agriculture
Vi antigen	Capsular antigen
WHO	World Health Organization
XLT4	Xylose Lysine Turgitol 4 Agar
χ^2-Test	Chi-square Test

1. INTRODUCTION AND OBJECTIVE

1.1 Introduction

Vietnam is a country in Southeast Asia. The country shares borders with China in the north, Laos in the West and the northwest, Cambodia in the southwest, and the South China Sea in the east and the southeast. Climatically, Vietnam is located within the tropical and sub-tropical areas. The latter are quite hot and humid.

Vietnam is a developing country with an old agricultural production system that is undergoing modernization. In recent years, the issue of food hygiene and safety has received special attention from many countries in the world, including Vietnam. According to the Ministry of Health of Vietnam, there are about 3.000-4.000 cases caused by food-borne infections with at least 100-200 fatalities annually. In 2004, there were 145 outbreaks with 3,584 cases and 41 deaths. A proportion of 55.8% of these cases were caused by several pathogens (MOH, 2005).

Hanoi, the capital city, has an estimated human population of three million. This population is ever increasing due to tourists and immigrants. As a result of this, Hanoi is continuously facing high demand for food, quantitatively and qualitatively. This has led to increases of food establishments, for example vendors, small shops, and services. However, the owners of these establishments have little knowledge or awareness of food hygiene and safety. Hence, a great majority of consumers buy food from vending or small shops at which food hygiene and safety conditions are not assured.

There are several causes of food-borne infections, for example by *Salmonella*. It is found in the intestinal tracts of both animals and humans. *Salmonella* is recognized worldwide as an important food-borne pathogen that causes salmonellosis in many people (Doyle and Cliver, 1990). For example it affects as many as 3.84 million Americans, and costs billions of dollars in lost productivity and medical costs

per years (Farmer and Kelly, 1991). In recent years the occurrence of this disease in humans has increased (NIAID Fact Sheet, 2005).

The infections caused by *Salmonella* serovars are implicated as important Public health problems worldwide (Van der Klooster and Roelofs, 1997; Workman *et al.*, 1999). The zoonoses, which occur most frequently in the industrialized world today, are food-borne infections caused by *Salmonela* and *Campylobacter* (Jørgensen *et al.*, 2002). In 2000, there were about 15,000 laboratory confirmed cases of *Salmonella* infection in the United Kingdom (Public Health Laboratory Service, 2002).

The vehicles indicated in these infections are mostly *Salmonella* contaminated foods (Cartwright and Evans, 1988). Poultry meat and its derivatives are among the food products that cause the most concerns to public health authorities, owing to the associated risks of bacterial food poisoning (Bäumler, 2000; Beli *et al.*, 2001). The most frequently reported and important source of *Salmonella* contamination is cross – contaminated or undercooked chicken meat (Todd, 1994). *Salmonella* and *Campylobacter* are the most important pathogens associated with poultry products in the world (Bryan and Doyle, 1995).

In Hanoi, there are so far no modern chicken slaughtering and processing facilities. Thus, small butchers in the markets provide most of the chicken meat. Live poultry markets are common not only in Hanoi, but also in all other parts of the country. Furthermore, street or vended food is very popular. However, food hygiene practices and food handling are still big problems in the city and in the country as a whole. Therefore, a study of the ***"Isolation and Identification of Salmonella from chicken meat in Hanoi- Vietnam"*** was necessary.

The result of this study will provide information necessary for the authorities to control and prevent future outbreak of salmonellosis in Hanoi.

1.2 Objectives

The objectives of this present study are as follows:

2.2.1 To determine the prevalence of *Salmonella* in chicken meat in an urban area in Vietnam.

2.2.2 To determine the serotypes of *Salmonella* found in chicken meat in Hanoi.

2.2.3 To determine some potential risk factors associated with chicken meat contamination with *Salmonella.*

2. LITERATURE REVIEW

2.1 Background of *Salmonella*

2.1.1 History

Before the nineteenth century, human enteric or typhoid fever was often confused with typhus, a riskettsial disease. The two diseases pathologically distinguished by P. Ch. A. Louis in France (1829) and William Jenner in the United States (Scherer and Miller, 2001). Further investigations by European workers led to the isolation and characterization of the typhoid bacillus responsible for typhoid fever and to the development of a serodiagnostic test for the detection of this serious human disease agent (D'Aoust, 1989; Le Minor, 1981). During the first quarter of the 20th century, great advances occurred in the serological detection of somatic and flagella antigens within the *Salmonella* group. *Salmonella* is a generic term coined by Lignieres in 1900 (Le Minor, 1981). An antigenic scheme for the classification of salmonellae was first proposed by White (1926) and subsequently expanded by Kauffman (1941) into the Kauffmann − White scheme, which currently includes more than 2,600 serovars (Portillo, 2000).

2.1.2 Taxonomy

There are many different references on the Taxonomy of *Salmonella.* According to the World Health Organization (WHO) collaborating centre for Reference and Research on *Salmonella* (Institute Pasteur, Paris) (D'Aoust *et al.*, 2001), the genus *Salmonella* contains two species: *S. enterica* and *S. bongori* (formerly subspecies V) (Table 1).

In the ninth edition of Bergey's Manual, all of *Salmonella* serovars belong to 2 species: *S. bongori* and *S. cholerasuis*. More than 2500 remaining serovars are all part

of *Salmonella cholerasuis*, which is divisible both phenotypically and genetically, into 6 subspecies (Holt *et al.*, 2002)

Table 1: Species within the *Salmonella* genus

***Salmonella* species and subspecies**	No. of serovars (source Popoff *et al.*, 2000)	No. of serovars (source Popoff, 2001)
S. enterica subsp. *enterica* (I)	1,454	1,478
S. enterica subsp. *salamae* (II)	489	498
S. enterica subsp. *arizonae* (IIIa)	94	94
S. enterica subsp. *diarizonae* (IIIb)	324	327
S. enterica subsp. *houtenae* (IV)	70	71
S. enterica subsp. *indica* (VI)	12	12
S. bongori (V)	20	21
Total	2,463	2,501

S. enterica is divided further into six subspecies, which are referred to by a Roman numeral and a name (I, *S. enterica* subsp. *enterica*; II, *S. enterica* subsp. *salamae;* IIIa, *S. enterica* subsp. *arizonae;* IIIb, *S. enterica* subsp. *diarizonae;* IV, *S. enterica* subsp. *houtenae;* V, *S. enterica* subsp. *indica*). *S. enterica* subspecies are differentiated biochemically and by genomic relatedness (Brenner *et al.*, 2000; Holt *et al.*, 2002).

Depending on this classification system, the correct names for the formerly, called *Salmonella enteritidis* and *Salmonella typhimuritum* are *S. enterica* subsp. *enterica* serovar Enteritidis and serovar Typhimurium, respectively. *S. bongori*, which was initially categorized as subspecies V, is generally considered a separate species due to its divergence from the other *Salmonella* (Reeves *et al.*, 1989).

According to Popoff and Le Minor (1997), the name of the *Salmonella* serotype is related to the geographical place where it was first isolated. The serotype name is written in roman (not italicized) letters (for example, *Salmonella* serotype

Typhimurium or *Salmonella* Typhimurium). Serotypes belonging to other subspecies are designated by their antigenic formulae following the subspecies name (for example, *Salmonella enterica* subsp. salamae ser. 50: Z: e,n,x or *Salmonella* serotype II 50: z: e,n,x).

Currently, the genus of *Salmonella* comprises more than 2,600 serovar of gram – negative facultative anaerobic bacilli (Portillo, 2000). Classification and detection of these bacteria is based in serology and phage susceptibility assays. New DNA-based typing methods, such as random amplified polymorphic DNA (RAPD) technique, ribotyping, pulsed field gel electrophoresis (PFGE), and multi locus enzyme electrophoresis (MLEE), have contributed to reclassification of the serovars of *Salmonella* into a new subspecies group's scheme (Scherer and Miller, 2001).

For epidemiological purposes the salmonellae can be classified into three groups (WHO Expert committee, 1988):

Those that infect humans only: including S. *typhi*, *S. paratyphi* A and *S. paratyphi* C. This group includes the agents of typhoid and the paratyphoid fevers, which are the most severe of all diseases caused by *Salmonella*.

The host-adapted serovars some of which are human pathogens and may be contracted from foods including *S. gallinarum* (poultry), *S. dublin* (cattle), *S. abortus-equi* (horses), *S. abortus-ovis* (sheep) and *S. cholerasuis* (swine).

Unadapted serovars, which have no host preference, these are pathogenic agents for humans. These groups include mostly food-borne serovars.

2.1.3 Morphology

Salmonella are small 0.7-1.5 x 2-5µm, gram-negative, facultative anaerobic, straight, rod-shaped bacteria belonging to the family Enterobacteriaceae. Members of this genus are usually motile by peritrichous flagella. The bacteria grow optimally at

37^0C. *Salmonella* are oxidase negative, catalase positive, indole and voges-proskauer negative, but methyl red and simmons citrate positive (Holt *et al.*, 2002).

A typical *Salmonella* isolate would produce acid and gas from glucose in a triple-sugar iron agar medium and would not utilize lactose or sucrose in differential plating media such as Brilliant Green, Xylose Lysine Deoxycholate. Additionally, typical salmonellae readily produce an alkaline reaction from the decarboxylation of lysine to cadaverine in lysine iron agar; generate hydrogen sulfide gas in triple sugar iron and lysine iron media, and fail to hydrolyze urea (D'Aoust and Purvis, 1998).

Although most salmonellae are motile, *S. gallinarum* or *S. pullorum* are always non-motile. Most salmonellae are aerogenic; however, *S. typhi* is an important exception, which never produces gas. Anaerogenic variants of normally gas – producing *Salmonella* serovar may occur, for example *S. dublin*. The majority of salmonellae produce hydrogen sulfide, but a few types do not form this gas (e.g., some strains of *S. cholerasuis* and most strains of *S. paratyphi* A) (Krieg and Holt, 1984).

2.1.4 Serotyping of *Salmonella*

Classification of these organisms by antigenic analysis is based on the original work of Kauffmann and White and it is often referred to as the Kauffmann-White scheme. Identification of the various serovars of *Salmonella* is historically based on the presence of lipopolysaccharide (somatic or O antigen), flagella (H antigen, phase I and II) and capsular (Vi) antigen on the bacterial cell surface as determined by serum agglutination. The use of O, H and Vi antigens as the basis of classification of *Salmonella* spp. is based on the fact that each antigen possesses its own genetically determined specificity (Jay, 1992)

The serological typing of salmonellae has led to the identification of a large number of types. In the Kaufman-White classification scheme, there are 2501 named serotypes or serovars, each one being defined primarily by two antigenic sites denoted

O (somatic) and H (flagellar) (Popoff, 2001). In addition, a few serovars, such as *S.* Typhi, *S.* Dublin and *S.* Hirschfeldii have a supplementary antigen denoted as Vi. This antigen is located in an external polysaccharide microcapsule and is associated with virulence in particular hosts. The O antigens consist of the lipopolysaccharide-protein chains exposed on the cell surface (Krieg and Holt, 1984). These are heterogeneous structures, and antigenic specificity is determined by the composition and linkage of the O group sugars. Mutations that affect the sugars may lead to new O antigens.

In many serovars the flagellar H antigens can switch between two types, called phase 1 and phase 2. This switching results in two alternative sets of H antigens. Because H antigens are less heterogeneous than the carbohydrate side chains, considerably fewer H antigenic serovars exist (Krieg and Holt, 1984). The H antigens of phase 1 are designated with small letters, and those of phase 2 are designated by Arabic numerals (Jay, 1992). The antigenic formulae for some salmonellae are shown in Table 2.

Table 2: Antigen of some *Salmonella* serotypes

Serotype	Serogroup	Somatic (O) antigens	Flagella (H) antigens	
			Phase 1	Phase 2
S. Paratyphi	A	1, 2, 12	a	(1,5)
S. Typhimurium	B	1, 4, (5), 12	i	1,2
S. Agona	B	4,12	f, g, s	-
S. Derby	B	1, 4, (5), 12	f, g	(1,2)
S. Typhi	D	9, 12, (Vi)	c	1,2
S. Enteriditis	D	1, 9, 12	g, m	(1,7)

(Krieg and Holt, 1984; Jay, 1992)

2.2 Salmonellosis

2.2.1 Epidemiology

The primary reservoir of salmonellae is in the intestinal tract of humans and animals, particularly in poultry and swine. As intestinal forms, the organisms are excreted in feces from which they may be transmitted by insects and other creatures to a large number of places such as to water, soils and kitchen surfaces. Egg, poultry and raw meat products are the most important food vehicles of *Salmonella* infection in human, with *S.* Typhimurium and *S.* Enteritidis being the most commonly isolated food-borne serovars (Jay, 1992).

Information about the incidence and serotype distribution of salmonellae in domestic animal populations is essential for understanding the relationships within and among reservoirs of salmonellae in animals and humans that are ultimately responsible for zoonotic disease transmission (Gast, 1997).

Salmonella infection is usually acquired by the oral route, mainly by ingesting contaminated food or drink. Any food product is a potential source of human infection. *Salmonella* can be transmitted directly from human to human or from animal to human without the presence of contaminated food or water, but this is not a common mode of transmission. The true incidence of *Salmonella* infection is difficult to determine. Reported cases represent only a small proportion of the actual number because it is only large outbreaks that are investigated and documented. Hence, sporadic cases are underreported because it is only patients with protracted diarrhea that report health care providers for microbiological evaluation (Hanes, 2003).

2.2.2 Public health and economic impacts

Infectious diseases spread through food or beverages are a common, distressing, and sometimes life-threatening problem for millions of people around the world. The Center for Disease Control and Prevention (CDC) estimates 76 million

people suffer food-borne illnesses each year in the United States, accounting for 325,000 hospitalizations and more than 5,000 deaths. Food-borne disease is extremely costly. Health experts estimate that the yearly cost of all food-borne diseases in this country is five to six billion dollars in direct medical expenses and lost productivity. Infections with *Salmonella* alone account for one billion dollars yearly, in direct and indirect medical costs (NIAID Fact Sheet, 2005).

Salmonella is one of the microorganisms most frequently associated with food-borne outbreaks of illness. Meat products in general and poultry in particular are the most common sources of food poisoning by *Salmonella* (D'Aoust, 1997; Antunes *et al.*, 2003).

Although many other pathogens have recently received considerable attention, salmonellae remain among the leading sources of food-borne illness throughout much of world (Gast, 1997).

Salmonella Typhimurium DT104 is an emerging pathogen detected in several countries worldwide including the United States, the United Kingdom, Canada, Germany, France, Austria, and Denmark. Illness has been associated with the consumption of pork sausages, chicken, unpasteurized dairy products, a brand of meat paste, and direct contact with ill animals. Much additional information is needed about the epidemiology of DT104 in the US (Hogue, 1997).

Typhoid and non-typhoid salmonellosis remain major public health problems and are clearly the most economically important food-borne disease. The incidence of typhoid salmonellosis is stable, with very low numbers of cases in developed countries, but cases of non-typhoid salmonellosis are increasing worldwide. Non-typhoid cases account for 1.3 billion cases of acute gastroenteritis/ diarrhea with 3 million deaths and for 16 million cases of typhoid fever with nearly 600,000 deaths (Pang *et al.*, 1995).

In the US 1997, the estimated annual incidence of salmonellosis was 13.8 cases per 100,000 people. However, most cases are unreported, and the true incidence may be much higher. Although the incidence is greatest among children, outbreaks are common among individuals who are institutionalized and residents of nursing homes. Far fewer cases of typhoid fever occur each year (0.2 per 100,000 people), and these are increasingly associated with travel to developing countries (currently 72% of cases) (Zapor, 2005). The Center for Disease Control and Prevention (CDC) in Atlanta, GA in 1999 estimated that there were about 1.5 million cases with 500 deaths associated with the consumption of food contaminated with *Salmonella*. The Food and Drug Administration (FDA) estimated that in 1995, salmonellosis from food-borne sources resulted in economic loses of $350 million to 1.5 billion dollars (Schneider *et al.*, 2003).

In many countries, the incidence of salmonellosis has markedly increased; however, a paucity of good surveillance data exists. In the Netherlands, which has a population of 15.8 million, 50,000 cases of salmonellosis are reported each year (incidence, 3 per 1,000 person-years) (Van Pelt and Valkenburgh, 2001). An estimated 12-33 million cases of typhoid fever occur globally each year, and the disease is endemic in many developing countries of the Indian subcontinent, South and Central America, and Africa (Zapor, 2005)

2.2.3 Antibiotic resistance

Another major public health concern is *Salmonella spp.* with resistance to antibiotics used in human medicine, thereby greatly reducing therapeutic options and threatening the lives of infected individuals (Tacket *et al.*, 1985). Antibiotic resistance in *Salmonella spp.* has been continuously reported since the early 1960s (Van Leeuwen *et al.*, 1979), when most of the reported resistance was to a single antibiotic (Cherubin, 1981). However, multidrug – resistant strains are emerging in India and Southeast Asia. In India 50- 70% of strains are resistant to chloramphenicol and other antibiotics (Pang *et al.*, 1995; Rowe *et al.*, 1997).

In some parts of the world, antibiotics are used increasingly in the agriculture and aquaculture industries as therapeutic, prophylactic, and growth-promoters to protect the vigor of reared animal species. New evidence indicates that growth-promoting drugs such as apramycin, avoparcin, and tylosin can engender bacterial resistance to gentamicin, vancomycin and erythromycin, respectively. The approved veterinary use of enrofloxacin in several European countries (1987-1994) and sarafloxacin in the US (1995) was most unfortunate because the agents used there led to the emergence and spread of fluoroquinolone-resistant *Salmonella*, *Campylobacter* and other bacterial pathogens in foods and in consumers (D'Aoust, 2001).

Recently, the emergence of antibiotic resistant *S.* Typhimurium strains, particularly the penta-resistant strain DT104, which is a more virulent than sensitive strain, is troublesome in the United States (Glynn *et al.*, 1998). This strain has been isolated from numerous species of animals, both wild and domesticate, and it is resistant to ampicilin, chloramphenicol, streptomycin, sulfonamides and tetracycline. In addition, there have been reports of resistance to two other antibiotics namely trimethoprim and fluoroquinolones, in Great Britain (Scherer and Miller, 2001).

2.2.4 Salmonellosis in Humans

Most persons infected with *Salmonella* develop diarrhea, fever, and abdominal cramps 12 to 72 hours after infection. Additionally, there may be chills, headache, nausea and vomiting. The illness usually lasts 4 to 7 days, and a majority of persons recover without any treatment. However, in some cases, the diarrhea may be so severe that the patient needs to be hospitalized. In these severe diarrhea patients, the *Salmonella* infection may spread from the intestine to the blood stream, and then to other parts of the body and can cause death unless the person is treated promptly with antibiotics. The elderly, infants, and those with impaired immune systems are more likely to have fatal illness (CDC, 2004).

2.2.4.1 Incidence

Human gastroenteritis is caused by many serotypes of *Salmonella*, the most common of which in the US are *S*. Enteritidis and *S*. Typhimurium (Altekruse *et al.*, 1997). Cases of gastroenteritis are usually due to contamination of food with animal rather than human waste. Undercooked meat, seafood, and eggs are common causes of salmonellosis, although the contamination of fresh produce with animal waste is also a significant problem (Tauxe, 1997).

In the US, approximately 2-4 million cases of *Salmonella* related gastroenteritidis occur per year. Children are the most likely to get salmonellosis. Young children, the elderly and the immuno-compromised people are the most likely to have severe infections. It is estimated that approximately 600 persons die each year with acute salmonellosis, and salmonellosis is more common in the summer than winter (CDC, 2004).

Salmonellosis may occur in small, contained outbreaks in the general population or in large outbreaks in hospitals, restaurants, or institutions for children or the elderly. While the disease is found worldwide, health experts most often report cases in North America and Europe. Every year, CDC receives reports of 40,000 cases of salmonellosis in the United States. The agency estimates that 1.4 million people in this country are infected and that 1,000 people die each year with salmonellosis. *Salmonella* Typhimurium and *Salmonella* Enteritidis are the two most commonly found in the United States (NIAID Fact Sheet, 2005).

2.2.4.2 Transmission

Salmonella bacteria can grow on just about any food, such as meat, poultry, seafood, eggs, and dairy products in particular, as well as vegetables and fruits, such as beans, grains, orange juice, cantaloupe, and sprouts. Food prepared on surfaces that previously were in contact with raw meat or meat products can, in turn, become contaminated with the bacteria. This is called cross-contamination. In recent years, the

CDC has received reports of several cases of salmonellosis from eating raw alfalfa sprouts grown in contaminated soil. *Salmonella* infection frequently occurs after handling pets, particularly reptiles like snakes, turtles, and lizards (NIAID Fact Sheet, 2005).

Infection occurs by ingestion of the organisms in food derived from infected animals or contaminated by the feces of an infected animal or person. This includes raw and undercooked (inadequate time for a given temperature) eggs and egg products, raw milk and raw milk products, contaminated water, meat and meat products, poultry and poultry products. Epidemics may also be traced to foods such as meat and poultry products that have been processed or prepared with contaminated utensils or on work surfaces or tables contaminated in previous use. *S.* Enteritidis infection of chickens and eggs has caused outbreaks and single cases, especially in the Northeastern US and Europe, and is responsible for the majority of cases of this serotype in the US. Temperature abuse of food during its preparation and cross contamination during food handling are the most important risk factors (Washington State Department of Health, 2002).

Salmonellosis can become a chronic infection in some people who may not have symptoms. Though they may have no symptoms, they can spread the disease by not washing their hands before preparing food for others. In fact, health care experts recommend that people who know they have salmonellosis not prepare food or pour water for others until a laboratory tests show they no longer carry *Salmonella* bacteria (NIAID fact sheet, 2005).

2.3 *Salmonella* in chicken meat

2.3.1 Distribution and importance in foods

Infections with bacteria of the genus *Salmonella* are responsible for a variety of acute and chronic diseases in poultry. Infected poultry, moreover, comprise one of the most important reservoirs of salmonellae that can be transmitted through food chains to humans. Isolations of *Salmonella* are reported more often from poultry and poultry products than from any other animal species (Gast, 1997).

Poultry can become colonized by pathogens via drinking water, feed or pecking in contaminated soil or litter (ICMSF, 1998).

Although the proportion of food poisoning outbreak and cases in which the sources of infections can be positively identified is small, poultry and poultry products are repeatedly implicated in human outbreaks. *Salmonella* organisms from poultry sources currently enter the human food chain mainly as a result of carcass contamination from infected fecal material or eggs. Several *Salmonella* serovars have been isolated from poultry. The exact number, however, is difficult to estimate, some serovars may be predominant for a number of years in a region or country.

The distribution of *Salmonella* serotypes from poultry sources varies geographically and changes over time. Although the frequency of isolation of various *Salmonella* serotypes from poultry changes from year to year, several serotypes are consistently found at a high incidence. Based on data from the US Department of Agriculture (USDA) National Veterinary Service Laboratory between July 1990 and June 2003, the most commonly identified serotypes in chickens in the US were (in descending order of incidence) *S*. Heidelberg, *S*. Enteritidis, *S*. Hadar, *S*. Montevideo, *S*. Kentucky and *S*. Typhimurium (Gast, 1997).

2.3.2 *Salmonella* Typhimurium and *Salmonella* Enteritidis in chicken meat

Human illness caused by infection with *Salmonella* enterica serovar Enteritidis (*S*. Enteritidis) increased worldwide beginning as early as the mid-1970s and, by 1990, this serovar displaced *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) as the primary cause of salmonellosis in the world (Bäumler *et al.,* 2000).

Occurrence of food poisoning related to *Salmonella* contaminated eggs and chicken meat has been frequent in humans (Mead *et al.,* 1999). Eating raw or undercooked eggs has also been considered a major risk factor for food poisoning with salmonellae in some situations (Molback and Neiman, 2002). *Salmonella* Enteritidis (SE) and *S.* Typhimurium (ST) are included among the most important paratyphoid salmonellae associated with chicken meat and eggs (Tavechio *et al.,* 2002, Taunay *et al.,* 1996). SE is the most frequently isolated *Salmonella* from poultry products in Brazil (Fiorentin, 2004).

Since the 1990's, a specific type of ST known as a definitive type DT 104 has become a problem in the UK, Western Europe and recently in the US. The primary route by which humans acquire ST infection is by consumption of contaminated food of animal origin. Unlike SE, which is mainly associated with poultry and eggs, multi-drug resistant ST DT104 can be found in a broad range of food. Outbreaks in the UK and Northern Ireland have been linked to poultry and unpasteurized milk (FSRIO, 2005).

ST DT104 is primarily associated with cattle but it has spread to a range of food animals, especially pigs and chickens (IFST, 1997). Recently, there has been little information as to whether foods sourced from other EU countries or elsewhere are also becoming increasingly contaminated with antibiotic-resistant *Salmonella* Typhimurium DT104. However, recently-published US statistics have shown a dramatic increase in the proportion of multi-resistant isolates of all *Salmonella*

Typhimurium from 7% in 1990 to 28% 1995, 83% of the later being *Salmonella* Typhimurium DT104 (Anon., 1997).

In many EU countries the salmonellae that most frequently cause human gastroenteritis are *S.* Typhimurium and, especially in more recent years, *S.* Enteritidis, particularly Phage Type 4 (PT4) (ACMSF, 2001; WHO, 2001). The other serotypes involved in human illness vary geographically but frequently include *S.* Agona, *S.* Hadar, *S.* Heidelberg, *S.* Infantis, *S.* Newport, *S.* Panama, *S.* Saint-paul, *S.* Thompson, and *S.* Virchow (WHO, 2001).

2.3.3 Studies of *Salmonella* in chicken meat in Vietnam and overseas

In Vietnam, there are very few published reports on *Salmonella* contamination in chicken meat as far as we know. A study that focused on chicken and duck showed that the prevalence of *Salmonella* in chicken and duck were 7.9% and 8.7% respectively (Phan *et al.,* 2004). In another study on contamination of *Salmonella* in retail meats and shrimp in the Mekong Delta, Vietnam (Phan *et al.,* 2005) showed that *Salmonella* was isolated from 21.0% of chicken meat samples.

Worldwide, there are many prevalence studies on *Salmonella* in poultry. Table 3 summarizes published reports on prevalence studies in many countries showing a prevalence for *Salmonella* in poultry ranging from 0% to >50%. Both fresh and frozen poultry have been contaminated by this pathogen at significant rates. The serotypes detected tend to be rather similar, with *S.* Enteritidis and *S.* Typhimurium being commonly isolated (Lake *et al.,* 2002).

Table 3: Prevalence of *Salmonella* in poultry and raw poultry products

Country	Samples tested	% positive	Serotype information	Reference
Albania	Chicken	30/461 (6.5)	S. Enteritidis 51.5%, S. Senftenberg 9.7% *S.* Serogroup C 9.7%, *S.* Newport 6.5%, *S.* Abony 3.2%, *S* Agona 3.2%, *S.* Banana 3.2%, *S.* Brancaster 3.2%, *S.* Infantis 3.2%, *S.* Oslo 3.2%, *S.* Serogroup B 3.2%	Beli *et al.*, 2001
Australia (ACT)	Chicken	109/266 (41.0)	*S.* Kiambu 19.4%, *S.* Sofia Subsp. II 58.1%, *S.* Subsp. II rough 2.2%, *S.* Typhimurium untypable 2.2%, *S.* Typhimurium RDNC 1.1%, *S.* Typhimurium 9 2.2%, *S.* Typhimurium 64 5.4%, *S.* Typhimurium 135 6.5%, *S.* Typhimurium 135a 1.1%, *S.* Typhimurium 193 1.1%, *S.* Zanzibar 1,1%	http://www.health. act.gov.au
Belgium	Chicken carcasses	45/133 (33.8)	*S.* Enteritidis 13.3%, Other serotypes 86.7%	Uyttendaele *et al.*, 1999
Spain	Chicken	71/198 (35.8)	*S.* Enteritidis 47.9%, *S.* Hadar 25.4%, *S.* Serotype 4,12:b:-(II) 19.7%, *S.* Mbandaka 2.8%, *S.* Virchow 1.4%, *S.* Derby 1.4%, *S.* Paratyphi B 1.4%	Dominguez *et al.*, 2002
UK	Chicken	74/325 (22.8)	*S.* Enteritidis 42.6%, *S.* Typhimurium 6.5%, Other serotypes 50.9%	Plummer *et al.*, 1995
Ireland	Poultry	28/106 (26.4)	*S.* Bredeney 46.4%, *S.* Kentucky 39.3%, *S.* Enteritidis 7.1%, *S.* London 3.6%, *S.* Schwartzangram 3.6%	Duffy *et al.*, 1999
Malaysia	Chicken portions	13/33 (39.4)	*S.* Blockley 33.0%, *S.* Enteritidis 26.7%, *S.* Chincol 13.3%, *S.* Paratyphi B var Odense 6.7, *S.* Kentucky 6.7, *S.* Welteverden 6.7, *S.* Virchow, 6.7	Arumugaswamy *et al.*,1995
USA	Retail chicken	9/212 (4.2)		Zhao *et al.*, 2001
Vietnam	Chicken meat	21%		Phan *et al.*, 2005
Thailand	Chicken meat	72%		Boonmar *et al.*, 1998

3. MATERIAL AND METHODS

3.1 Study area and period

This research focused on retail markets in Hanoi city. Most of the poultry meat (chicken, goose, and duck) would be sold on the retail market and it is very popular in the north part of Vietnam. The rest was sold in supermarkets around Hanoi. Actually, people still have a habit of buying ready-to-eat food and ready-to-cook food in the retail shops.

Samples collection was divided into two periods: during the first sampling (from December, 2004 to February, 2005) it was winter in the north of Vietnam. The second sampling (from March, 2005 to April, 2005) took place during springtime.

3.2 Sample selection

The sample selection was based on the geographical position. During the study period, Hanoi was divided into 5 urban districts. From each of these districts, 4 markets (in District 1) and 3 markets (in District 2, 3, 4 and 5) were randomly selected for sample collection (Table 4).

Table 4: Sampling frame

Sampling time	District	Market	Shop	n/shop	Total
1st sampling	D1	4	14	5	70
2nd sampling	D2	3	12	4	48
	D3	3	12	4	48
	D4	3	12	4	48
	D5	3	12	4	48
Total					**n = 262**

In each market, 4 shops were conveniently selected and 4 chicken pieces were collected from each shop (5 chicken pieces were collected from each shop in District 1).

3.3 Sampling

The samples were collected in the morning – when the open market started. Three hundred gram chicken breast was purchased and stored at 4^0C. Samples were brought to the laboratory and processed within 6 hours after collection.

3.4 Sample size

The expected prevalence of *Salmonella* in chicken meat in Hanoi was assumed at a percentage of 50%, and in chickens sold per day more than 10.000; a level of confidence of 90% and accepted error of 5% were used in sample size determination. A sample size (n) of 240 was obtained using the program Win-Episcope 2.0 (Dawson and Trapp, 2004).

3.5 Laboratory methods

In this study, we followed ISO 6579 “Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.” and DIN method (Germany), see Figure 1.

There are five steps for the detection of *Salmonella*

- Pre-enrichment
- Enrichment
- Selective culture

- Preliminary confirmation using biochemical tests
- Confirmation by serological testing

Twenty-five grams of each sample was taken from chicken breast by knife and scissors using aseptic technique. Homogeneous samples with Buffer Pepton Water by stomacher machine then samples were incubated at 37^0C for 18h-24 hours.

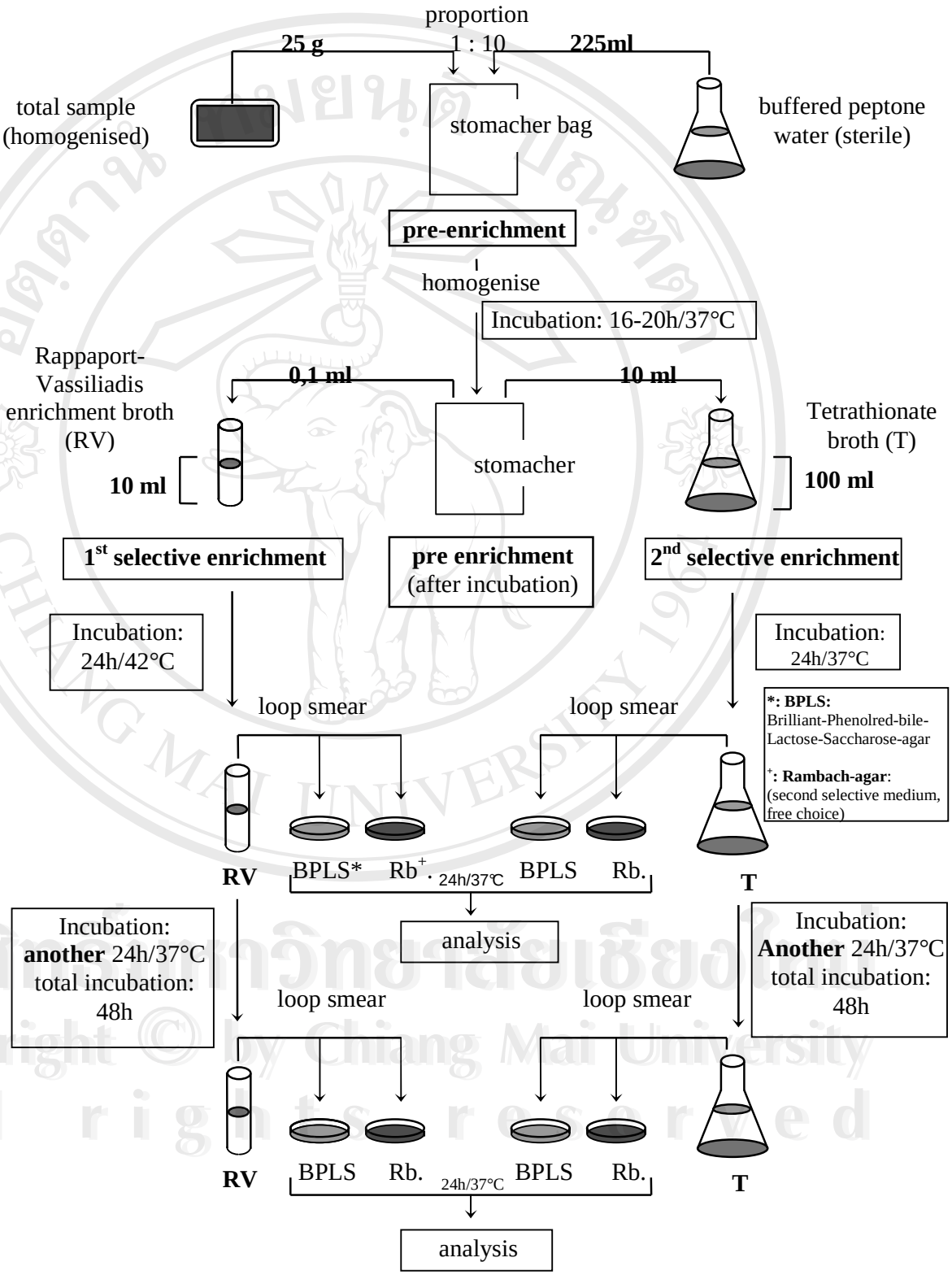
In this study, Rappaport Vassiliadis and Tetrathionate broth were used as selective enrichment media. The pre-enrichment suspension was transferred from the stomacher bag to Rappaport Vassiliadis (0,1ml) and Tetrathionate broth (10ml). Rappaport Vassiliadis was incubated at 42^0C, while Tetrathionate broth was incubated at 37^0C.

Then, from the selective enrichment broth, a loop of the inoculum was transferred to a selective agar. In this case, Rambach agar and XLT4 agar (Xylose Lysine Turgitol 4 Agar) were used. Both were incubated at 37^0C /18h-24 hours.

Salmonella colonies in the XLT4 agar showed black colour on orange-coloured agar and *Salmonella* colonies in the Rambach agar showed red colour on pink agar.

From each plate, up to 5 colonies with *Salmonella* characteristics were transferred to nutrient agar for further test.

Figure 1: Flow of *Salmonella* Chart Conventional Culture Methods



Biochemical confirmation

The Kligler Iron Agar (KIA) was used. Kligler iron agar is based on double sugar fermentation and hydrogen sulphide production. Smear the surface of a Kligler Iron Agar slope and stab the butt with a colony picked off one of the solid media. There are three reactions to record when interpreting a KIA tube, reactions after 18 - 24 hours at 35°C (Oxoid, 2004).

1- Carbohydrate utilization

(i) slant reaction	**(ii) butt reaction**
acidity: yellow colour	*acidity*: yellow colour
alkalinity: red colour	*alkalinity*: red colour

2- Gas production

aerogenic	**anaerogenic**
bubbles or splitting of agar	no gas production

3- H_2S production: Blackening in whole or part of butt

Table 5: Kligler reactivity of *Salmonella*

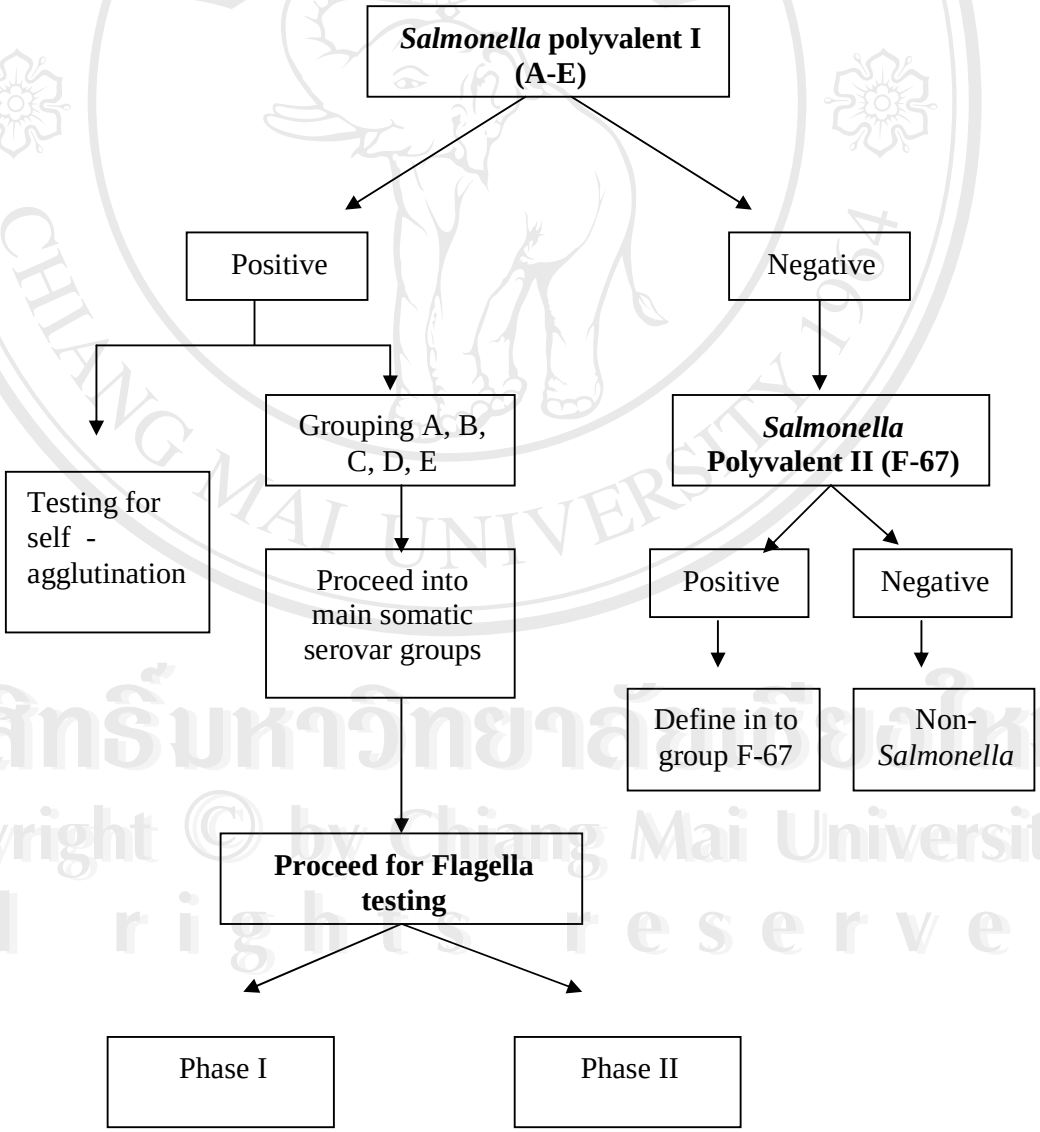
Organism	**Slope**	**Butt**	**Gas**	**H_2S**
Shigella sonnei	red	yellow	-	-
Shigella dysenteriae	red	yellow	-	-
Salmonella typhi	red	yellow	-	+
Salmonella species	red	yellow	+	+
Enterobacter species	red	yellow	+	-
Klebsiella species	yellow	yellow	+	-

V = variable, + = positive, - = negative.

Serological confirmation

All isolates growing on solid media indicating to be *Salmonella* and showing a reaction on KIA as *Salmonella* were serotyped by agglutination according to the Kauffmann-White scheme, using *Salmonella* polyvalent I, II (A-E; F-67). Then, typing was completed with *Salmonella* somatic antigen (O) and *Salmonella* flagella antigen (H).

Figure 2: Serological procedure



3.6 Information from the questionnaire survey

Information about possible risk factors was collected using a questionnaire. The author collected all of the information. Thirteen factors were obtained as follows (Table 6).

Table 6: List of Factors

List	Factors	Description
1	Chicken/source	Source of chicken
2	Chicken/slaughter by	How the chicken was slaughtered
3	Chicken/eviscerated	How the chicken was eviscerated
4	Water/source	Source of water
5	Water/chlorinate	Was water chlorinated?
6	Water/storage	How water was stored
7	Shop/knife	Number of knives used in shop
8	Shop/chopper	Number of choppers used in shop
9	Shop/worker	Number of workers in shop
10	Shop/surface	Type of table surface (where the chicken was placed on)
11	Hygiene/market	The hygiene status of market
12	Hygiene/shop	The hygiene status of shop
13	Hygiene/human	The hygiene status of workers

3.7 Data management and data analysis

Field, laboratory and questionnaire data were managed using MS excel. Databases were prepared for each type of data and later merged into one. Chi-square (corrected/Pearson) test was used to compare the prevalence of *Salmonella* according to seasons, districts and shops within markets.

Univariate analysis was performed to relate the potential risk factors, (derived from the questionnaire responses), to *Salmonella* outcomes (present or not present) in samples and shops. The categorical variables were analyzed using the Chi-square (Corrected/Pearson) test while the continuous variables were assessed using either the T-Test or ANOVA depending on the factor levels. Overall, 13 risk factors (Appendix B) were used in this study. Factors that showed significant ($P<0.1$) association with the outcome were offered for multivariate analyses. The two multivariate analyses used were linear multiple regression and logistic regression. A backward elimination algorithm was used for the parameter estimation in the final model.

4. RESULTS

4.1 *Salmonella* isolation

A total of 262 samples of chicken meat from 62 shops in 16 markets in 5 districts of Hanoi were collected for *Salmonella* isolation. Of these samples, 128 were positive for *Salmonella* giving an overall sample prevalence of 48.9% (Table 7). Seasonally, 41.43% of the samples gathered during winter were positive while 51.56% of spring samples were positive for *Salmonella*. However, these two seasonal proportions were not significantly ($p = 0.1894$) different.

Numerically, the percent of district-specific *Salmonella* contamination was different with the highest recorded in district 2 (62.5%) and the lowest in district 4 (37.5%). No statistically significant difference was observed among proportions ($p=0.0698$) (Table 7).

Similarly, the different markets had different *Salmonella* percent contamination levels. The highest proportion (81.2%) was recorded in Market 2 (M2) located in District 2 (D2) and the lowest (30%) in Market 4 (M4) in District 1 (D1). Nevertheless, there was no significant difference among the proportions of *Salmonella* contamination among and within markets in each district (Table 7).

Table 7: Proportion of *Salmonella* positive sample

Prevalence of *Salmonella* contaminated			**n**	**No. of positive**	**Percent**	**P-value**
Overall			262	128	48.9	
By season						
- Winter time			70	29	41.43	**p=0.1894**
- Spring time			192	99	51.56	
By districts (n=5)						
- D1			70	29	41.42	
- **D2**			48	30	**62.5**	**p=0.0698**
- D3			48	27	56.25	
- **D4**			48	18	**37.5**	
- D5			48	24	50	
By markets in district	**D1**	M1	20	10	50	**p=0.7584**
		M2	20	8	40	
		M3	20	8	40	
		M4	10	3	**30**	
	D2	M1	16	10	61.2	**p=0.0907**
		M2	16	13	**81.2**	
		M3	16	7	43.7	
	D3	M1	16	11	68.7	**p=0.4667**
		M2	16	8	50	
		M3	16	8	50	
	D4	M1	16	6	37.5	**p=0.7659**
		M2	16	7	43.7	
		M3	16	5	31.2	
	D5	M1	16	8	50	**p=0.7788**
		M2	16	9	56.2	
		M3	16	7	43.75	

(D= District; M= Market)

Of the 62 shops participating in the study, there was one shop with 100% percent *Salmonella* contamination (D2 M2 S1) and one shop with no *Salmonella* contamination (D4 M3 S1) (Table 8).

Table 8: Proportion of *Salmonella* positive samples by shop

Market	**Shop**	**District**				
		D1**	D2*	D3*	D4*	D5*
M1	S1	40	75	75	25	50
	S2	40	75	50	50	50
	S3	60	50	75	25	50
	S4	60	50	75	50	50
M2	S1	40	**100**	50	50	50
	S2	60	75	75	25	50
	S3	40	75	50	75	50
	S4	20	75	25	25	75
M3	S1	20	25	50	**0**	25
	S2	80	50	50	50	50
	S3	40	25	25	25	25
	S4	20	75	75	50	50
M4	S1	40	-	-	-	-
	S2	20				

D= District; M= Market; S= Shop

* 4 samples per shop

** 5 samples per shop

4.2 Serogroups and serotypes

A total of 128 *Salmonella* positive samples were tested for sero-grouping using polyvalent antisera I and II. Out of these samples 129 isolates (Table 9) were obtained (2 isolates from sample 44- D2M1S2). All the 129 *Salmonella* isolates belonged to 5 somatic groups. The main somatic groups were B (42.6%), C (27.9%) and E (25.6%).

Table 9: Serogroups of *Salmonella* isolated from chicken meat

Group	No. of isolates in group	Percent (%)
Group B	55	42.6
Group C	36	27.9
Group E	33	25.6
Group D	2	1.6
Group F-67	3	2.3
Total	**129**	**100**

Table 10 shows that members of *Salmonella* group B were most frequently found in the Districts 1, 4 and 5 in the following descending order: 100% in D4, 58.33% in D5 and 48.28% in D1. *Salmonella* group B was found in all markets in D1, D4 and D5. In particular, this serogroup accounts for the majority of isolates that were isolated from all markets of District 4 (100%), following by Market 4 (D1) and Market 2 (D5) with 66.7%

Whereas the most commonly found isolates in D2 and D3 were *Salmonella* Group C (54.84%) and E (48.14%), respectively. Within D2, *Salmonella* group C was found with the highest percentage of 71.44% of isolates from M3. Similarly, in D3, *Salmonella* group E accounts for 75% of isolates from M2.

Table 10: *Salmonella* serogroups distributed by market and district

Districts	Markets	Group B		Group C		Group E		Group D		Polyvalent II		Total
		n	%	n	%	n	%	n	%	n	%	
D1	M1	3	30	3	30	4	40					10
	M2	4	50	3	37.5	1	12.5					8
	M3	5	62.5	2	25	1	12.5					8
	M4	2	66.7			1	33.33					3
	Σ	**14**	**48.28**	**8**	**27.8**	**7**	**24.14**					**29**
D2	M1	2	18.18	5	45.45	3	27.27			1	9.09	11
	M2			7	53.85	6	46.15					13
	M3	1	14.28	5	71.44	1	14.28					7
	Σ	**3**	**9.67**	**17**	**54.84**	**11**	**35.48**					**31**
D3	M1	1	9.09	5	45.45	5	45.45					11
	M2			1	12.5	6	75	1	12,5			8
	M3	5	62.5			2	25	1	12,5			8
	Σ	**6**	**22.22**	**6**	**22.22**	**13**	**48.14**	**2**	**7.41**			**27**
D4	M1	6	33.3									6
	M2	7	38.9									7
	M3	5	27.8									5
	Σ	**18**	**100**									**18**
D5	M1	5	62.5	2	25	1	12.5					8
	M2	6	66.67	1	11.11	1	11.11			1	11.11	9
	M3	3	42.86	2	28.56	1	14.28			1	14.28	7
	Σ	**14**	**58.33**	**5**	**20.83**	**3**	**12.5**			**2**	**8.33**	**24**

Most (67.74%) of the shops were contaminated with *Salmonella* of Group B (Table 11). However, only 3.22% of the shops were contaminated with S*almonella* belonging to Group D and 4.84% shops had *Salmonella* of Group F-67. As the table shows, 40.31% shops were contaminated with two serogroups of *Salmonella* and 8.06% with three serogroups.

Table 11: Distribution of *Salmonella* serogroups by shops (n=62)

Serogroups	Number of shops/ sero-group	Percent
Group B	42	67.74
Group C	26	40.625
Group E	24	38.7
Group D	2	3.22
Group F-67	3	4.84
Two groups		
Overall	**25**	**40.31**
B + E	7	11.29
B + C	7	11.29
C + E	8	12.9
C + F-67	1	1.61
E + D	2	3.23
Three groups		
Overall	**5**	**8.06**
B + C + E	3	4.84
B + E + F-67	1	1.61
B + C + F-67	1	1.61

Table 12 shows the distributions of the numbers of isolates of each *Salmonella* serotype by district and market. Overall, twelve serotypes were identified from 129 isolates. Most (31.01%) isolates were *S.* Agona, followed by *S.* London (18.6%) and *S.* Emek (17.83%). Other serotypes of *Salmonella* detected belong to *S.* Typhimurium (7.75%), *S.* Brunei (6.2%), *S.* Senftenberg (3.87%), *S.* Derby (3.87%), *S.* Weltevreden (3.1%), *S.* Haardt (3.1%), somatic group F-67 (2.33%), *S.* Enteritidis (1.55%), and *S.* Newport (0.78%).

There was only one serotype distributed in District 4 (*S.* Agona), whereas eight serotypes were distributed on District 5. *S.* Enteritidis (two isolates) and *S.* Typhimurium (10 isolates) were found only in D3 and D1, respectively.

S. Agona was found in all markets of D4 and D5. *S.* London was detected in all markets of D2 and D3. *S.* Emek was found in all markets of D2. However, these serotypes were not found in D1.

Similarly, *S.* Typhimurium and *S.* Senftenberg were found in all markets of D1 only (in the winter time), and are meanwhile not found in other districts (in the spring time). In addition, the *S.* Newport serotype was detected only in M1 of D5.

Table 12: Number of isolates in each serotype of *Salmonella* by Markets and Districts

SEROTYPES	Group	D1				D2			D3			D4			D5			Total	Percent
		M1	M2	M3	M4	M1	M2	M3	M1	M2	M3	M1	M2	M3	M1	M2	M3	n	%
S. Agona	B					2		1	1		5	6	7	5	5	6	2	**40**	**31.01**
S. London	E					3	6	1	5	6	1				1	1		**24**	**18.6**
S. Emek	C					5	5	4	5	1					1		2	**23**	**17.83**
S. Typhimurium	B	3	2	4	1													**10**	**7.75**
S. Brunei	C	3	3	2														**8**	**6.2**
S. Senftenberg	E	2	1	1	1													**5**	**3.87**
S. Derby	B		2	1	1												1	**5**	**3.87**
S. Wetevreden	E	2									1						1	**4**	**3.1**
S. Haardt	C						2	1								1		**4**	**3.1**
S. F-67	F-67					1										1	1	**3**	**2.33**
S. Enteritidis	D									1	1							**2**	**1.55**
S. Newport	C														1			**1**	**0.78**
No. of serotypes		**4**	**4**	**4**	**3**	**4**	**3**	**4**	**3**	**3**	**4**	**1**	**1**	**1**	**4**	**4**	**5**		**100**
No. of isolates		**10**	**8**	**8**	**3**	**11**	**13**	**7**	**11**	**8**	**8**	**6**	**7**	**5**	**8**	**9**	**7**	**129**	

D= District; M= Market;

4.3 Results from the questionnaire

4.3.1 Shop level

The distributions of proportions of *Salmonella* contaminations per levels of each risk factor and number of shops are shown in Table 13. Eight of 13 factors were significantly associated with *Salmonella* proportions in the univariated analysis.

Summary results of the multiple linear regression analysis are shown in Table 14. The results indicate that “number of knives used” was marginally (p= 0.0632) associated with *Salmonella* contamination.

However, it should be noted that the number of shops which used only one knife were twice the number of shops that used more than one knife (table 13). But the mean prevalence was higher (53.3) than those (40.75) that used more than one knife. These two mean proportions were significant (p=0.0235) at the univariate analytical level.

In addition, the proportion of *Salmonella* contamination in shop was significantly (p<0.0001) associated with the level of “The hygiene status of shop”, whether the shop hygiene level is clean or dirty.

Table 13: Summary results of univariate analysis of potential risk factors for *Salmonella* contamination in chicken shops (continuous variable)

Factors	Level	No. of shop	Mean of proportion	P-value
Chicken/source	Household	55	47.818	0.12037
	Farm	7	60.714	
Chicken/slaughter by	Others	18	50.833	0.7069
	Retailer	44	48.636	
Chicken/eviscerated	at home	46	49.782	0.8293
	at retail	16	47.8125	
Water/source	Well	21	59.048	0.0482
	Tap	41	44.268	
Water/chlorinate	No	21	57.857	0.0178
	Yes	41	44.878	
Water/storage	Closed	2	62.5	0.3612
	Open	60	48.833	
Shop/knife	>1	20	40.75	0.0235
	=1	42	53.333	
Shop/chopper	>1	17	36.765	0.0026
	=1	45	54	
Shop/worker	>1	27	44.63	0.1205
	=1	35	52.857	
Shop/surface	Ceramic	3	26.666	0.0142
	Stainless	40	46.125	
	Steel	8	56.25	
	Wood	11	61.818	
Hygiene/market	Dirty	54	51.296	0.0441
	Clean	8	35.625	
Hygiene/shop	Dirty	34	62.941	<0.0001
	Clean	28	32.678	
Hygiene/human	None	25	59	0.0017
	Apron	37	42.702	
	Mask	0		
	Glove	0		

Table 14: Variables in final model of Multivariate analysis of risk factors associated with proportion of *Salmonella* contamination in shops

Factors	P-value
Shop/knife	0.0632*
Hygiene/shop	<0.0001

*significant at p = 0.1000

4.3.2 Sample level

Number of *Salmonella* positive samples in each level of risk factor is shown in Table 15. There were seven out of 13 factors that were significantly (p= 0.1000) associated with sample prevalence in univariate analysis.

Table 15: Summary results of the assessment of associations between sample prevalence of *Salmonella* with potential risk factors (univariate analysis)

Factors	Level	No. of sample examined	n (+)	n (-)	% (+)	*P-value**
Chicken/source	Household	234	113	121	48.29	0.2927
	Farm	28	17	11	60.71	
Chicken/ slaughter by	Others	73	37	36	50.68	0.9388
	Retailer	189	93	96	49.2	
Chicken/ eviscerated	at home	197	99	98	50.25	0.8297
	at retail	65	31	34	47.69	
Water/source	Well	90	54	36	60	0.0214
	Tap	172	76	96	44.186	
Water/ chlorinated	No	90	53	37	58.88	0.0413
	Yes	172	77	95	44.76	
Water/storage	Close	8	5	3	62.5	0.7032
	Open	254	125	129	49.21	
Shop/knife	>1	85	35	50	41.18	0.0781
	= 1	177	95	82	53.67	
Shop/ chopper	>1	72	27	45	37.5	0.0228
	= 1	190	103	87	54.21	
Shop/worker	>1	111	50	61	45.04	0.2525
	= 1	151	80	71	52.98	
Shop/surface	Ceramic	15	4	11	26.66	0.0908
	Stainless steel	164	76	88	46.34	
	Steel	36	20	16	55.55	
	Wood	47	30	17	63.82	
Hygiene/market	Dirty	228	118	110	51.75	0.1081
	Clean	34	12	22	35.29	
Hygiene/shop	Dirty	142	91	51	64.08	<0.0001
	Clean	120	39	81	32.5	
Hygiene/human	None	117	69	48	58.97	0.0094
	Apron	145	61	84	42.06	
	Mask					
	Glove					

*P-value from Chi-square test

Of the seven factors, only four were found significantly ($p<0.05$) associated with the sample prevalence (Table 16). Four factors associated with sample prevalence of *Salmonella* were "number of knives used", "number of choppers used", "type of table surface" and "the hygiene status of shop".

Notably, the odds ratios of the number of choppers per shop, type of table surface (steel, stainless steel and wood) in the shop were greater than one. Thus they were strongly associated with the presence of *Salmonella* in the samples.

Table 16: Logistic regression of the risk factors associated with sample prevalence of *Salmonella*

Factors	Level	OR	P-value	95% CI
Shop/knife	>1	1	-	0
	=1	0.456347819	<0.001	[-1.0668, 0.3262]
Shop/chopper	>1	1		0
	= 1	2.150069141	<0.001	[0.4082, 1.1228]
Shop/surface	Ceramic	1	-	0
	Stainless steel	1.771629	0.0002	[0.2693, 0.8745]
	Steel	2.01980	0.0016	[0.2652, 1.1407]
	Wood	2.552568	0.0002	[0.4525, 1.4218]
Hygiene/shop	Dirty	1	-	0
	Clean	0.313893978	<0.001	[-1.5045, -0.8130]

Note:

OR = Odds ratio

OR = 1: no association exits between presence of *Salmonella* and factor

OR > 1: the factor is positively associated with the presence of *Salmonella* (risk factor)

OR < 1: the factor is negatively associated with the present of *Salmonella* (protective factor)

5. DISCUSSION AND CONCLUSIONS

5.1 Discussion

5.1.1 The aim of the study

It was the aim of this study to get a picture of *Salmonella* from highly populated urban/ suburban areas of South East Asia, where overlapping production/ stocking of animals as well as consumption would be observed. Here, data on the prevalence of salmonellae in chicken meat ready for selling was obtained from popular markets. In the different districts, a different pattern was obtained, possibly reflecting a different origin of the birds and the products.

5.1.2. Aspects of sampling

There were 262 samples taken from 16 markets in 5 districts of the capital of Hanoi. A total of 62 shops were visited, offering pieces for sale according to the convenience of the customers. During sampling, the samples were kept in plastic bags. The samples were investigated for their presence (presence/ absence test). A quantitative result was not intended.

5.1.3 Level of contamination

In this study, the prevalence of *Salmonella* in chicken meat from retail markets in Hanoi was 48.9%. The results are comparable to the findings reported in the US (Bokanyi, 1990) with 43% of broiler carcasses being contaminated with *Salmonella* or with results from Spain with 60% (Carraminana *et al.*, 1997) or Portugal (Antunes, 2003), 36% in Malaysia (Rusul *et al.*, 1996) and 34% in Belgium (Uyttendaele *et al.*, 1999).

However, in studies from other countries, the prevalence of *Salmonella* in chicken meat was lower than here: 8% in Albania (Beli *et al.*, 2001), 25% in the UK (Jorgensen *et al.*, 2002), 26% in Ireland (Duffy *et al.*, 1999), 16.4% in Austria (Mayrhofer *et al.*, 2004), 15% Denmark (Bager, 2000), 5.7% in UK (Food standard agency, 2001). Moreover, this study shows a lower prevalence of *Salmonella* in chicken meat when compared with countries such as Thailand with 72% (Boonmar, 1998) and Greece with 69% (Arvanitidou *et al.*, 1998).

For Vietnam, there are only a few reports on the prevalence of *Salmonella* in chicken meat. A study from the south part of Vietnam shows that 21% of the chicken meat samples were positive with *Salmonella* (Phan *et al*., 2005).

5.1.4 The Districts, Markets and Shops

In samples of one of the shops visited (District 4), no *Salmonella* was found, and at one of the shops visited (District 2), 100% of the samples were *Salmonella* positive. The high percentage of positive samples in some markets confirms the major role of salmonellae in poultry products, which had been expected from the production and marketing patterns in these markets.

However, there is still a difference: from District 4, a uniform pattern was obtained (*S.* Agona), which should be scrutinized more thoroughly. Possibly, the results reflect the same origin of the raw material or a sort of “market flora”. Also, the percentage of positive samples was quite different: in District 2, the highest percentage (62.5 %) and District 4, the lowest percentage (37.5 %) was obtained. The hygienic status of the shops promotes the transfer of salmonellae, once they are in or on the birds.

All of *Salmonella* Typhimurium (10 isolates) have been found in District 1 during winter time. On the other hand, some serotypes were common in the spring time - *S.* Agona. *S.* Emek or *S.* London could not be found during the winter time.

5.1.5 The serotypes

Mainly *S.* Agona (group B), *S.* Emek (group C), *S.* London (group E), and *S.* Typhimurium (group B) were obtained. *S.* Agona (31%) has been obtained most frequently in this study. In a similar study, Phan *et al.*, (2005) collected samples from different species from markets in the Mekong Delta, Vietnam. Predominant serotypes were *S.* Weltevreden (group E), *S.* Derby (group B), *S.* London (group E), *S.* Lexington (group E) and *S.* Tennessee (group C). Isolates from chicken meat were more broadly distributed, in this study among them *S.* Emek (group C), *S.* Typhimurium (group B) and *S.* Dessau (group E).

Data from the EU clearly show a different pattern of *Salmonella* serotypes: From the Zoonoses Report (EC, 2005), the range of predominant serotypes was *S.* Enteritidis (group D), *S.* Typhimurium (group B), *S.* Saintpaul (group B), and *S.* Heidelberg (group B). Also, in the EU, a higher proportion of group D types were obtained.

Salmonella Enteritidis (SE) and *Salmonella* Typhimurium (ST) are known as the most important non-typhoidal salmonellae associated with chicken meat and eggs (Taunay *et al.*, 1996). Many studies indicate a high prevalence of *S.* Enteritidis: 44% in Portugal (Antunes, 2003), 28% in Thailand (Boonmar, 1998), 54.35% in Austria (Mayrhofer *et al.*, 2004). But, in this study, *S.* Enteritidis was isolated only is 1.55% of isolates.

In Germany, the sero- pattern is different from the data obtained here; much more of Group D (*S.* Enteritidis 58%) was isolated, followed by Group B (*S.* Typhimurium 28%) (SIFIN, 2000).

From the different seropattems, it is concluded that serovares from chicken for international trade should be investigated in order to get a picture of upcoming global strains.

5.1.6 Geographic and local aspects

In the northern part of Vietnam, there are four seasons (summer, winter, spring and autumn). In the winter time, the temperature is low, cold and humid; during the first sampling from December, 2004 to January, 2005, the temperature was at a range 13-18^{0}C. The second sampling (spring time) from March to April 2005, the temperature was at 20-25^{0}C. The proportion of *Salmonella* contaminated in winter time was lower (41.43%) than in spring time (51.56%). However, the different contaminated proportion was not significant.

At present, there is no modern chicken processing line in Hanoi yet. Most of the poultry is slaughtered by the retailer. Others would be slaughtered in some wholesale chicken market. This might explain why the prevalence of *Salmonella* contamination in chicken meat in Hanoi is high.

5.1.7 Risk factor

The results from the questionnaire show that several factors can be considered risk factors, which increase the risk of presence of *Salmonella,,* such as chicken source, hygiene status, and shop surfaces. This study indicates that the “number of knives used”, “number of choppers used”, “hygiene status of shop” and “type of table surface” were significant risk factors of *Salmonella* contamination in chicken. Odds ratios showed the strong relation of exposure and a presence of *Salmonella.*

Distribution and trade patterns on the markets support the spread of salmonellae from the place of origin via markets to the consumers.

Finally, the high percentage of positive samples in some markets in an urban area in Vietnam confirms the major role of salmonellae in poultry products, which was to be expected from the production and marketing patterns on these markets.

5.2 Conclusions

262 samples from chicken meat in Hanoi, Vietnam were investigated for salmonella. The contamination rate of *Salmonella* was 48.9%. Season, district and market were not significantly associated with contamination of the poultry meat.

The main somatic group pattern was B (43 %), C (28 %) and E (26 %), predominant serotypes were *S.* Agona, S. Emek, *S.* London.

The proportions of *S.* Enteritidis and *S.* Typhimurium contamination were low 1.55% and 7.75%, respectively.

Some handling pattern ("Number of knives used", "Number of choppers used") as well as several aspects ("Hygiene status of shop" and "Type of table surface") were significant risk factors of *Salmonella* contamination.

The time of data collection represented only a short duration, the sample size was small. So, the data cannot stand for the prevalence in the entire area of the capital of Hanoi. However, these data may reflect other areas in Hanoi as well.

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APPENDICES

APPENDIX A: EQUIPMENTS, MATERIALS, MEDIA AND REAGENTS

Equipments and Materials

- Sterile 100, 200, 500, 1000, 2000 and 5000ml Erlenmeyer flasks
- Electric balance
- Incubator, 37^0C and 42^0C
- Refrigerator 4^0C
- Water bath
- Eppendoff tube 1,5µl
- Vortex mixer
- Sterile scissors, forceps, knifes, spoons
- Bunsen burner
- Autoclave
- Plastic bag, cotton pad, Aluminum foil
- Sterile plastic plate, plastic loop

Media and reagents

- Buffered Pepton Water (Merck - Germany)
- Rappaport – Vasilliadis medium (RV) (Merck - Germany)
- Tetrathionate broth (Merck - Germany)
- Kligler agar (Merck - Germany)
- Xylose Lysine Turgulor 4 Agar (XLT4) (Merck - Germany)
- Nutrient agar (Merck - Germany)
- Rambach Agar (Merck - Germany)
- Ethanol 70% and 90% (Vietnam)
- Sterile distilled water (Vietnam)
- NaCl (Sigma - US)
- Gram staining set (OXOID – UK)
- Salmonella polyvalent antiserum: I, II (SIFIN - Germany)
- Salmonella somatic antiserum (SIFIN - Germany)
- Salmonella flagella antiserum (SIFIN - Germany)

APPENDIX B: QUESTIONNAIRE FORM

QUESTIONNAIRE

Questionnaire number ……

Isolation and Identification *Salmonella* from chicken meat in Hanoi – Vietnam

No.	Item	Question		Response	Code
1	**Collection**	1.1	**Time**		
		1.2	**Collector**		
2	**Retailer**	2.1	**Name**		
		2.2	**Market**		
		2.3	**District**		
3	**Chicken**	3.1	**Source**	□ Farm □ Household	
		3.2	**Slaughter by**	□ Retailer □ Others	
		3.3	**Eviscerated**	□ At retail □ At slaughter	
4	**Water**	4.1	**Source**	□ Tap □ Well	
		4.2	**Chlorinated**	□ Yes □ No	
		4.3	**Storage**	□ Open □ Close	
5	**Shop**	5.1	**Number of knives**	□ = 1 □ >1	
		5.2	**Number of choppers**	□ = 1 □ >1	
		5.3	**Number of workers**	□ = 1 □ >1	
		5.4	**Table surface**	□ Wood □ Steel □ Stainless □ Ceramic	
6	Hygiene	6.1	**Market hygiene**	□ Clean □ Dirty	
		6.2	**Shop hygiene**	□ Clean □ Dirty	
		6.3	**Human hygiene**	□ Glove □ Mask □ Apron □ None	

CURRICULUM VITAE

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I, the undersigned, declare that the thesis is my original work and has not been presented for a degree at any University.

Name **LUU QUYNH HUONG**

Signature …….

Date of Submission ……...