# PREVALENCE OF *SALMONELLA* AND *CAMPYLOBACTER SPP.* FROM BROILER MEAT IN ABATTOIRS AT HO CHI MINH CITY, VIETNAM

VO NGOC BAO

MASTER OF SCIENCE
IN VETERINARY PUBLIC HEALTH



CHIANG MAI UNIVERSITY AND FREIE UNIVERSITÄT BERLIN
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VO NGOC BAO

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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EXAMINING COMMITTEE

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Assoc.Prof. Dr. Renu Pinthong



23 September 2005

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|---|---|
| **Thesis Title** | Prevalence of *Salmonella* and *Campylobacter spp.* from Broiler Meat in Abattoirs at Ho Chi Minh City, Vietnam |
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| **Degree** | Master of Science (Veterinary Public Health) |
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**ABSTRACT**

Over the past 20 years poultry meat production worldwide has increased rapidly with an annual growth rate of 6%. In Ho Chi Minh City, the animal husbandry has rapidly developed, especially in poultry production. The increase has been in both the number the farms and flock sizes. Fifty five poultry abattoirs are operated in this city. This enables poultry processors to slaughter large number of animal. However, there was very little information about the contamination of *Salmonella* in broiler carcasses. Similarly, there was paucity of data about *Campylobacter* in broiler meat. Poultry and poultry products are important vehicles of food- born illnesses in humans, especially salmonellosis and campylobacteriosis.

Therefore, this study was done to establish the prevalence of *Salmonella* and *Campylobacter* spp. in chicken carcasses in 15 abattoirs (large and small). Abattoir were categorized as large if the daily slaughter was between 1200- 2000 chickens, and small if less than 1200 chickens. From November 2004 to May 2005, 319 chicken carcass- rinse samples were collected. All were examined for the presence of *Salmonella* and *Campylobacter*. The samples were obtained from the final product at the inside –outside shower stage of the slaughter processing and were collected using the procedure described in USDA (2002). [Briefly, the carcass was put into a plastic bag (30 cm ×60 cm) and four hundred ml of Buffered Peptone Water (Oxiod, CM 509) was added into the bag. The isolation procedure followed ISO and serotyping identification for *Salmonella* followed the instruction from manufacture (Sifin,

Germany)]. Out of 319 samples, 136 chicken carcasses were *Salmonella*- positive giving a prevalence of 42.63%. In the small abattoirs a prevalence of 47.96% was abtained, while, in large abattoirs a prevalence of 34.15% was recorded. These two proportions were different (p = 0152). Overall, *S.* Emek (33.3 %), *S.* Haardt (18.42%), *S.* Typhimurium (7.89%), and *S.* London (7.02%) were the most prevalent serotypes. Nine *Salmonella* isolates of *S.* Typhimurium were found in five abattoirs.

*Campylobacter* spp. was isolated from 35.11% of the 319 chicken carcasses. The occurrence *Campylobacter* spp. was marginally higher (36.58%) in the large abattoirs than in the small abattoirs (34.18%) (p= 0.6618). Overall, the combined proportion of the occurrence of *Salmonella* and *Campylobacter* in 319 chicken carcasses was 17.87%. In conclusion, presence of *Salmonella* and *Campylobacter* spp. in chicken carcasses pose potential sources of foodborne hazards to humans. Therefore, based on these findings it is strongly recommended that effective hygienic standards along the poultry slaughter line be implemented. In addition, further studies should be designed to establish the specific critical points in whole poultry production chain (farm to table).

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| ชื่อเรื่องวิทยานิพนธ์ | ความชุกของเชื้อซัลโมเนลลาและเชื้อแคมไพโรแบคเตอร์จากเนื้อไก่ในโรงฆ่าสัตว์ที่นครโฮจิมินท์ ประเทศเวียดนาม |
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**บทคัดย่อ**

ในช่วง 20 ปีที่ผ่านมานี้ ได้มีการผลิตผลิตภัณฑ์สัตว์ปีกเพิ่มขึ้นทั่วโลกอย่างรวดเร็วในอัตราการเจริญเติบโต 6 % ที่เมืองโฮจิมินมีการเลี้ยงสัตว์ปีกเพิ่มขึ้นอย่างรวดเร็ว มีการเพิ่มทั้งจำนวนฟาร์มและขนาดฝูงสัตว์ มีโรงฆ่าสัตว์ 55 แหล่งในเมืองนี้ ทำให้สามารถชำแหละสัตว์ปีกได้เป็นจำนวนมาก อย่างไรก็ตามยังมีข้อมูลการปนเปื้อนจาก Salmonella และ Campylobacter ในเนื้อไก่กระทง (Broiler) น้อยมาก

สัตว์ปีกและผลิตภัณฑ์จากสัตว์ปีกเป็นพาหะสำคัญในการนำโรคทางเดินอาหารมาสู่คน โดยเฉพาะโรค Salmonellosis และ Campylobacteriosis

การศึกษานี้ได้ทำการตราวจหา Salmonella และ Campylobacter spp. ในซากไก่จากโรงฆ่าสัตว์ขนาดเล็ก 5 แห่ง และขนาดใหญ่ 10 แห่ง โรงฆ่าสัตว์ที่ชำแหละไก่จำนวน 1,200-2,000 ตัว เป็นประจำทุกวันจัดเป็นโรงฆ่าสัตว์ขนาดใหญ่ และ ถ้าชำแหละเนื้อไก่วันละต่ำกว่า 1,200 ตัวจัดว่าเป็นโรงฆ่าสัตว์ขนาดเล็ก ได้ทำการเก็บน้ำล้างไก่ทั้งตัวเพื่อทำการตราวจหา Salmonella และ Campylobacter spp. ตั้งแต่เดือนพฤศจิกายน 2004 ถึงพฤษภาคม 2005 จำนวน 319 ตัวอย่าง ได้เก็บตัวอย่างผลิตภัณฑ์ไก่สดทั้งตัว หลังจากได้ผ่านขั้นตอนการล้างโดยการพ่นน้ำครั้งสุดท้ายตามวิธีของ USDA (2002) โดยนำซากไก่แต่ละตัวใส่ถุงพลาสติกขนาด (30ซ.ม.* 60ซ.ม.) แล้วเติมน้ำบัฟเฟอร์เปปโตน (Oxford, CM509) ลงไปจำนวน 400 มิลลิลิตรในถุง ทำการตรวจเชื้อ Salmonella ตามวิธี ISO และทำการแยกชนิด ซีโรวาร์(Serotyping) ตามวิธีของบริษัทซิฟิน (Sifin, Germany) ประเทศเยอรมัน ผลปรากฏว่าจากจำนวนตัวอย่าง ทั้งหมด 319 ตัวอย่าง พบซากไก่จำนวน 136 ตัวอย่าง หรือ 42.63 % ปนเปื้อน Salmonella โดยพบตัวอย่างจากโรงฆ่าสัตว์ขนาดเล็กปนเปื้อน Salmonella 47.96 % ตัวอย่างจากโรงฆ่าสัตว์ทั้ง 2 ขนาดมีความแตกต่างกัน (p=0.0152) สายพันธุ์

Salmonella ที่พบบ่อยที่สุดเป็น S. Emek 33.3 % , S. Haardt 18.42 % , S.Typhimurium 7.89 % และ S. London 7.02 % พบ S.Typhimurium จำนวน 9 ครั้ง จากโรงฆ่าสัตว์ 5 แห่ง

ได้พบ Campylobacter spp. จำนวน 35.11 % จากซากไก่ 319 ตัวอย่าง พบ Campylobacter จากตัวอย่างจากโรงฆ่าสัตว์ขนาดใหญ่ 36.58 % ซึ่งมากกว่าตัวอย่างจากโรงฆ่าสัตว์ขนาดเล็กจำนวนเล็กน้อย คือจากโรงฆ่าสัตว์ขนาดเล็กพบ 34.18 % (p=0.6618) ตัวอย่างที่พบทั้ง Salmonella และ Campylobacter จากตัวอย่างทั้งหมด 319 ตังอย่าง คือ 17.87 % สรุปได้ว่าทั้ง Salmonella และ Campylobacter spp.ในซากไก่ ต่างมีแนวโน้มที่จะเป็นตัวอันตรายทำให้เกิดโรคอาหารเป็นพิษในคน ดังนั้นจากผลการศึกษานี้จึงต้องแนะนำอย่างแรงกล้าให้มีการนำระบบมาตรฐานด้านสุขอนามัยไปใช้ในกระบวนการผลิตของโรงฆ่าสัตว์ประเภทสัตว์ปีกอย่างมีประสิทธิภาพ ในการศึกษาครั้งต่อไปควรวางแผนการศึกษาการตรวจหาจุดควบคุมวิกฤติโดยเฉพาะของห่วงโซ่ของกระบวนการผลิตสัตว์ปีก (จากฟาร์มถึงโต๊ะอาหาร)

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## ABBREVIATIONS AND SYMBOLS

| | | |
|---|---|---|
| CCP | = | Critical Control Point |
| Cm | = | Centimetre |
| $CO_2$ | = | carbon dioxide |
| *C.* | = | *Campylobacter* |
| h | = | hours |
| $^0C$ | = | degree Celsius |
| LDC | = | lysine dercarboxylase |
| l | = | litter |
| ISO | = | International Organization for Standardration |
| ml | = | Milliliters |
| mg | = | milligram |
| NCSS | = | Number Cruncher Statistical System |
| $N_2$ | = | nitrogen |
| $O_2$ | = | oxygen |
| p- value | = | probability value |
| ppm | = | parts per million |
| *S.* | = | *Salmonella* |
| TSI | = | triple sugar iron agar |
| UI | = | unit |
| USDA | = | United States Department of Agriculture |
| XLD | = | xylose lysine desoxycholate |
| µg | = | microgram |
| $\chi^2$ | = | Chi- square |

# 1. INTRODUCTION AND OBJECTIVES

## 1.1 Introduction

Over the past 20 years poultry meat production worldwide has increased rapidly with an annual growth rate of 6%. This has led to intensive animal production with increases in both the farms and flock size. Both have raised specific problems, such as contamination with human and animal pathogens, animal welfare and environment problems (Mulder, 1993). In poultry meat processing there has been a very rapid transition from handcraft operation of the 1950 and 1960 to an almost fully automated and mechanized process today. This development enables poultry processors to slaughter large number of animal without much handling labor.

Poultry and poultry products are important vehicles of food- born illnesses in humans with certain serotypes of *Salmonella* and thermophilic *Campylobacter*. spp being commonly involved. Products are perceived to be safe when microbiology and chemical hazards are absent. Poultry for meat production are normally raised on litter floors. This may lead to contamination of poultry with human pathogens, such as *Salmonella, Campylobacter, Listeria, Escherichia coli, Clostridium* and *staphylococcus aureus.* As long as these pathogens are not excluded from animal husbandry, poultry and poultry product may be contaminated. The carcasses may also be contaminated with enteric organisms if the bung or the cut end of the intestines is allowed to make contact with the carcass during evisceration. Such contamination is in the processing of mammals commonly avoided by enclosing the freed bung in a plastic bag when the large intestine is pulled from the body cavity, and by retaining the bag in place during the removal of the intestine (Nesbakken *et al.*, 1984). Scalding loosens feathers, their removal depend on the water temperature and time

combination. The incidence of *Salmonella* and *Campylobacter* can be influenced by the scalding temperatures (Slavik *et al*., 1994).

Poultry can be infected by *Salmonella*, *Staphylococcus aureus*, and *Campylobacter* spp. at the breeding and /or fattening farm. From the time the poultry leaves the farm to slaughterhouse, poultry meat has several opportunities to be infected or contaminated with bacteria during slaughtering or transport from the slaughterhouse to the market.

Broiler carcasses can be infected by bacteria from the equipment of the slaughterhouse. However, almost all developing countries have low quality poultry slaughterhouses that contain old facilities and an unsuitable processing chain.

During poultry processing, the contamination level can be controlled by taking hygiene measures, based on the HACCP principles, to avoid cross contamination, both between product and between equipment and product. Complete eradication of pathogens from poultry products seems impossible without additional decontamination treatments. By applying “the critical control point” for checking poultry processing, we can detect the main points of contamination during poultry processing.

Ho Chi Minh City consists of 25 districts and is located in the south- eastern area of Vietnam. It has an estimated population 8.5 million inhabitants (Statistic, 2002). The animal husbandry in Ho Chi Minh City has developed over the years, especially poultry production. The city has about 55 poultry slaughterhouses. This enables poultry processors to slaughter large numbers of animals. However, there is very little information about the contamination of broiler carcasses with *Salmonella.* Similarly, no data about *Campylobacter* from broiler meat is currently available.

In the present study, *Salmonella* and *Campylobacter* contamination in the broiler carcasses was investigated.

**1.2 The objectives of this study**

- To estimate the prevalence of the contamination of *Salmonella* and *Campylobacter* spp. in broiler carcases in different abattoirs.
- To identify *Salmonella* and *Campylobacter* strains isolated from carcasses.

# 2. LITERATURE REVIEW

## 2.1 *Salmonella*

### 2.1.1 Microbiology

*Salmonella* bacteria belong to the family *Enterobacteriaceae.* They are generally motile straight rods with peritrichous flagella. They grow on nutrient agar and aero-anaerobes, ferment glucose and often producte of gas, reduce nitrate into nitrite and give oxidase test a negative. Thiosulphate and iron salt allow the production and detection of $H_2S$ unless the pH is acid. Thus, in *Salmonella- Shigella* (SS) agar, selective agents used bile salt and brilliant green. Typically, *Salmonella* strains produce colourless colonies with black centers. The optimal growth temperatures of *Salmonella* ranges between 35-43$^0$C, optimal pH of between 7-7.5, and the $a_w$ of 0.99.

The genus *Salmonella* consists of two species*: S. bongori and S. enterica* (Le minor and Popoff, 1987).

The *S. bongori* contains less than 10 serovars that are extremely rare. Whereas *S. enterica* species has more than 2500 serovars (Kauffmann –White- Scheme) and is divided into six subspecies: *S. enterica* ssp. *enterica* which highly pathogenic to warm blooded animals has 1435 serotypes. The *S. enterica* ssp. *salamae* (485 serotypes found), *S. enterica* ssp. *arizonae* (94 serotypes founds), *S. enterica* ssp. *diarizonae* (321 serotypes found), *S. enterica* ssp. *houtenae* (69 serotypes found), *S. enterica* ssp. *indica* (11 serotypes found). All *Salmonella* strains belong to a serovar based on the analysis of somatic O-antigen. This antigen is lipopolysaccharide (heat stable) and flagella H-antigens of protein nature (heat labile). Each antigenic variant is a serovar in the Kauffmann –White- scheme. The genus *Salmonella* of the family *Enterobacteriaceae* can roughly be classified into three categories or group.

- Group 1: The highly host adapted and invasive serovars include species restricted and invasive *Salmonella* such as *S.* Pullorum, *S.* Gallinarum, and *S.* Typhi in humans.

- Group 2: The non host adapted and invasive serovars consist of approximately 10-20 serovars that are able to cause an invasive infection in poultry and may capable of infecting human. Currently, the most important serovars are *S.* Typhimurium, *S.* Hadar, *S.* Arizonae and *S.* Enteritidis.

- Group 3: The non-host adapted and non-invasive serovars include most serovars of the genus *Salmonella*. They are pathogenic for animal and human.

### 2.1.2 Salmonellosis in humans

Salmon and Smith reported the isolation of the bacteria responsible for “hog cholera” or “swine fewer” in 1885. As with most other enteric infection, the very young, the elderly and those who are immuno-compromised or who have underlying disease are more at risk from infection. *Salmonella* infection is only possible if large numbers of cells were consumed. Minimal infective doses required vary with age and state of health and dose of at least 100.000 cells is required to cause infection. The common symptoms of *Salmonella* infection are shown in Table 1.

Table 1: Symptoms of *Salmonella* infection (Humphrey, 2000)

| Symptom | % of case |
|---|---|
| Diarrhea | 87 |
| Abdominal pain | 84 |
| Feeling feverish | 75 |
| Nausea | 65 |
| Muscle pain | 64 |
| Vomiting | 24 |
| Headache | 21 |
| Blood in stools | 6 |

The incubation period ranges between 12-72 hours but occasionally may extend up to a week. In some outbreaks, where large numbers of organisms consumed, incubation period may be as short as 2.5 hours (Humphrey, 1989).

### 2.1.3 Poultry meat and poultry products-important source of *Salmonella* human infections

Members of the genus *Salmonella* pose a serious threat to the domestic food- animal industry. These organisms are responsible for significant morbidity and mortality in these hosts (Bullis, 1977), as well as causing substantial disease in humans. Human infections are commonly associated with contaminated chicken meat or eggs. Human salmonellosis originating from the consumption of meat or poultry products is a big problem and has been dealt with for decades (St Louis *et al.*, 1988). The main risk factors incriminated in the transmission of *S.* Enteritidis PT4 and *S.* Typhimurium DT04 infection in England and Wales are show in Table 2.

Table 2: Food vehicles in outbreaks of *S.* Enteritidis PT4 and *S.* Typhimurium DT04 infection in England and Wales (Wray and Wray, 2000)

| Food vehicle | PT4 (1989-1996) | DT04 (1992-1995) |
|---|---|---|
| Egg and egg dishes | 103 | 2 |
| Desserts | 98 | - |
| Poultry | 75 | 12 |
| Red meat and meat products | 39 | 10 |
| Fish /shelfish | 18 | 1 |
| Salad/fruit/vegetables | 17 | - |
| Sauces | 9 | - |
| Milk/milk products | 9 | 5 |
| Miscellaneous foods | 130 | 3 |

Concerning the vertical transmission, the most important vehicle of *Salmonella* infection is eggs laid by infected carriers. Lateral spread of infection takes place through contaminated feed, water, equipment, and environment.

Outbreaks are related predominantly to the consumption of contaminated eggs and egg products (Haeghebaert *et al.*, 1998). Nevertheless, because of the many forms in which chicken meat is consumed and the risk of cross contamination to other foods, poultry has long been an important source of *Salmonella* infection in humans (Hird *et al.*, 1993).

In a British study, *S.* Enteritidis PT4 appears to be one of the most predominant serotypes in broiler chickens. The cross contamination of broiler carcasses most likely occurs in the scalding tank, the plucking machines and during evisceration procedures. In Turkey, cross-contamination with the incidence of *Salmonella* during processing increased from 33.3% to 60% at two plants in all broilers carcasses. Two incidences of 36.6 % and 31.1 % were recorded in the plants (Goksoy *et al.*, 2004**).**

In Australia, there were 1153 *Salmonella* isolations. The most frequent serovars from poultry were *S.* Sofia (36.6%), S. Virchow (11.3%), *S*. Infantis (10.9%) and *S.* Typhimurium PT 64 (3.4%), *S*. Typhimurium PT 108 (3.2%) (Sumner *et al.*, 2004). In Argentina, the prevalence of *Salmonella* in chicken carcasses following evisceration was 20.8 % and 20 % of the visibly uncontaminated carcasses (Jimenez *et al.,* 2002).

In Vietnam, *Salmonella* spp. was isolated from almost 20%. Tran *et al.*, (2005) reported that *Salmonella* was isolated from 21.0% of chicken meat. In another study, Tran, *et al.* (2004) recovered *Salmonella* from 7.9% (24/302) of faecal sample in adult chicken in a slaughterhouse. In Thailand, Boonmar *et al.* (1998) isolated *Salmonella* 72 % of retail chicken meat samples and 10% from chicken meat samples in slaughterhouse. They also isolated *Salmonella* in 80 % of samples from open markets and 64% in supermarkets. In Malaysia, *Salmonella* was isolated from 35.5% of broiler carcasses (Rusul *et al.,* 1996).

### 2.1.4 Epidemiology

*Salmonella* can infect a diverse range of animal hosts including man, insects, reptiles and birds, and can be present and persist in the environment. All *Salmonella* serovars are considered potential pathogens in most animal species. However, the pathogenicity of some serovars appears to be limited to a narrow range of animal hosts and are considered “host adapted”, such as *S.* Dublin in cattle.

Table 3: *Salmonella* serovars according to their host adaptation and importance for animals and humans (Kleer, 2004)

| Main characteristics | Serovars | Important for animal | Important for human |
|---|---|---|---|
| Adapted to man | *S.* Typhi<br>*S.* Paratyphi | unimportance | typhoid or enteric fever |
| Adapted to certain species of animal | *S.* Dublin<br>*S.* Choleraesuis<br>*S.* Ganillarum<br>*S.* Abortusovis | typical infection<br>severe epidemics | sometimes, but seldom salmonellosis<br>severe infection possible |
| Not adapted to certain species of animal, but invasive | *S.* Enteritidis<br>*S.* Typhimurium | from severe epidemics to symptomless carrier state | main cause for salmonellosis |
| Not adapted to certain species of animal , not invasive | more than 2.000 other serovars | in general latent infection, but disease possible | (seldom) cause for salmonellosis |

Table 4: Serovars of *Salmonella* isolation from animals

| Serotype | Vietnam* | South East Asia** | Europe*** |
|---|---|---|---|
| *S.* Aberdeen | 1.3 | - | - |
| *S.* Aantum | 1.3 | - | - |
| *S.* Bovismorbificans | 2.5 | - | - |
| *S.* Branenderup | 1.3 | - | - |
| *S.* Derby | 6.3 | - | 0.7 |
| *S.* Dublin | 1.3 | - | - |
| *S.* Choleraesuis | - | 4 | - |
| *S.* Emek | 10.0 | | |
| *S.* Enteritidis | 1.3 | 3 | 58 |
| *S.* Hadar | 2.5 | - | 0.7 |
| S. infantis | - | - | 1 |
| *S.* Java | - | 10 | - |
| *S.* Javiana | 21.3 | - | - |
| *S.* Lexington | 3.8 | 3 | - |
| *S.* Senftenberg | 3.8 | 2 | - |
| *S.* Saintpal | - | 2 | - |
| *S.* Typhimurium | 12.5 | 13 | 28 |
| *S.* Weltevreden | 12.5 | 13 | - |
| *S.* Virchow | 1.3 | 4 | 0.5 |
| *S.* Tyresoe | 1.3 | - | - |
| *S.* Tennessee | 1.3 | - | - |
| *S.* wagenia | 1.3 | - | - |
| *S.* Singapore | 1.3 | - | - |
| *S.* London | 1.3 | - | - |
| *S.* Newport | 1.3 | - | - |

*. Tran *et al.* (2004)

**, *** RK Institute, Berlin (Fries, 2005)

### 2.1.5 Public health concern

In the USA, over 150 different *Salmonella* serotypes have been isolated from poultry. Evidence of disease in birds is most common in chicken, poultry or ducklings under 2 weeks of age. The main significance of *Salmonella* infection is as a zoonosis. The Zoonoses Directive (92/117/EEC) contains provisions for community controls measures for *Salmonella* in domestic fowl and the poultry and the poultry breeding flocks and hatcheries.

In 2003, in a total of 15,600 laboratory-diagnosed cases in surveillance areas, 6,017 *Salmonella* isolates were identified. Of the 5,455 (91%) *Salmonella* isolates serotyped, five serotypes accounted for 59% of infection, as follows: Typhimurium (20%), Enteritidis (12%), Newport (6%), and Heidelberg, (6%). The incidence of *Salmonella* infection, defined as the number of laboratory isolation per 100,000 persons, was 122.7 for infants and 50.6 young children. (MMWR, 2004) In 2004, laboratory-diagnosed cases of infections in food- surveillance areas were identified *Salmonella* 6,464. Overall incidence per 100,000 persons was 14.7 *Salmonella.* Of the 5,942 (92%) *Salmonella* isolates serotyped, five serotypes accounted for 56% of infection, as follows: Typhimurium (20%), Enteritidis (15%), Newport (10%), Javiana (7%), and Heidelberg (5%) (MMWR, 2005).

There was an increased incidence of *Salmonella* Enteritidis phage type 4 around 1990 but this has then decreased, probably owing to increased surveillance, and subsequent control measure. But, *Salmonella* Enteritidis phage type 4 (PT4) has become a major problem in chicken in many areas of Europe, emerging as the major of salmonellosis in humans. Nevertheless, Salmonellosis due to PT4 has not been reported in the United Stateds and Canada. (Humphrey, 2000)



In Denmark the incidence of human salmonellosis has been increasing with poultry and poultry products being the major sources for human salmonellosis (Olsen *et al.*, 1992).

Vietnam experienced a more than six- fold increase cases of typhoid fever from 1990 (4,859 cases) to 1995 (30,901ccases) (Lin *et. al.*, 2000). Most cases (about 90%) were reported from the southern region, which consists of 17 provinces with about 39% of the total population in Vietnam, Between 1995 to 2002, there were 81 reports that were of *S. enterica* serovar Typhi isolates from sporadic cases and minor outbreaks in Vietnam (Le *et al.*, 2004). In three rural communes of Dong Thap province in southern Vietnam, 8.5% (56/658cases) were positive for *Salmonella* Typhi with an overall accidence for 198 per $10^5$ population (Lin., *et al* 2000).

## 2.2. *Campylobacter*

### 2.2.1 Microbiology

*Campylobacter* organisms were recognized in early decades of 20$^{th}$ century as causes of infectious abortion and infertility in sheep and cattle. The pathogenicity of these organisms in human was suggested in 1946 and described in an epidemic of gastroenteritis in two institutions in Illinois, associated with the consumption of raw material. In that epidemic a woman suffered from septic abortion (Blaser and Reller, 1981). Over the next decade, *Campylobacter* organisms have been occasionally isolated from blood, cerebral spinal fluid, and other human body fluids and were believed to be opportunistic pathogens.

Genus *Campylobacter*, a gram-negative bacteria, has a curved rod and spiral conformation. At one or both ends of the cell, a polar flagellum can be found, which makes the microorganism highly motile. These curved rods display darting or corkscrew motility, and joined, form zigzag or gull, spiral-shaped (Weijtens, 1996). These bacteria are 0.2-0.5 µm wide and 0.2-0.8 µm long. They have cell membrane, which is a typical rough cell wall with polar pits and unsheathed bipolar flagella (Goodwin *et al.*, 1985). The optimal for growth is 42-43$^0$C. Therefore, the organism is called thermophilic (optimum 5-7% $O_2$, 10% $CO_2$ and 85% $N_2$) (Quinn *et al.*, 1998). *Campylobacter jejuni* also requires a microaerobic atmosphere consisting of 3-5%

oxygen, 3-15% carbon dioxide and 85% nitrogen, for optimal growth. Because of these characteristics, *Campylobacter jejuni* adapts well in the bird intestinal tract where the temperature is about $42^0$ C. The optimal pH range between 6.5- 7.5 and the $a_w$ 0.997.

The biochemical reactions of the organism are nitrate reduction, $H_2S$ production, catalase and oxidase positive, and non- fermentation of carbohydrates. *Campylobacter jejuni* is unique in its ability of hydrolysing sodium hippurate (Quinn *et al*., 1998).

The taxonomical classification of *Campylobacter* has constantly been reviewed since the beginning of the $20^{th}$ century (Vandamme and Goossens, 1992). Vandamme and Goossens, (1992) introduced the new eubacterial family *Campylobacteriaceae*, grouping the genus *Campylobacter* and its closest related genus, the genus *Arcobacter*. Microorganisms belonging the genus *Campylobacter* are slender, spirally curved, gram- negative rods that are 0.5 to 0.8 µm long and 0.2 to 0.5 µm wide. At present, the genus *Campylobacter* mainly consists of the following *Campylobacter* species: *C. hyointestinalis, C. fetus, C. consisus, C. mucosalis, C. sputorum, C. curvus, C. rectus, C. showae, C. gracilis, C. upsaliensis, C.helveticus, C. hyoilei, C. jejuni, C. coli,* and C. *lari* (On 1996).

### 2.2.2 Campylobacterosis in Humans

The *Campylobacter* organisms were recognized likely causal agents of enteric disease. *Campylobacter jejuni* was isolated from human diarrhea stools in 1972 (Dekeyser *et al.,* 1972). Subsequent development of selective stool-culture media (Butzler *et al.,* 1973; Skirrow *et al.,* 1977) led to the recognition of *Campylobacter* as a common cause of human diarrhea in most parts of the world (Allos and Blasser, 1995). Thermophilic *Campylobacter* species have been recognized as the major cause of bacterial gastrointestinal human infections in the USA (Altekruse *et al*., 1999), and in England and Wales (Forst *et al.,* 1998).

Human volunteer studies have shown that ingestion of *Campylobacter* can produce infection at a variety of doses ranging from 500 organisms (the lowest dose)

to $10^6$ organisms (Keener *et al*., 2004). The rates of infection did not vary in importance with the dose, being generally about 10 % (Robinson, 1981).

The most important clinical symptom of human infection with *Campylobacter* is diarrhoea. The incubation period ranges from 3 to 7 days. Diarrhea may vary from very mild to massive watery or grossly bloody stools. In addition to diarrhea, most patients have fever, abdominal pain, nausea, and malaise (Keener *et al*., 2004). The diagnosis is made when the organism is isolated from stools (Butzler and Skirrow, 1979; Griffiths and Park, 1990; Allos and Blaser, 1995). The most important non-suppurative extra-intestinal complication of *Campylobacter* infections is reactive arthritis and an acute demyelization disease from reactive arthritis, but much less from Guillain- Barre syndrome (GBS) (Kosunen *et al.*, 1981; Rhodes and Tattersfield, 1982). *Campylobacter* enteritis is a self-limiting infection in mild cases. Mostly symptoms resolve within one week without antimicrobial therapy being indicated. However, symptoms of *Campylobacter* may persist for 1-3 weeks in up to 20% of cases (Keener *et al*., 2004). Antimicrobial therapy is indicated in severe cases with prolonged illness and bacteraemia. The mean duration of excretion of *Campylobacter* after acute enteritis is 2-3 weeks. In immuno-deficient patients, excretion may persist up to one year (Endtz, 1993; Allos and Blaser, 1995).

The clinical features of *Campylobacter* infections in human range from an absence of symptoms to sepsis and death. Twenty- five percent of person with culture proven infections (in feces) contracted in large outbreaks does not show clinical symptoms. Death due to *Campylobacter* infection is rare, approximately 3 per 10,000 cases of *Campylobacter*iosis (Tauxe, 1992).

*C*. jejuni *and C. coli* have also been implicated in extra-intestinal disease. These may include meningitis, endocarditis, septic arthritis, osteomyelitis and neonatal sepsis (Allos, 1997; Nachamkin *et. al*., 1998

### 2.2.3 Poultry meat and poultry products as important sources of *Campylobacter* in humans

*Campylobacter* commonly lives in the intestinal tract of a wide range of birds and chicken. *Campylobacter* can survive in the environment for several weeks at temperatures around $4^0$C, but also can be present in surface water with higher temperatures. Therefore, many potential pathways of infection exist. Chicken is often contaminated with *Campylobacter*. In many industrialized countries, this figure is even higher. Besides direct infection by consumption of chicken, cross-contamination from raw chickens to other foods during storage and preparation has also been a cause of infection. Sources of the infection are associated with handling raw or eating uncooked poultry products contaminated with *Campylobacter* (Hopkins *et al.*, 1984; Harris *et al.*, 1986, Kapperud *et al.*, 1992).

The prevalence of *Campylobacter*- positive poultry flock in different countries, varies among countries as summarized Table 5.

Apparently, the rate of contamination from poultry products in retail or in ready-to-eat chicken meat with *Campylobacter* is enormously high (Harrison *et al.*, 2001; Dickins *et al.*, 2002; Moore *et al.*, 2001). Consumption of raw milk and unchlorinated water were proven to be the sources of infection in a large number of cases (Tauxe, 1992).

Table 5: Prevalence of *Campylobacter* in broiler flocks from selected countries (Newell *et al.*, 2003)

| Country | Sample type | Prevalence (%) |
|---|---|---|
| United States | Cecal/feces | 87.5 |
| United Kingdom | - | 76 |
| | Cloaca | >90 |
| | Cloaca | 45 |
| Denmark | Cloaca | 42.5 |
| | - | 39.6 (*C. jejuni*) |
| | | 5 *(C. coli*) |
| Norway | - | 18 |
| Sweden | - | 27 |
| Germany | - | 41.1 |
| Italy | Cloaca | 80 |
| France | Feces | 42.7 |
| Canada | Coloaca or ceca | 44.4 |
| Chile | Feces | 19,7 (*C. jejuni*) |
| | | 6 (*C. coli*) |
| Taiwan | Cloaca | 24.1 |
| Malaysia | - | 53.7 (*C. jejuni)* |
| | | 28.3 *(C. coli)* |
| Japan | - | 45 |

In the study of *Campylobacter* spp. isolated from poultry carcasses in big poultry slaughterhouses in Switzerland, the prevalence of *Campylobacter* from chicken carcasses was 24.37% (195/800) (Frediani-Volf, and Stephan, 2003). A cross-sectional survey of broiler flocks in England and Wales found that 45% (95% confidence limits: 37±53%) of flocks were colonised with *Campylobacter* when the birds were 5 weeks of age (Evans, 1997).

In a study about the reservoirs of *Campylobacter*, the likely sources of human infection were identified as chicken (94.2%), pig (90.5%), dog (46.9%), cats (37.3%), sheep (4.2%), wild birds (39.6%) and monkey (17.1%), while chicken meat had 58.4% contamination with *Campylobacter*. *Campylobacter jejuni* was identified in humans in 63.6% of samples. It was the most commonly (86.6%) identified species from chicken feces, dog (51.5%) and chicken meat (79.8%). Chicken meat is the likely vehicle for transmission of *Campylobacters* to humans (Workmam *et al.*, 2005). However, inadequately cooked meat, particularly poultry, unpasteurized milk and contaminated drinking water are the most common sources for epidemic and sporadic food-borne cases (Alterkuse *et al.*, 1999). Furthermore, cross contamination of other foods caused by raw poultry meat during food preparation is also important. Such events are difficult to control at this stage and lead to an increased risk of contamination of carcasses at the end of the slaughtering process (Oosterom *et al.*, 1983).

### 2.2.4 Epidemiology

*Campylobacter* is spread mainly by the animal reservoirs and is commonly found in livestock and domestic animals (Rosef *et al.*, 1983, Wolfs *et al.*, 2001) where they generally reside in the intestinal tract without causing clinical symptoms. Basically, chickens are suitable hosts of *Campylobacter* bacteria because the body temperature is about $41^0$C, which is about the optimal temperature for *Campylobacter* (Quinn, *et al.*, 1998). Moreover, in the caecum of chicken, there is a complete anaerobic atmosphere. Furthermore, the conformation of villi, which contain plenty of mucine with fucose meets the requirements of *Campylobacter* well. So, without showing any clinical signs, chicken are potential reservoirs transmitting the infection to other warm blooded animals. Commercially raised poultry very often carries *Campylobacter* in the intestinal tract. Other domestic animals, such as cattle, swine, sheep, dog and cats are often intestinal tract carriers of *Campylobacter*. Many wild animals are carriers of *Campylobacter* (a number of avian species like crows, pigeons, ducks, and seagulls) (Blaser *et al.*, 1997).

The presence of *Campylobacter* in food and water is most frequently due to feacal contamination. Products, uncooked meat, poultry (20-100%) and seafood, widely distributed among feral animals or food that has been contaminated during processing or preparation, accounts for 70% of *Campylobacter*–related illnesses each year. The ecological habitant of *Campylobacter spp* is the intestinal tract of wild and domestic animals. *C. jejuni* is predominant in broilers and cattle but is infrequent in pigs (Aarestrup *et al.*, 1997).

Broiler houses are usually depopulated over a number of days and the risk of infection to remaining birds in the flock might be increased by the presence of processing-plant personnel or equipment when birds are collected in batches for slaughter (Jacobs-Reitsma *et al.*, 1994; Berndtson *et al.*, 1996a; Evans, 1997; van de Giessen *et al.*, 1998). The carriage of *Campylobacter* in chicken is influenced by season: a high infection rate of *Campylobacter* occurs around June to September (Jacobs- Reitsma *et al.*, 1994, Wedderkopp *et al.*, 2000). In a longitudinal study in broiler farms in the U.K., the carriage of *Campylobacter* in poultry was obviously associated with temperature and sunlight hours (Wallace *et al.*, 1997). Moreover, the infection rate of *Campylobacter* in broilers is associated with the chicken age.

Chickens have been implicated in about 50 to 70% of human cases. The most common species in 90% of cases is *Campylobacter jejuni* (Anon, 1993).

Table 6: Known sources and disease associations of *Campylobacter* species (Weijtens, 1996)

| Taxon | Known source(s) | Human disease | Animal disease |
|---|---|---|---|
| *C. fetus* subsp.fetus | cattle, sheep | septicaemia, gastroenteritis abortion, meningitis | bovine and ovine spontaneous abortion |
| *C. fetus* subsp.venerialis | cattle | septicaemia | bovine infectious infertility |
| *C. hyointest* subsp hyoint | pigs, cattle, hamsters, deer | gastroenteritis | porcine and bovine enteritis |
| *C. hyointest* subsp.*lawsonii* | pigs | none at present | unknown |
| *C. consisus* | humans | periodontal disease, gastroenteritis | none at present. |
| *C. mucosalis* | pigs | none at present | porcine necrotic enteritis and ileitis |
| *C. sputorum* bv.*sputorum* | humans, cattle, pigs | abscesses, gastroenteritis | none at present |
| C. *sputorum* bv fecalis | sheep, cattle | none at present | none at present |
| *C. curvus* | humans | periodontal disease, gastroenteritis | none at present |
| *C. rectum* | humans | periodontal disease | none at present |
| *C. showae* | humans | periodontal disease | none at present |
| *C. upsaliensis* | dog, cat | gastroenteritis, septicemia, abscesses | canine and feline gastroenteritis |
| *C. helveticus* | cat, dog | none at present | feline and canine gastroenteritis |
| *C. hyoilei* | pigs | none at present | porcine proliferative enteritis |

Table 6 (Con.)

| Taxon | Known source(s) | Human disease | Animal disease |
|---|---|---|---|
| *C. coli* | pigs, poultry, cattle, sheep, birds, dogs, cats, rodents, insects, environment | gastroenteritis, septicemia | gastroenteritis |
| *C. jejuni* | poultry, pigs, cattle, sheep, birds, dogs, cats, milk rodents, insects | gastroenteritis, septicemia, arthritis, meningitis, abortion guillain- barre, etc | gastroenteritis avian hepatitis, abortion |
| *C. lari* | poultry, dogs, cats, birds, monkeys, environment | gastroenteritis, septicemia | avian gastroenteritis |

2.2.5 Public health concern

Food-borne infections caused by species of *Campylobacter* occur most frequently in developing countries and represent a considerable drain on economic and public health resources. In developing countries, most reported *Campylobacter* infections are in children (Keener *et al.*, 2004). Peaks in *Campylobacter* infection rates have been reported in children less than one year of age. Moreover, *Campylobacter* is known as the leading bacteria in food-borne pathogens causing human enteritis for the part 3 decades worldwide, when compared with other pathogenic diarrhea agents like *Salmonella* and *E. coli.*



During the last 25 years, reported cases of *Campylobacter* have risen greatly. There were approximately 44,000 laboratory reports of these infections in 1995 in England and Wales and this figure continued to rise to 58,000 cases by 1998. Poultry is an important reservoir of infection. Broiler flocks are frequently infected with *Campylobacters,* mainly *C. jejuni* (Prescott and Munroe, 1982; Hood *et al.*, 1988; Humphrey *et al.,* 1993). The consumption or handling of chicken is a major risk

factor for human *Campylobacter*iosis (Harris *et al.*, 1986; Deming *et al.*, 1987). The annual incidence exceeds 2.4 million cases in North America. At times the infection may lead to complications, including reactive arthritis and a postinfective polyneuropathy called Guillain- Barre syndrome.

In 2003, among the total of 15,600 laboratory- diagnosed cases of infections in food of surveillance areas, 5,215 were due to *Campylobacter* spp.. (MMWR, 2004) and in 2004, 5,665 out of 15,806 laboratory- diagnosed cases were due to *Campylobacter* spp.

In Ha Noi, Vietnam, during June 2000 to December 2001, the 104 *Campylobacter* isolated were from 1159 diarrheal patients. These were 72 *Campylobacter jejuni* isolates (69.2%) and 32 *Campylobacter coli* isolates (30.8%) (Phung, *et al.*, 2002).

## 2.3 *Salmonella* and *Campylobacter* contamination in poultry processing

### 2.3.1 Transportation

Stress can cause a disturbance of intestinal functions and may lower the resistance of animals and increase the spread of intestinal bacteria. For example, *Campylobacter* detection has been shown to increase during transport and holding before slaughter (Stern *et al.*, 1995). If the crates are stacked, the birds in the lower cages will be contaminated with the feces of birds in the cages above them.



### 2.3.2 Pre-slaughter inspection

*Campylobacter* detection on the feathers of cooped and transported birds is 10-fold greater than that of those remaining on the farm (Stern *et al.*, 1995). A Stern *et al.*,

*(*2001) also found that many coops were not properly cleaned between flocks, which might contribute to increased contamination levels observed at the plant. Some *Campylobacter* – negative flocks reach the abattoir but the carcasses from such flocks are rapidly contaminated by various *Campylobacter* subtypes during processing. Negative flocks, *Campylobacter* of the same subtype as those recovered from the carcasses were isolated from the crates used to transport the birds (Newell and Fearnley, 2003).

### 2.3.3 Handling prior to dressing

Fed chicken do not bleed well and are harder to eviscerate. However, withholding feed for more than 12 hours will cause a marked loss in dressing yield. Also over-heated or over- excited birds will bleed poorly, producing carcasses of higher blood content and lower keeping quality (Keener *et al.*, 2004).

### 2.3.4 Scalding

The scalding procedure is used to open the feather follicles to facilitate the removal of feathers. The potential for bacterial cross-contamination during scalding and picking is well recognized (Bailey *et al.* 1990).

A study on the number of *Campylobacter* and *Salmonella* on chicken carcasses scalded at three different temperatures ($52^0$C, $56^0$C and $60^0$C) found that the higher the temperature of scalding the greater probability of the contamination (Slavik *et al.*, 1994). Lower bacterial contamination was obtained with spray scalding and plucking in a single operation. *Campylobacter* has been periodically recovered from scald water (Stern *et al.*, 2001). Cason *et al.* (1999) examined the microbiological effect of removing feathers from the carcasses between the tanks of a multiple scalding tank. The data showed no reduction in populations of aerobic bacteria, *Escherichia coli*, or *Campylobacter* on carcasses during scalding and defeathering.

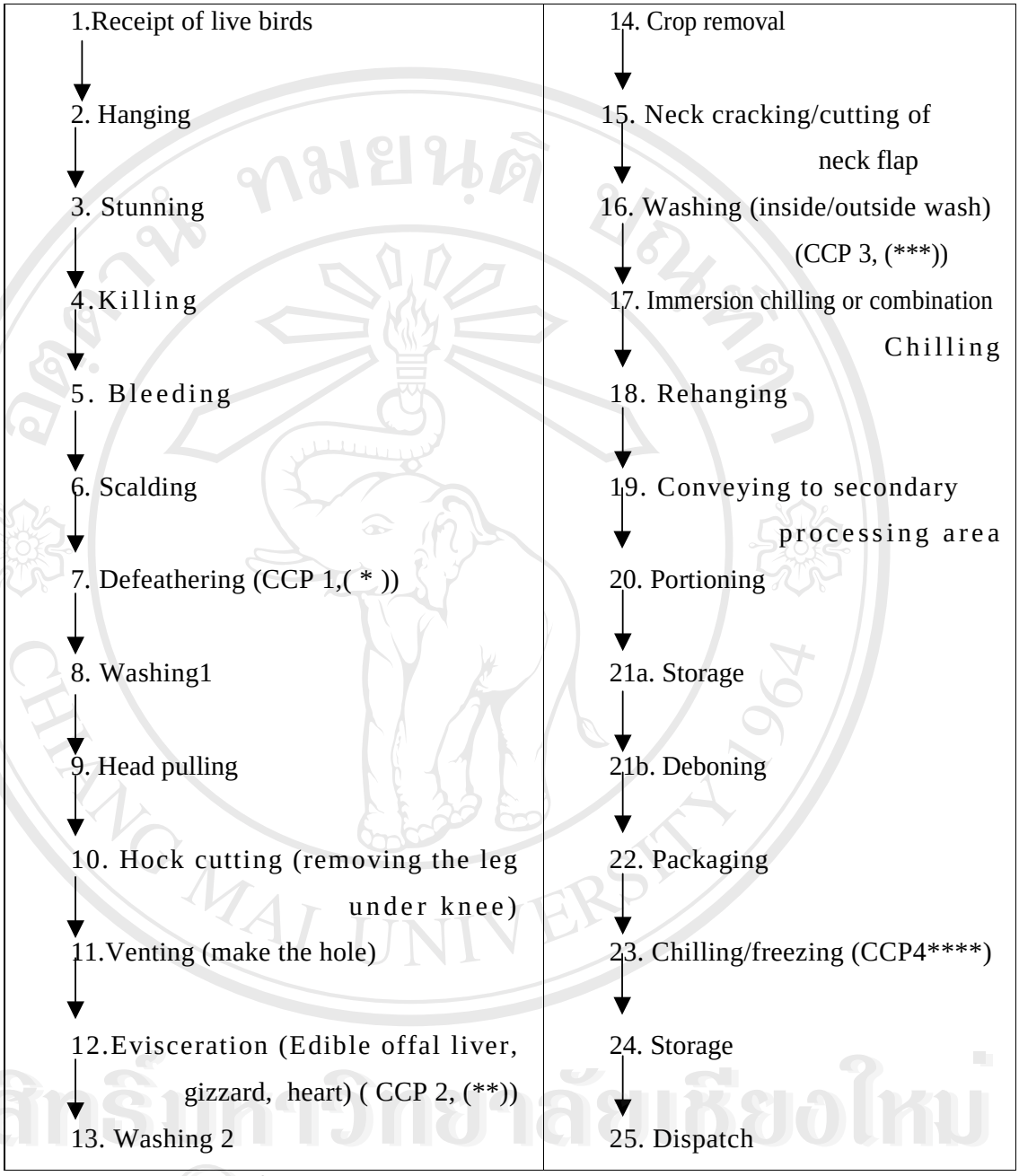


Fig.1. Diagram of standard poultry slaughter process

CCP: Critical Control Points

(*) the first CCP, after defeathering

(**)The second CCP, after evisceration

(***)The third CCP, after inside-outside shower

(****)The four CCP, after chilling

### 2.3.5 Defeathering

Wempe *et al.* (1983) isolated *C. jejuni* from 94.4% of the feather, picker drip and water samples, and the population of organisms present was high. This is an area where cross-contamination occurs, since the rubber fingers in the mechanical picker beat the feathers from the bird, become contaminated and pass the organism from bird to bird. They observed that the water used in rinsing physically removed the *Campylobacter* organism and thus reduced the number of organisms on the edible parts. They recovered *C. jejuni* also from all recycled water samples. The use of recycled water to clean the gutters may further contaminate the receiving room with *C. jejuni*. Further distribution of *C. jejuni* may also occur through movement of plant personnel from the receiving area to other areas of the plant. Berrang and Dickens (2000) found that after de-feathering, the counts increased significantly (3.70 log10). An increase in *Campylobacter* counts after de-feathering has been previously reported (Acuff *et al.,* 1986; Izat *et al.,* 1988). It was suggested that the rubber fingers in the mechanical picker act to cross-contaminate birds that previously had low or undetectable levels of *Campylobacter* (Acuff *et al.,* 1986; Stern *et al.,* 1995).

### 2.3.6 Evisceration

Chicken skin has been shown to harbour and support the survival of *C. jejuni* (Lee *et al.,* 1998). Berrang *et al.* (2001) studied the presence and level of *Campylobacter, Coliforms, E. coli,* and total aerobic bacteria recovered from broiler parts with and without skin. Samples were taken from de-feathered carcasses before evisceration. No *Campylobacter* were recovered from meat collected from the breasts or thighs, and only 2 of 10 drumstick meat samples had detectable levels of *Campylobacter*. However, 9 of 10 breast skin, 10 of 10 thigh skin, and 8 of 10 drumstick skin samples were positive for *Campylobacter,* with levels between 2 log10 and 3 log10 CFU/g of *Campylobacter* after evisceration. In a related study, Altmeyer *et al.,* (1985) collected 50 muscle samples from broilers and found no *Campylobacter*. Kotula and Pandya (1995) found higher counts on breast tissue of broiler meats than

on the thigh or drumstick. The high incidence of contaminated neck flaps and breast tissue suggest that the crop contents may be an important source of *Campylobacter* contamination during processing. The crop has been found to be a significant source of *Campylobacter*, thus potentially contributing to carcass contamination (Byrd *et al* ., 1998). Berrang *et al.,* (2000) reported that 100% of the crops of 18 broilers were positive for *Campylobacter*. The study also showed that *Campylobacter* could be found on the skin of carcasses in the early stages of processing even with no contamination from internal organs. The heart, liver and gizzard (the giblets) are often pooled and inserted into the body of the chicken. Giblets are more frequently contaminated with *Salmonella* than other sample sites and chickens which contain them are more often contaminated than those without giblets. Carcass and skin of these chickens are frequency contaminated with *Salmonella* Enteritidis PT4 than sites not containing giblets (Gracey, 2001). Another study showed that 20% of carcasses after the evisceration visibly uncontaminated with feces harboured *Salmonella* and 20.8% of the visibly contaminated carcasses were positive for *Campylobacter* (Jimenez *et al.,* 2002). Removal of skin before processing reduces *Campylobacter* levels by 0.7 log10 CFU/carcass (Berrang *et al.,* 2002). Jeffery *et al.* (2001) studied the prevalence of *Campylobacter* from skin, crop, and intestines of commercial broiler chicken carcasses at processing and found positive percentages of 78%, 48%, and 94%, respectively. Berndtson *et al.* (1992) isolated *Campylobacter* in 89% of neck skin samples, 93% of peritoneal cavity swab samples, and 75% of subcutaneous samples. They also found that muscle samples were only very sparsely contaminated, and concluded that the feather follicles were the orifices where *Campylobacter* is introduced into the subcutaneous layer. Overall, *Campylobacter* counts dropped as the flocks moved through the plant (Berrang and Dickens, 2000).



### 2.3.7 Carcass washing

Carcass wash systems use 20 to 50 ppm of chlorine as an anti-microbial agent and generally consume 25 to 50 gallons/min (GPM) of water. Washer systems currently used for inside and outside surface cleaning of chicken carcasses have

shown a limited effectiveness for *Campylobacter* removal (Bashor *et al.*, 2004). The primary reason is that washing with cold water, regardless of pressure and flow volume, does not lower water surface tension, an important factor in bacterial/fecal removal. Some plants use more than 9 L of water per bird for carcass washing with a minimal of (0.5 log10) reduction in *Campylobacter* levels (Bashor *et al.*, 2004).

### 2.3.8 Chilling

The type of chilling used can have an impact on the type and quantity of microbial contamination of the end product. Many poultry processors use water chillers for rapid cooling of carcasses. Recent studies on *Campylobacter* document its potential for cross- contamination in the water chiller (Sancherz *et al*., 2002; Whyte *et al.*, 2002).

### 2.3.9 Water of washing

A study by Li *et al.* (2002) found that the 55°C and 60°C water spray treatments significantly reduced *C. jejuni* by more than 0.78 log cfu/carcass compared with the 20°C water spray treatment. Purnell *et al.* (2004) found that a 70 °C, 40-s rinse showed no detrimental effect on chicken skin and produced a 1.6 log10 reduction in *Campylobacter*/ml. It is suspected that warm water rinsing kills bacteria directly and also reduces the surface tension of the water, which may enhance removal of bacteria and fecal removal.

# 3. MATERIALS AND METHODS

## 3.1 Time and location of study

The study was carried out during the dry season between November 2004 and May 2005 in 15 abattoirs located in 6 districts of the 25 districts in Ho Chi Minh City. These abattoirs accounted for about 27% of all abattoirs (n=55) in Ho Chi Minh City. They were categorized as large, if the daily slaughter was between 1200- 2000 chickens, and small, if less than 1200 chickens were slaughtered. The slaughtering was performed during night time. Chickens slaughtered in these abattoirs are from Ho Chi Minh City farms and 7 southeast provinces of Vietnam (Dong Nai, Binh Duong, Binh Phuoc, Long An, Tien Giang, Tay Ninh, and Vung Tau provinces). The age of the chickens from intensively managed farms ranged from 42 to 45 days old and 75 to 90 days old from backyard farms.

Bacterial isolation was performed at the Center Laboratory of the Sub-Department of Animal Health, Ho Chi Minh City. *Salmonella* serotyping was carried out at the Region Centre for Veterinary Public Health in Chiang Mai University, Thailand.

## 3.2 Slaughter process

The slaughtering process was as depicted in Figure 2. In large abattoirs, electrical stunning is used where chickens were hanged and their heads dragged across an electrically- charged water-bath. The amount of electricity used is 120mA for 15 seconds. Following stunning, the necks are immediately sliced with a knife for the purpose of bleeding. The blood is passed through a tunnel into a holding tank. The birds are scalded by immersing into hot water of temperatures ranging between $56^0$C and $58^0$C, pH 6 for 2-2.5 minutes. After scalding feathers are mechanically removed by a series of online plucking machines.

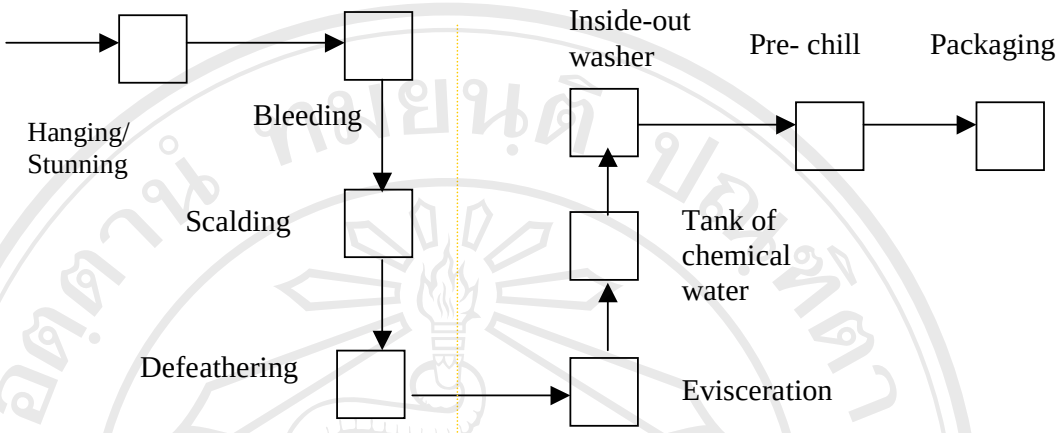


Fig. 2 Plant of the poultry abattoir

They consist of counter-rotating, stainless steel domes with attached rubber "fingers". Then continuous water-sprays are usually incorporated within machines for flushing out feathers. Following de-feathering, evisceration was carried out: while still suspended, the chicken is cut and the internal organs are removed. The de-feathering area was physically separated from the evisceration area.

In the small abattoirs**,** the stunning step was skipped (involvment of chickens in the normal mechanics). The chickens were killed by bleeding with a knife after cutting the head. Chickens were scalded in a scalding tank of about 58 $^{0}$C to 65$^{0}$C within 2 to 3 minutes, defeathering was carried out with an automatic de-feathering machine with rubber fingers. The evisceration was performed by hand with a knife to open the carcass.

Of the 15 abattoirs, 3 abattoirs out of 15 abattoirs used chemicals such as chlorine (100 ppm) in water to wash the chicken carcass.



### 3.3 Sample size

Sampled size of the study was estimated based on the population of the chicken slaughtered in Ho Chi Minh City and an estimated prevalence of *Salmonella* and *Campylobacter* in broiler meat (20 %), at a 95% confidence level and a standard error (SE) of 5%. Win Episcope 2 software was used. A total of 319 samples of broiler

carcasses were collected from 15 abattoirs (3 large abattoirs and 12 small abattoirs). Samples were collected twice to three times from each abattoir. The number of samples per abattoir is shown in Table 7.

Table 7: Distribution and number of samples per abattoir

| Group of abattoir | Large | | | Small | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Abattoir ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | |
| No. of samples | 39 | 45 | 39 | 18 | 20 | 20 | 21 | 12 | 15 | 10 | 10 | 12 | 22 | 16 | 20 | 319 |

### 3.4 Sample collection

Applying the critical control points for checking the poultry processing, it was found out that the main points of contamination during poultry processing. CCP were the de-feathering, the evisceration point and the inside-outside shower stages. Since the purpose of this study was to find the prevalence of *Salmonella* and *Campylobacter* in broiler carcasses, the samples were obtained from the final product at the inside – outside shower stage of the slaughter processing. Ten to fifteen samples were taken per day from each abattoir. One month later, samples were again collected one more time. The total number of samples obtained from abattoirs was shown in Table 7.

Samples were collected using the procedure described in USDA (Sparling, 2002). Briefly, the carcass was put into a plastic bag (30 cm ×60 cm), and four hundred ml of Buffered Peptone Water (Oxiod, CM 509) was added. The carcass was rinsed inside and outside with a rocking motion for one minute. This was done by grasping the carcass in the bag with one hand and the closed top of the bag with the other. The carcass was then removed. The remaining fluid was kept in an icebox and sent to the laboratory. Samples were analyzed for *Campylobacter* and *Salmonella* as soon as possible, but not more than 24 hours later. The information of the samples was collected using the questionnaire.

### 3.5 Questionnaire survey

A questionnaire survey was carried out in abattoirs involved in this study. The questionnaire included the province where the chicken came from, the types of the chicken production (intensive farm or backyard farm), risk factors of contamination in the abattoir where the chickens were slaughtered, and data on the hygiene conditions of the abattoir (Appendix 4).

### 3.6 Microbial analysis

#### 3.6.1 *Salmonella* isolation and identification

*Salmonella* isolation and identification was done based on the instructions of ISO 6579 (2002) (Figure 3 and Table 8). Thirty ml of carcass-rinse fluid were added into 30 ml Buffer Peptone Water (Oxiod, CM 509) and mixed well using a stomacher and then incubated overnight at $37^0$C. One ml of the culture was transferred to 10ml Tetrathionate Broth (Oxiod, CM 29), and another 0.1 ml of the culture was added to 10ml Rappaport Vassilialis broth (Oxiod, CM 669). Both were incubated for 24 h at $42^0$C. A loopful culture from both Tetrathionate and Rappaport broth was streaked on Brilliant Green Agar (BGA, Oxiod, CM 329) and Xylose Lysine Desoxycholate agar (XLD, Oxiod, CM 469) and incubated at $37^0$C for 24h. Five typical colonies from BGA or XLD were inoculated into Triple Sugar Iron agar (Oxiod, CM277), and one colony was streaked on Nutrient Agar and incubated at $37^0$C for 24h. Suspected colonies were inoculated into Urea agar, Lysin Decarboxylase broth (Oxiod, CM 308) and incubated at $37^0$C for 18 -24 h. Colonies considered positive in biochemical tests (Table 8) were chosen for serological testing. A smooth *Salmonella* colony was emulsified in a drop of antiserum on a clean microscopic slide and was well mixed. The slide was rocked gently for about 30 seconds and the antigen- antibody mixture examined for agglutination.

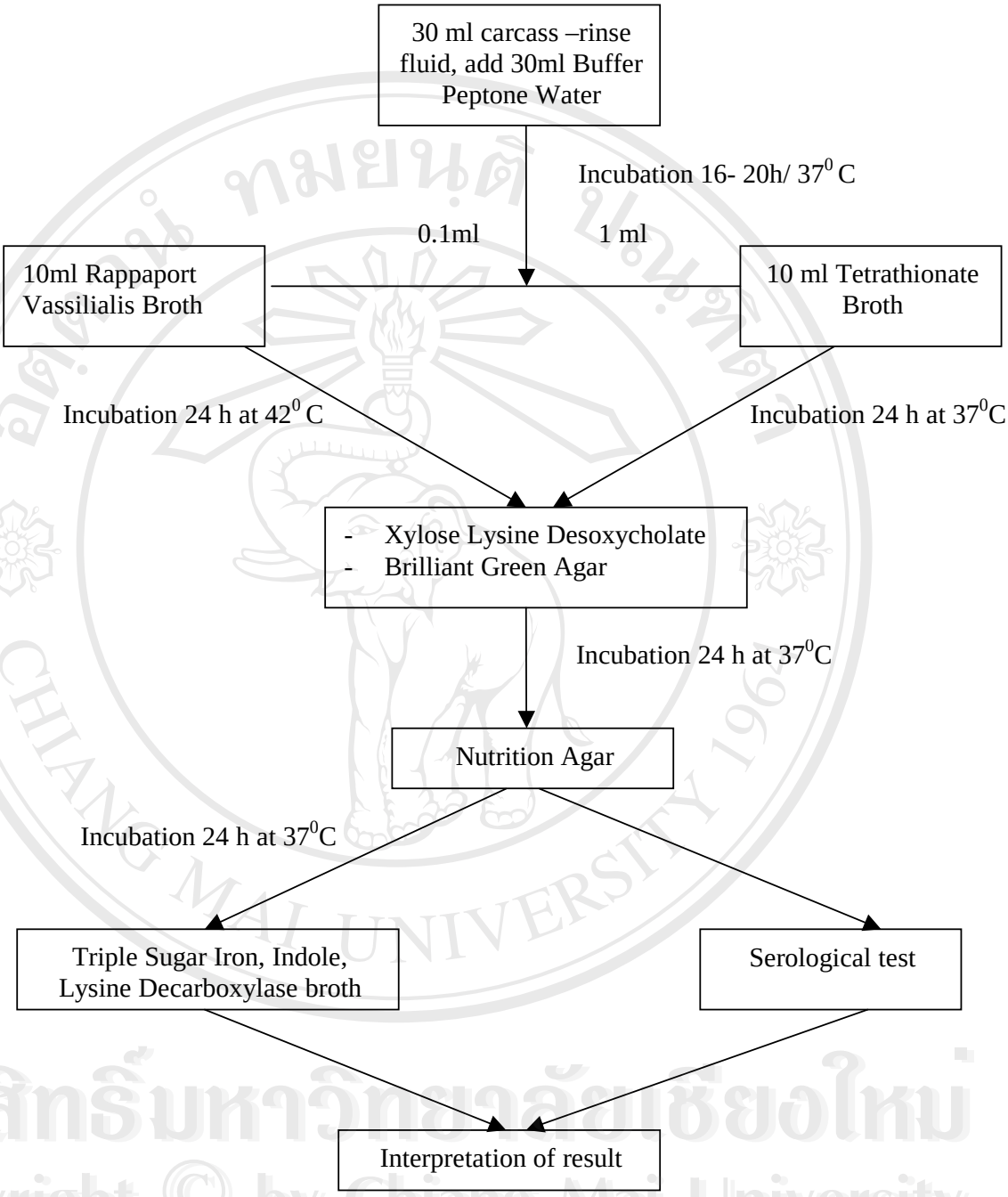


Fig.3. *Salmonella* isolation and identification (ISO 6579:2002)

Table 8: Interpretation for biochemical test of *Salmonella* (FU Berlin, Germany, 2004)

| Reactions in case of Salmonella | |
|---|---|
| Glucose | + |
| Gas | + |
| Lactose | - |
| Sacchar | - |
| $H_2S$ | + |
| Urease | - |
| Lysine Decarboxylase broth | + |
| Voges- Proskauer | - |
| Indol | - |

(+) = Positive (-) = Negative

Samples were tested first against polyvalent antisera (Group A –I and F -67) at the Center Laboratory of the Sub-Department of Animal Health, Ho Chi Minh City, Vietnam, then against each antisera group at the Region Center for Veterinary Public Health, Chiang Mai, Thailand. The full antigenic formulas were determined by the somatic (O) antigen agglutination test, and the flagellar (H) antigen agglutination tests (Figure 4).

**Somatic (O) antigen agglutination test**: At a minimum, isolates should be tested with polyvalent O antiserum reactive with serogroups A through I. Test for O group A through I should encompass the majority of the *Salmonella* serotypes commonly recovered from meat and poultry products. If there is agglutination with the saline control alone (autoagglutination), identify such a culture by biochemical reactions only. If the saline control does not agglutinate and the polyvalent serum does, test the culture with *Salmonella* O grouping antisera. Record positive results and proceed to H agglutination test.

**Serological test**: A smooth colony of *Salmonella* was serotyped by emulsifysing in a drop of 0.85 % saline on a clean microscope slide. A drop of antiserum was well mixed with one drop of *Salmonella* suspension. The slide was rocked gently for about 30 seconds and the antigen- antibody mixture examined for agglutination. The

*Salmonella* is first tested against antisera to the O (somatic) antigens and then the H (flagella) antigens. The test was performed first with polyvalent O antiserum. A saline control was always used in each isolate.

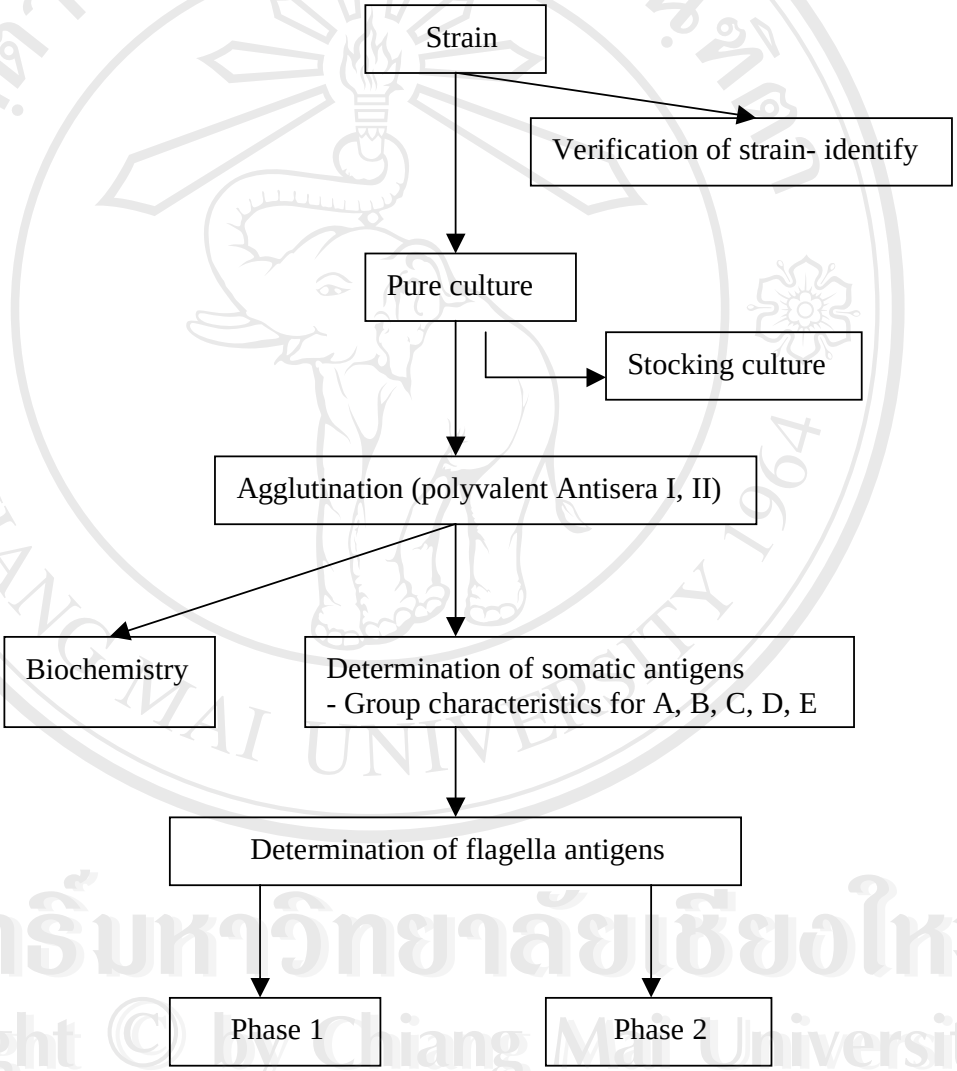




Fig.4 Flow chart for serotyping (Fries, 2005)

**Flagellar (H) Antigen Agglutination Tests**

10ml liquefied warm agar (Nutrient Agar, Sifin, Berlin) was poured into a petri dish of 6 cm diameter. The medium was used for isolate incubation at 35 ± 1°C overnight. A drop of antiserum of the H (flagella) antigens was well mixed with one drop of *Salmonella* suspension. The slide was rocked gently for about 30 seconds and the antigen- antibody mixture examined for agglutination.

### 3.6.2 *Campylobacter* isolation and identification

*Campylobacter* isolation was done as described by ISO10272 (1995) (Figure 5 and Table 9). Briefly, 30 ml of carcass-rinse fluid was added to 30 ml of enriched *Campylobacter* selective medium (Bolton broth, Oxoid, CM 983) with 5% lysed horse blood, polymyxin B (10,000IU/l), rifampicin (20mg/l), trimethoprim (20mg/l), cycloheximide (0,2mg/l) and mixed well. The whole process was always done under micro-aerophilic conditions (7% $O_2$, 10% $CO_2$ and 83% $N_2$) at $42^0$ C for 24h. One ml of the culture was then transferred to Karmali Agar (Oxoid, CM 935) with Sodium pyruvate (0,1mg/l), Cefoperazone (0,032mg/l), Vancomycin (0,02mg/l), Amphotericine (0,01mg) and incubated under micro-aerobic conditions for 1 to 5 days at $42^0$C. The growth of bacteria was checked daily. Typical colonies from Karmali Agar (round or irregular-shaped, white to clear with smooth edges) were harvested and examined with oil under dark field or with oil under 1000× phase-contrast microscope. The colony was first emulsified in a drop of saline or buffer and then placed on a slide covered with slip. The typical colony was also streaked onto Brucella Medium Base agar (Oxiod, CM169) with 5% of inactivated sheep blood and incubated under the above mentioned conditions for 24h. A characteristic colony was examined under a phase- contrast microscope for typical spiral-shaped cells with rapid motility. Gram staining and biochemical tests (catalase, Triple Sugar Iron, oxidase, Hippurat-Hydrolysis) and a test of resistance against nalidixic acid were performed.

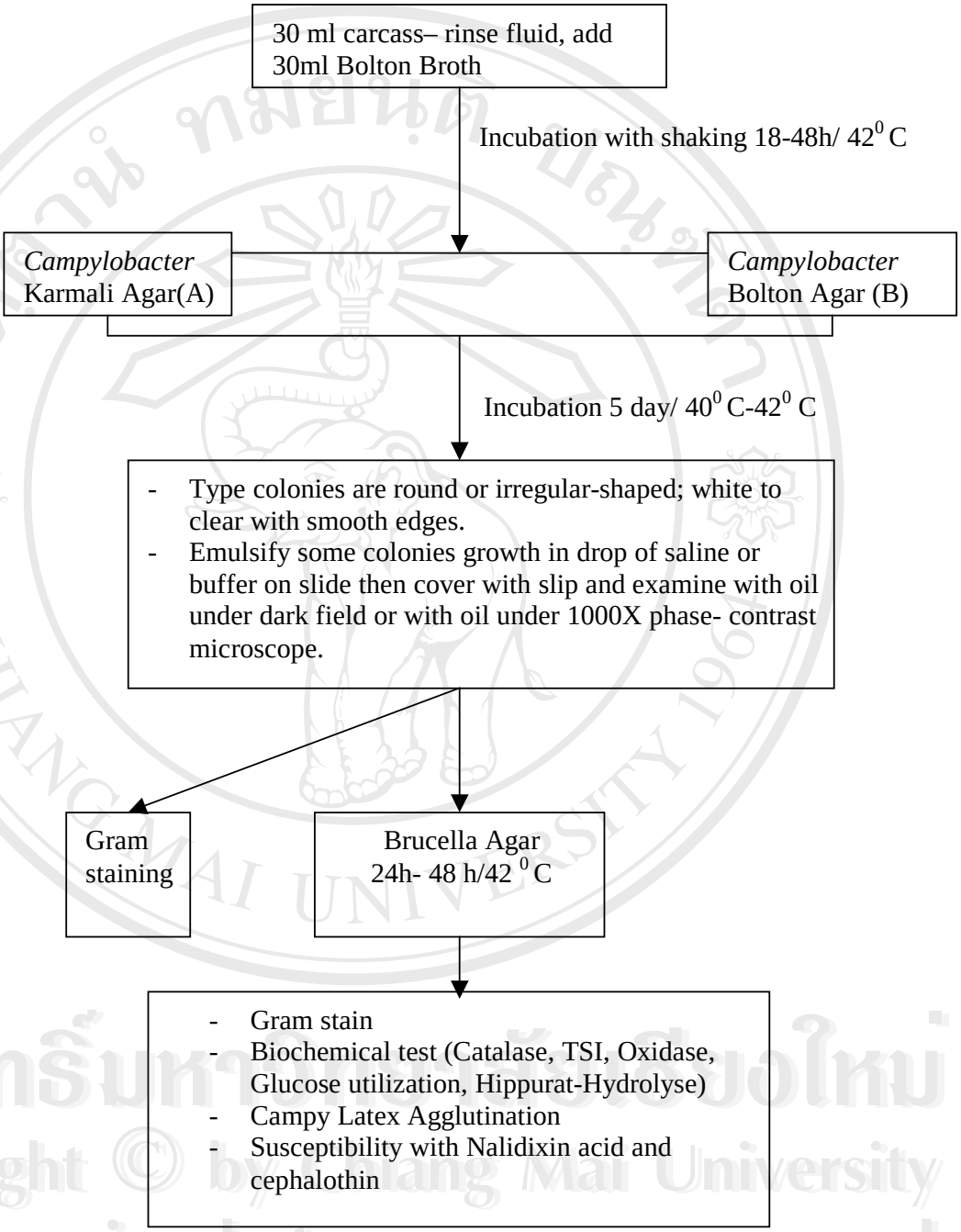


Fig.5 *Campylobacter* isolation and identification (ISO 10272) ISO10272: 1995)

Table 9: Differentiation of *Campylobacter* species (Quinn *et al*., 1998)

| Species | Growth at | | | $H_2S$ production | | | Susceptibility to (30mg/ disc) | |
|---|---|---|---|---|---|---|---|---|
| | $42^0$ C | Catalase | Oxidase | Lead acetate | TSI | Hydrolysis hippurate | Nalidixic acid | Cephalothin |
| *C. jejuni* | + | + | + | + | - | + | S | R |
| *C. coli* | + | + | + | + | + | - | S | R |
| *C. laridis* | + | + | + | + | - | - | R | R |
| *C. upsaliensis* | + | V | + | | - | - | S | S |
| *C. mucosalis* | | - | + | + | + | | V | S |
| *C. cryaerophila* | - | | + | + | - | | S | R |
| *C. fefus subsp veneealis* | - | + | + | - | - | | R | S |
| *C. sputorum boivar fecalis* | - | + | + | + | + | | R | S |

Key: + =Positive - = Negative S = Sensitive R = Resistant

The Campy Latex Agglutination test (Oxiod, Dryspot *Campylobacter* test) containing *Campylobacter jejuni, Campylobacter coli, Campylobacter lari, Campylobacter upsaliensis* antiserum was used. A loopful of a colony with morphology suggestive for *Campylobacter* spp. from Brucella agar after 48 hours of incubation was mixed with one drop of extraction reagent 1 (acetic acid 1.2M) in a tube and let to stand for 3 minutes. Two drops of the extraction reagent 2 (a neutralising reagent of Tris buffer containing 0.09% sodium azide as a preservative) was added to the mixture and made homogenous. Fifty µl of the mixture were added and mixed onto the test circle containing blue latex particles sensitized with rabbit antibody reactive with 4 selected species of *Campylobacter* cell surface antigens. A control solution containing a neutralized acid extract of appropriate *Campylobacter* organisms in a buffer containing 0.09% sodium azide as a preservative was placed and mixed onto the control circle. A result was recorded as positive if agglutination of latex particles occurred within 3 minutes in both the control and the test circle.

The *Campylobacter* isolates were stored by mixing the overnight Brain Heart Infusion Broth (Oxiod, CM 225) with glycerine 67% (1Vol/ 1Vol) in Eppendorf tubes at – $70^0$C.

### 3.7 Data management and analysis

Laboratory and questionnaire data were entered and stored in database management software MS Excel 2003.

The prevalence estimates of *Salmonella* and *Campylobacter* were determined using the standard formula (i.e. the number of positive carcasses divided by the number of samples examined). The exact binomial confidence limits of prevalence were determined using the Fishers exact Chi-square. Characteristics and distribution of *Salmonella* serotypes were determined and presented in Table and graphs. The data from questionnaires was analysed by analysis of variances in NCSS (version 1997).

# 4. RESULTS

## 4.1 Prevalence of *Salmonella* in chicken carcasses

### 4.1.1 Prevalence of *Salmonella* in chicken carcasses from all abattoirs (small and large abattoirs)

The prevalences of *Salmonella* in 319 broiler carcasses examined during the study period from November 2004 to May 2005 are shown in Table 10. Out of all the samples 136 were found contaminated with *Salmonella* giving an overall prevalence of 42.63%. The prevalence of *Salmonella* contaminations in the large abattoirs of 34.15% was lower than that in the small abattoirs of 47.96%. These two prevalences were significantly ($p = 0.0152$) different. In general, the proportions of *Salmonella*-positive carcasses ranged from 0% (in abattoir 15) to 100% (in abattoir 10). The proportions of *Salmonella* contamination among abattoirs 1 to 15 were significantly different ($p= 0.0001$).

Table10: Prevalence of *Salmonella* in chicken carcasses from abattoirs in Ho Chi Minh City, Vietnam

| Abattoir size | Abattoir ID | No. of samples examined | No. of positive samples | Sample prevalence (%) | 95% Confidence interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower limit | Upper limit |
| Large abattoirs | 1 | 39 | 17 | 43.58 | 27.81 | 60.38 |
| | 2 | 45 | 21 | 46.66 | 31.66 | 62.13 |
| | 3 | 39 | 4 | 10.25 | 2.87 | 24.22 |
| Small abattoirs | 4 | 18 | 7 | 38.88 | 17.30 | 64.25 |
| | 5 | 20 | 12 | 60.00 | 36.05 | 80.88 |
| | 6 | 20 | 7 | 35.00 | 15.39 | 59.22 |
| | 7 | 21 | 14 | 66.66 | 43.03 | 85.41 |
| | 8 | 12 | 10 | 83.33 | 51.59 | 97.91 |
| | 9 | 15 | 10 | 66.66 | 38.38 | 88.18 |
| | 10 | 10 | 10 | 100.00 | 69.15 | 100.00 |
| | 11 | 10 | 4 | 40.00 | 12.16 | 73.76 |
| | 12 | 12 | 6 | 50.00 | 21.09 | 78.91 |
| | 13 | 22 | 7 | 31.81 | 13.86 | 54.87 |
| | 14 | 16 | 7 | 43.75 | 19.75 | 70.12 |
| | 15 | 20 | 0 | 0.00 | 0.00 | 16.84 |
| Total large abattoirs | | 123 | 42 | 34.15 | 25.84 | 43.24 |
| Total small abattoirs | | 196 | 94 | 47.96 | 40.79 | 55.19 |
| Overall | | 319 | 136 | 42.63 | 37.14 | 48.26 |

### 4.1.2 Prevalence of *Salmonella* in chicken carcasses from intensive and backyard farmed chickens

The chicken carcasses were categorized by two types of rearing practices chickens from intensive and from backyard farms (Table 11). The sample prevalence of *Salmonella*-positive carcasses from backyard raised chickens was 22.53% while that observed in carcasses from intensively raised chickens was 48.39%. These two proportions were significantly (p= 0.0001) different.

Table 11: Prevalence of *Salmonella* in chicken carcasses from intensive and backyard farmed chicken in Ho Chi Minh City, Vietnam

| Type of chicken farm | No. of samples examined | No. of positive samples | Sample prevalence (%) | 95% Confidence interval | |
|---|---|---|---|---|---|
| | | | | Lower limit | Upper limit |
| Intensive farm | 248 | 120 | 48.39 | 42.00 | 54.80 |
| Backyard farm | 71 | 16 | 22.53 | 13.46 | 34.00 |

### 4.1.3 Prevalence of *Salmonella* in chicken carcasses by different provinces in South Vietnam

The prevalences of *Salmonella* in 319 carcass samples examined distributed by provincial sources of the chickens are shown in Table 12. These prevalences ranged from 0% (0/4) to 100% (10/10). The *Salmonella* positive-carcass rate was significantly different between provinces 1 to 9 of South Vietnam (p= 0.0001).

Table12: Prevalence of *Salmonella* in chicken carcasses from different provinces in South Vietnam

| Province ID | No. of samples per abattoir size | | | No. of positive samples | Sample prevalence (%) | 95% Confidence interval | |
|---|---|---|---|---|---|---|---|
| | Large | Small | Overall | | | Lower limit | Upper limit |
| 1 | 26 | 20 | 46 | 15 | 32.61 | 19.53 | 48.02 |
| 2 | 43 | 73 | 116 | 49 | 42.24 | 33.13 | 51.76 |
| 3 | 34 | 41 | 75 | 41 | 54.67 | 42.75 | 66.21 |
| 4 | 16 | 0 | 16 | 2 | 12.50 | 1.55 | 38.35 |
| 5 | 0 | 10 | 10 | 10 | 100.00 | 69.15 | 100.00 |
| 6 | 0 | 10 | 10 | 6 | 60.00 | 26.24 | 87.84 |
| 7 | 0 | 30 | 30 | 9 | 30.00 | 14.73 | 49.4 |
| 8 | 4 | 0 | 4 | 0 | 0.00 | 0.00 | 60.24 |
| 9 | 0 | 12 | 12 | 6 | 50.00 | 21.09 | 78.91 |
| Total | 123 | 196 | 319 | 136 | 42.63 | 37.14 | 48.26 |

4.1.4 Prevalence of *Salmonella* contamination in carcasses by abattoirs that used machine (automatic) or manual stunning and scalding (and evisceration)

The prevalences of *Salmonella*-positive chicken carcasses from the abattoirs using different methods of stunning, scalding and evisceration are shown in Table 13. In the two abattoirs that used automatic machines a prevalence of 45.24% was observed while, in the 13 abattoirs that used manual (hand), a prevalence of 41.70% was observed. There was no significant (p =0.5738) difference between these two proportions.

Table 13: Prevalence of *Salmonella* isolates in chicken carcasses in two types of processing (stunning, scalding and evisceration)

| Methods of stunning/scalding/evisceration per abattoir | No. of samples examined | No. of positive samples | Sample prevalence (%) | 95% Confidence interval | |
|---|---|---|---|---|---|
| | | | | Lower limit | Upper limit |
| Automatic machine (n = 2) | 84 | 38 | 45.24 | 34.34 | 56.48 |
| Manual (n =13) | 235 | 98 | 41.70 | 35.33 | 48.29 |

n= Number of abattoirs

4.1.5 Distribution of prevalence of *Salmonella* contamination chicken carcasses in abattoirs by frequency of cleaning during slaughter

The prevalence of *Salmonella* contamination in chicken carcasses was 34.58% in abattoirs that were cleaned at least twice during slaughtering (Table 14). But, it was 59.05% in all those that were only cleaned once. The two percentages were significantly ($\chi^2$ = 17.24, df = 2, p = 0.0001) different.

Table 14: Prevalence of *Salmonella* contamination in chicken carcasses in abattoirs by frequency of cleaning

| Frequency of cleaning abattoir | No. of samples per abattoir size | | | No. of positive samples | Prevalence (%) | 95% Confidence interval | |
|---|---|---|---|---|---|---|---|
| | Large | Small | Overall | | | Lower limit | Upper limit |
| At least twice (n= 8) | 123 | 91 | 214 | 74 | 34.58 | 28.23 | 41.37 |
| Once (n= 7) | 0 | 105 | 105 | 62 | 59.05 | 49.02 | 68.55 |

n= Number of abattoir

### 4.1.6 Prevalence of *Salmonella* contamination in chicken carcasses by abattoirs using either chlorinated or unchlorinated in washing water

There were only three abattoirs that used chlorinated water at 100ppm out of the 15 abattoirs (Table 15). A prevalence of 24.04% of *Salmonella* contaminated chicken carcasses was observed in them. In the rest (12) of the abattoirs that did not use chlorinated water for washing a prevalence of 51.63% was obtained. A significant (p = 0.0001) difference between these two proportions was obtained.

Table 15: Prevalence of *Salmonella* contamination of chicken carcasses in abattoirs using either chlorinated or unchlorinated washing water

| Chlorinated water for washing | No. of samples per abattoir size | | | No. of positive samples | Sample prevalence (%) | 95% Confidence interval | |
|---|---|---|---|---|---|---|---|
| | Large abattoirs | Small abattoirs | Overall | | | Lower limit | Upper limit |
| Yes (n =3) | 84 | 20 | 104 | 25 | 24.04 | 16.20 | 33.41 |
| No (n = 12) | 39 | 176 | 215 | 111 | 51.63 | 44.73 | 51.63 |

n = Number of abattoirs

4.1.7 *Salmonella* serotypes obtained from chicken carcasses in abattoirs, Ho Chi Minh City, Vietnam

The overall distribution of *Salmonella* serogroups in chicken carcasses is shown in Table 16. One hundred and sixteen *Salmonella* isolates out of 136 (20 isolates could not be re-cultured after transportation from Vietnam to Thailand) belonged to four somatic serogroups (B, C, E and F- 67). A proportion of 65.52% of the isolates belonged to group C followed by 25% in serogroup B, 7.76% in serogroup E and only 1.72% in serogroup F-67.

Table 16: *Salmonella* serogroups from chicken carcasses from abattoirs in Ho Chi Minh City, Vietnam

| Serogroup | | | | No. of *Salmonella* isolates | Prevalence (%) | 95% Confidence interval | |
|---|---|---|---|---|---|---|---|
| B | C | E | F- 67 | | | Lower limit | Upper limit |
| + | - | - | - | 29 | 25.00 | 17.40 | 33.90 |
| - | + | - | - | 76 | 65.52 | 56.10 | 74.10 |
| - | - | + | - | 9 | 7.76 | 3.61 | 14.22 |
| - | - | - | + | 2 | 1.72 | 0.21 | 6.09 |
| Overall | | | | 116 | 100 | | |

(+): Positive

The distributions of *Salmonella* serotypes obtained from the 319 chicken carcasses are shown in Table 17 and 18. All the 116 *Salmonella* isolates obtained belonged to 19 serotypes. The *S.* Typhimurium, *S.* Derby, *S.* Schwarzengrund, *S.* Stanley and *S.* Agona belonged to serogroup B, *S.* Galiema, *S.* Mbandaka and *S.* Virchow to serogroup $C_1$, *S.* Alminko, *S.* Bardo, *S.* Corvallis, *S.* Emek, S. Haardt, *S.* Hindmarsh, *S.* Reubeuss, and *S.* Thompson to serogroup $C_3$ and *S.* London and *S.* Nchanga belonged to serogroup $E_1$. No specific serotype belonged to F- 67.

Table 17: Serovars of *Salmonella* isolated from chicken carcasses in abattoirs, Ho Chi Minh City, Vietnam

| Somatic (O) Serogroups | Serotype (Serovar) | Abattoir | | | | | | | | | | | | | | Total | Proportion (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | | |
| B | *S.* Agona | - | - | 1 | - | 1 | - | - | 1 | - | - | - | 1 | - | - | 4 | 3.45 |
| B | *S.* Derby | 3 | 1 | - | - | - | - | 5 | - | - | - | - | - | 1 | - | 10 | 8.62 |
| B | *S.* Schwarzengrund | 1 | - | - | - | 1 | - | - | - | 1 | - | - | - | - | - | 3 | 2.59 |
| B | *S.* Stanley | - | - | - | - | - | - | 1 | - | - | 1 | - | - | - | - | 2 | 1.72 |
| B | *S.* Typhimurium | 1 | 1 | - | - | 1 | - | 3 | 3 | - | - | - | - | - | - | 9 | 7.76 |
| B | O 4,5,12:b: | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | 1 | 0.86 |
| C1 | *S.* Galiema | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | 0.86 |
| C1 | *S.* Mbandaka | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | 1 | 0.86 |
| C1 | *S.* Virchow | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | 1 | 0.86 |
| $C_3$ | *S.* Corvallis | - | - | - | 2 | 2 | - | - | - | - | - | - | - | - | - | 4 | 3.45 |
| C3 | *S.* Alminko | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | 1 | 0.86 |
| C3 | *S.* Bardo | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | 1 | 0.86 |
| C3 | *S.* Emek | 8 | 5 | - | 3 | 5 | - | 1 | | 4 | 2 | | 3 | 5 | 2 | 38 | 32.76 |
| C3 | *S.* Haardt | - | 6 | - | - | - | 4 | 2 | 3 | 1 | 3 | 1 | - | - | 2 | 22 | 19.0 |
| C3 | *S.* Hindmarsh | - | 1 | - | - | - | - | - | - | - | - | 1 | - | - | - | 2 | 1.72 |
| C3 | *S.* Reubeuss | - | - | - | 1 | - | - | - | - | 1 | - | - | - | - | 1 | 3 | 2.59 |
| C3 | *S.* Thompson | - | - | - | - | 1 | - | 1 | - | - | - | - | - | - | - | 2 | 1.72 |
| E | *S.* London | - | 2 | - | - | 1 | 2 | - | - | - | 1 | 1 | 1 | - | - | 8 | 6.90 |
| E | *S.* Nchanga | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | 1 | 0.86 |
| F- *67* | | 1 | - | - | - | - | - | 1 | - | - | - | - | - | - | - | 2 | 1.72 |

Proportionally, the serovars isolated from chicken carcasses were *S.* Emek (32.76%), *S.* Haardt (19.0%), *S.* Derby (8.82%)*S.* Typhimurium (7.76%), *S.* London (6.90%), *S.* Agona (3.45%), *S.* Corvallis (3.45%), *S.* Reubeuss (2.59%), *S.* Schwarzengrund (2.59%) *S.* Hindmarsh (1.72 %), *S.* Stanley (1.72 %) and *S.* Thompson (1.72 %). Serotypes such as *S.* Alminko, *S.* Bardo, *S.* Mbandaka, *S.* Nchanga and *S.* Galiena, *S.* Virchow had only one isolate. Overall, *S.* Emek and *S.* Haardt had high proportions in both small and large abattoirs.

Table18: Distribution of *Salmonella* serotypes in chicken carcasses by abattoir sizes, Ho Chi Minh City, Vietnam

| Somatic (O) serogroups | Serotypes (Serovar) | No. of serotypes per abattoir size | | Total | (%) |
|---|---|---|---|---|---|
| | | Large abattoirs | Small abattoirs | | |
| B | *S.* Agona | 1 | 3 | 4 | 3.45 |
| B | *S.* Derby | 4 | 6 | 10 | 8.82 |
| B | *S.* Schwarzengrund | 1 | 2 | 3 | 2.59 |
| B | *S.* Stanley | 0 | 2 | 2 | 1.72 |
| B | *S.* Typhimurium | 2 | 7 | 9 | 7.76 |
| B | O 4,5,12:b: | 1 | 0 | 1 | 0.86 |
| $C_1$ | *S.* Galiena | 1 | 0 | 1 | 0.86 |
| $C_1$ | *S.* Mbandaka | 0 | 1 | 1 | 0.86 |
| $C_1$ | *S.* Virchow | 1 | 0 | 1 | 0.86 |
| $C_3$ | *S.* Corvallis | 0 | 4 | 4 | 3.45 |
| $C_3$ | *S.* Alminko | 0 | 1 | 1 | 0.86 |
| $C_3$ | *S.* Bardo | 0 | 1 | 1 | 0.86 |
| $C_3$ | *S.* Emek | 13 | 25 | 38 | 32.76 |
| $C_3$ | *S.* Haardt | 6 | 16 | 22 | 19.0 |
| $C_3$ | *S.* Hindmarsh | 1 | 1 | 2 | 1.72 |
| $C_3$ | *S.* Reubeuss | 0 | 3 | 3 | 2.59 |
| $C_3$ | *S.* Thompson | 0 | 2 | 2 | 1.72 |
| $E_1$ | *S.* London | 2 | 6 | 8 | 6.90 |
| $E_1$ | *S.* Nchanga | 1 | 0 | 1 | 0.86 |
| F- 67 | | 1 | 1 | 2 | 1.72 |
| Total | | 35 | 81 | 116 | 100 |

## 4.2 Prevalence of *Campylobacter* in chicken carcasses

### 4.2.1 Prevalence of *Campylobacter* in chicken carcasses from small and large abattoirs in Ho Chi Minh City

The prevalences of *Campylobacter* from 319 broiler carcasses are shown in Table19. A total of 112 samples were contaminated with *Campylobacter* giving an overall sample prevalence of 35.11%. In general, the proportions of positive carcasses ranged from 0% (abattoir 11) to 50% (abattoirs 4, 10 and 12). However, no significant ($p = 0.1302$) differences were found among the abattoir-specific prevalences. In the large abattoirs the overall sample prevalence was 36.58% and in small abattoirs 34.18%. These two values were not different ($p = 0.6618$). There was no significant ($p= 0.194$) differences between the prevalences of *Campylobacter* in carcasses within the 3 large abattoirs. Similarly, no significant ($p = 0.1175$) differences were found within small abattoir prevalences.

Table 19: Distribution of prevalences of *Campylobacter* in chicken carcasses by abattoir sizes in Ho Chi Minh City, Vietnam

| Abattoir size | Abattoir ID | No. of samples examined | No. of samples positive | Sample prevalence (%) | 95% Confidence Interval of sample prevalence | |
|---|---|---|---|---|---|---|
| | | | | | Lower limit | Upper limit |
| Large abattoir | 1 | 39 | 10 | 25.64 | 13.04 | 42.12 |
| | 2 | 45 | 20 | 44.44 | 29.64 | 60.00 |
| | 3 | 39 | 15 | 38.46 | 23.36 | 55.38 |
| Small abattoir | 4 | 18 | 9 | 50.00 | 26.02 | 73.98 |
| | 5 | 20 | 9 | 45.00 | 23.06 | 68.47 |
| | 6 | 20 | 3 | 15.00 | 3.21 | 37.89 |
| | 7 | 21 | 8 | 38.09 | 18.11 | 61.56 |
| | 8 | 12 | 3 | 25.00 | 5.49 | 57.19 |
| | 9 | 15 | 6 | 40.00 | 16.34 | 67.71 |
| | 10 | 10 | 5 | 50.00 | 18.71 | 81.29 |
| | 11 | 10 | 0 | 0.00 | 0.00 | 30.85 |
| | 12 | 12 | 6 | 50.00 | 21.09 | 78.91 |
| | 13 | 22 | 8 | 36.36 | 17.20 | 59.34 |
| | 14 | 16 | 6 | 37.50 | 15.20 | 64.57 |
| | 15 | 20 | 4 | 20.00 | 5.73 | 43.66 |
| All large abattoirs | | 123 | 45 | 36.58 | 28.09 | 45.75 |
| All small abattoirs | | 196 | 67 | 34.18 | 27.57 | 41.28 |
| Overall | | 319 | 112 | 35.11 | 29.87 | 40.62 |

### 4.2.2 Prevalence of *Campylobacter* in chicken carcasses from intensive and backyard farmed chickens

The sample prevalence of *Campylobacter* in carcasses of chickens from intensive farms was 34.68 %, while that obtained from carcasses of chickens from backyard farms was 36.62 % (Table 20). There was no significant (p= 0.7624) difference between these two percentages.

Table 20: Prevalence of *Campylobacter* in chicken carcasses from intensive farms chicken and backyard farms chicken

| Type of chicken farm | No. of samples examined | No. of samples positive | Sample prevalence (%) | 95% Confidence interval | |
|---|---|---|---|---|---|
| | | | | Lower limit | Upper limit |
| Intensive farm | 248 | 86 | 34.68 | 28.77 | 40.96 |
| Backyard farm | 71 | 26 | 36.62 | 25.49 | 48.89 |

4.2.3. Prevalence of *Campylobacter* isolates in chicken carcasses of chickens from different provinces in South of Vietnam

Prevalences of *Campylobacter*-positive carcasses distributed by different provincial sources of chickens are shown in Table 21. In general, the proportions ranged from 0% (0/10) to 66.67% (8/12). There was no significant (p= 0.1108) differences among these prevalences.

Table 21: Prevalence of *Campylobacter* from chicken carcasses from different provinces in the South of Vietnam

| Province ID | No. of samples per abattoir size | | | No. of positive samples | Sample prevalence (%) | 95% Confidence interval | |
|---|---|---|---|---|---|---|---|
| | Large | Small | Overall | | | Lower limit | Upper limit |
| 1 | 26 | 20 | 46 | 17 | 36.96 | 23.21 | 52.45 |
| 2 | 43 | 73 | 116 | 36 | 31.03 | 22.77 | 40.29 |
| 3 | 34 | 41 | 75 | 27 | 36.00 | 25.23 | 47.91 |
| 4 | 16 | 0 | 16 | 6 | 37.50 | 15.20 | 64.57 |
| 5 | 0 | 10 | 10 | 5 | 50.00 | 18.71 | 81.29 |
| 6 | 0 | 10 | 10 | 0 | 0.00 | 0.00 | 30.85 |
| 7 | 0 | 30 | 30 | 11 | 36.67 | 19.93 | 56.14 |
| 8 | 4 | 0 | 4 | 2 | 50.00 | 6.759 | 93.24 |
| 9 | 0 | 12 | 12 | 8 | 66.67 | 34.89 | 90.08 |
| Total | 123 | 196 | 319 | 112 | 35.11 | 29.88 | 40.62 |

4.2.4 Prevalence of *Campylobacter* contamination in chicken carcasses by abattoir that used machine (automatic) or manual stunning and scalding (and evisceration)

The prevalences of *Campylobacter*-positive chicken carcasses from the abattoirs using different methods of stunning, scalding and evisceration are shown in Table 22. In the two abattoirs that used automatic machines, a prevalence of 35.71% was observed, while in the 13 abattoirs that used manual (hand) power a prevalence of 34.89%, was observed. There was no significant (p= 0.8924) difference between these two proportions.

Table 22: Prevalence of *Campylobacte*r isolates in chicken carcasses in two types of processing (stunning, scalding and evisceration)

| Methods of stunning/scalding/evisceration per abattoir | No. of samples examined | No. of positive samples | Sample prevalence (%) | 95% Confidence interval | |
|---|---|---|---|---|---|
| | | | | Lower limit | Upper limit |
| Automatic machine (n = 2) | 84 | 30 | 35.71 | 25.55 | 46.92 |
| Manual (n =13) | 235 | 82 | 34.89 | 28.81 | 41.36 |

n= Number of abattoirs

4.2.5. Distribution of prevalence of *Campylobacter* contamination chicken carcasses in abattoirs by frequency of cleaning during slaughter

The prevalence of *Campylobacter* contamination in chicken carcasses was 27.57% in abattoirs that were cleaned at least twice during slaughtering (Table 23). But, it was 50.48% in all those that were only cleaned once. The two percentages were significantly (p = 0.006) different.

Table 23: Prevalence of *Campylobacter* contamination in chicken carcasses in abattoirs by frequency of cleaning

| Frequency of cleaning abattoir | No. of samples per abattoir size | | | No. of positive samples | Sample prevalence (%) | 95% Confidence interval | |
|---|---|---|---|---|---|---|---|
| | Large | Small | Overall | | | Lower limit | Upper limit |
| At least twice (n= 8) | 123 | 91 | 214 | 59 | 27.57 | 21.70 | 34.08 |
| Once (n= 7) | 0 | 105 | 105 | 53 | 50.48 | 40.55 | 60.38 |

n= Number of abattoirs

### 4.2.6 Prevalence of *Campylobacter* contamination in chicken carcasses by abattoirs using either chlorinated or unchlorinated in washing water

There were only three abattoirs that used chlorinated water at 100ppm out of the 15 abattoirs (Table 24). A prevalence of 37.50% of *Campylobacter* contaminated chicken carcasses was observed in them. In the rest (12) of the abattoirs that did not use chlorinated water for washing a prevalence of 33.95% was obtained. No significant (p = 0.5338) difference between these two proportions was obtained.

Table 24: Prevalence of *Campylobacter* contamination of chicken carcasses in abattoirs using either chlorinated or unchlorinated washing water

| Chlorinated water for washing | No. of samples per abattoir size | | | No. of positive samples | Prevalence (%) | 95% Confidence interval | |
|---|---|---|---|---|---|---|---|
| | Large abattoirs | Small abattoirs | Overall | | | Lower limit | Upper limit |
| Yes (n =3) | 84 | 20 | 104 | 39 | 37.50 | 28.19 | 47.53 |
| No (n = 12) | 39 | 176 | 215 | 73 | 33.95 | 27.65 | 40.70 |

n= Number of abattoirs

### 4.3 Combined *Campylobacter* and *Salmonella* contamination of chicken carcasses

The occurrences of *Campylobacter* and *Salmonella* in 319 chicken carcass samples examined in this study are shown in Tables 25 and 26. In Table 25, 17.87% of the samples were contaminated with both *Salmonella* and *Campylobacter.* Singly, *Salmonella* was found in 42.63% of the samples while, *Campylobacter* was found in 35.11% of them. In general, these percentages were significantly ($p = 0.0001$) different. The difference occurred due to the low proportion (17.87%) of the combined *Salmonella* and *Campylobacter* contamination of the carcasses. The other two proportions (42.63% and 35.11%) were marginally ($p = 0.05735$) significantly different at significance level of $\alpha = 0.05$.

Table 25: Prevalence of *Campylobacte* and *Salmonella* in chicken carcasses from abattoirs in Ho Chi Minh City, Vietnam

| Code | *Salmonella* | *Campylobacter* | No. of samples | Sample Prevalence (%) | 95% confidence interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower limit | Upper limit |
| 1 | + | + | 57 | 17.87 | 13.82 | 22.52 |
| 2 | + | - | 136 | 42.63 | 37.14 | 48.26 |
| 3 | - | + | 112 | 35.11 | 29.87 | 40.62 |

(1): Carcasses positive both *Campylobacter* and *Salmonella*

(2): Carcasses positive *Salmonella*

(3): Carcasses positive *Campylobacter*



The proportions of contaminations of carcasses with combined *Salmonella* and *Campylobacter* ranged from 0% (abattoirs 11 and 15) to 50% (abattoir 10). Overall, there was significant ($p= 0.000328$) difference among these abattoir-specific proportions. But, no significant ($p =0.1349$) difference was observed between the proportions of the large and small abattoirs.

Table 26: Distribution of sample prevalences of combined *Salmonella* and *Campylobacter* in chicken carcasses by abattoirs in Ho Chi Minh City, Vietnam,

| Type of abattoir size | Abattoir ID | No. of samples examined | No. of samples positive | Sample prevalence (%) | 95% Confidence interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower limit | Upper limit |
| Large abattoir | 1 | 39 | 5 | 12.82 | 4.30 | 27.43 |
| | 2 | 45 | 12 | 26.67 | 14.60 | 41.94 |
| | 3 | 39 | 1 | 2.56 | 0.06 | 13.48 |
| Small abattoir | 4 | 18 | 4 | 22.22 | 6.41 | 47.64 |
| | 5 | 20 | 6 | 30.00 | 11.89 | 54.28 |
| | 6 | 20 | 2 | 10.00 | 1.23 | 31.70 |
| | 7 | 21 | 4 | 19.05 | 5.45 | 41.91 |
| | 8 | 12 | 3 | 25.00 | 5.49 | 57.19 |
| | 9 | 15 | 6 | 40.00 | 16.34 | 67.71 |
| | 10 | 10 | 5 | 50.00 | 18.71 | 81.29 |
| | 11 | 10 | 0 | 0.00 | 0.00 | 30.85 |
| | 12 | 12 | 5 | 41.67 | 15.17 | 72.33 |
| | 13 | 22 | 2 | 9.09 | 1.12 | 29.16 |
| | 14 | 16 | 2 | 12.50 | 1.56 | 38.35 |
| | 15 | 20 | 0 | 0.00 | 0.00 | 16.84 |
| All large abattoir | | 123 | 17 | 13.82 | 8.26 | 21.20 |
| All small abattoir | | 196 | 40 | 20.41 | 14.99 | 26.74 |
| Overall | | 319 | 57 | 17.87 | 13.82 | 22.52 |

### 4. 4 Questionnaire results

The questionnaire results are summarized in Table 27. In 14 out of 15 abattoirs the transportation crates used for chickens from farm to abattoir were cleaned 93.3% of the time after unloading. Furthermore, all (100%) abattoirs were cleaned and disinfected after work using chlorine of about 2-3% in water.

In addition, workers in all abattoirs used protective clothing (100%). Before working, they were trained to implement hygiene in the abattoir. The workers were checked for their health condition.

Table 27: Distribution of variables from abattoir in Ho Chi Minh City, Vietnam

| Variable | Type of abattoir | | |
|---|---|---|---|
| | Large (n= 3) | Small (n=12) | All (n=15) |
| Cleaning and disinfection the crate | | | |
| Yes | 2 (20%) | 11 (73.3%) | 14 (93.3%) |
| No | 0 (0%) | 1 (6.7%) | 1 (6.7%) |
| Stunning | | | |
| Electricity | 2 (13.3%) | 0 (0%) | 2 (13.3%) |
| Knife | 1 (6.7%) | 12 (80%) | 13 (87%) |
| Scalding | | | |
| Controlled (56- 58$^0$C) | 2 (13.3%) | 0 (0%) | 2 (13.3%) |
| Uncontrolled (55- 68$^0$C) | 1 (6.7%) | 12 (80%) | 13 (87%) |
| Evisceration | | | |
| On- line | 2 (13.3%) | 0 (0%) | 2 (13.3%) |
| On table | 1 (6.7%) | 12 (80%) | 13 (87%) |
| Washing water using chemicals | | | |
| Yes | 2 (13.3%) | 1 (6.7%) | 3 (20%) |
| No | 1 (6.7%) | 11 (73.3%) | 12 (80%) |
| Workers using protective- clothing | 3 (20%) | 12 (80%) | 15 (100%) |
| Cleaning before and after working | 3 (20%) | 12 (80%) | 15 (100%) |
| Cleaning during working time | | | |
| ≥ Twice | 3 (20%) | 5 (33.3%) | 8 (53%) |
| < Twice | 0 (0%) | 7 (46.7%) | 7 (46.7%) |
| Disinfection | | | |
| Once | 3 (20%) | 12 (80%) | 15 (100%) |
| Twice | 0 (0%) | 0 (0%) | 0 (0%) |

### 4.5 Identification of protecting or risk factors associated with contaminated carcasses with focussed agents

The contamination of carcasses with *Salmonella* was dependent on several risk factors (Table 28). The contamination level in the chicken carcasses from intensive farming was more than 0.31 times the chicken carcasses from backyard farming (p= 0.001). The contamination level in the small abattoirs was more than 0.56 times greater than the large abattoirs (p= 0.0016).

Three abattoirs used chlorine (100ppm) in the water for washing the chicken carcasses. The percentage of *Salmonella*- positive chicken carcasses was significantly different (p= 0.0001), the contamination level in the abattoirs without chemicals in the washing water was more than 3.37 times higher than in the abattoirs using chlorine.

Use of water to clean the floor at least twice during slaughter time or once: the percentage of *Salmonella*- positive chicken carcasses from these different procedures was significantly different (p= 0.0001). The probability of *Salmonella* contamination in chicken carcasses from abattoirs using the water to clean the floor only once was higher than 2.73 times in carcasses from abattoirs cleaning the floor at least twice cleaning

Table 28: Summary results of Logistic regression of potential risk factors for contamination of chicken carcasses with *Salmonella* from abattoirs in Ho Chi Minh City, Vietnam

| Risk factor | Odds Ratio | 95% Confidence interval | | P- value |
|---|---|---|---|---|
| | | Lower limit | Upper limit | |
| Type of farm chicken (Intensive and backyard) | 0.31 | 16.0 | 59.0 | 0.001 |
| Provinces | 1.072 | 0.964 | 1.192 | 0.0001 |
| Abattoir factors | | | | |
| Type of abattoir (Large and small) | 0.56 | 0.34 | 0.92 | 0.016 |
| Type of plant | 1.15 | 0.68 | 1.77 | 0.547 |
| Hygiene factors | | | | |
| Using the chlorinated in washing water | 3.37 | 1.94 | 5.90 | 0.0001 |
| Cleaning during working | 2.73 | 1.64 | 4.54 | 0.0001 |

# 5. DISCUSSION

## 5.1 *Salmonella*

### 5.1.1 *Salmonella* overall

*Salmonella* was isolated in 42.63% of chicken carcasses from abattoirs in Ho Chi Minh City, Vietnam. The prevalence was highly variable and ranged from 34.15 % in small abattoirs to 47.45 % in the large abattoirs, the contamination among all abattoirs varied between 0 % and 100 %.

The rate of *Salmonella* contamination was higher than it was in other studies in Vietnam but lower than those from countries such as Thailand, Malaysia, and Taiwan. In a survey done in Vietnam (Tran *et al*., 2005) from slaughtered chickens, farm chickens, and retail meat, *Salmonella* spp. was isolated from almost about 20% of the samples. Tran, *et al*. (2005) reported that *Salmonella* was isolated from 21.0% of chicken meat samples. Tran *et al*. (2004) recovered *Salmonella* in 7.9% (24/302) of chicken fecal samples from adult chickens in slaughterhouses. One of the reasons for the higher isolation rate in the present study than in that study might be due to different sampling methods. In this study, the carcass rinse of chicken was used to isolate the *Salmonella*.

In Thailand, Boonmar *et al.* (1998) reported that *Salmonella* was isolated from 72% of retail chicken meat samples, and from 10% chicken meat samples in the slaughterhouse from 80% of samples from open markets and from 64% of samples in supermarkets.



In Malaysia, *Salmonella* was isolated from 35.5% of broiler carcasses (Rusul *et al*., 1996). Bryan *et al.* (1968) and Bailey *et al.* (1990) concluded that the presence of *Salmonella* on live poultry could lead to the introduction of *Salmonella* into processing plants. Olsen *et al.* (2003) confirmed, that the slaughtering of *Salmonella*-

positive birds leads to contamination of the processing line, of the equipment and subsequently to cross-contamination to non-infective chicken.

In studies from developed countries such as the United States, the percentage of *Salmonella* isolated from chicken carcasses was also relatively high. It ranged from 0% to 36% in samples from post-chill carcasses in the United States (Bailey *et al*., 2001) *Salmonella* was found in 40.4% of chicken neck skin samples after the defeathering step in Germany (Fries, 2002). In Japan, the percentage of *Salmonella* was 14.3% of the cecal contents of broiler chickens from commercial farms (Limawongranee *et al*., 1999). In Argentina, the prevalence of *Salmonella* in chicken carcasses after evisceration in commercial slaughter practice was 20.8 % and 20 % from visibly uncontaminated carcasses (Jimenez *et al*. 2002).

### 5.1.2 Type of farming

The higher prevalence of *Salmonella* in carcasses from intensive farming compared to backyard farm chickens may be due to differences of the density of chickens in flocks. Broiler houses contain many thousands of birds. This concentration of potential hosts gives *Salmonella* a better opportunity for infection (Humphrey, 2000) and spread can be rapid through infected flocks. Almost all intensive farm chicken were from the southern provinces, which are located far from the abattoirs. In this study, the occurrence of *Salmonella* in chicken carcasses was significantly different between the provinces where the animal came from ($p= 0.0001$).



### 5.1.3 Technical equipment

Poultry abattoirs in Ho Chi Minh City run with few machines and a great number of workers. This may be one reason for the relatively high occurrence of *Salmonella* contamination compared to other reports. The standard cleaning procedure was not the way to eliminate or to reduce this contamination.

Chicken were transported from the farm to the plant in crates that can hold between 20 and 30 birds each. Crates are usually stacked, meaning that birds in lower cages will become contaminated with the feces of birds in the cages above them. In the present study, the crates were recycled during the working day. In another study, transporting crates were reused with high frequency and so were still contaminated with *Salmonella* and *Campylobacter*. So, crates are to be considered a potential route of infection (Slander *et al*, 2001).

### 5.1.4 Hygienic measures

Statistical analysis showed that the hygiene in the slaughterhouses and the hygiene of the slaughter process in this study were also important for the *Salmonella* built-up. The prevalence of *Salmonella*- positive chicken carcasses was significantly higher in the abattoir using automatic machinery (large abattoirs) than in abattoir using manuel power (small abattoirs). The cause may be an inappropriate handling of the machinery.

Application of an anti-microbial spray in an inside- outside washer has been proposed as a means of treating the interior and exterior of pre- chilled carcasses (Li *et al*., 1997). In the present study, prevalence of *Salmonella* in chicken carcasses was significantly lower in the abattoirs using chlorine in water to wash pre-chilled carcasses.

### 5.1.5 Serotypes of *Salmonella*

Results of the present study indicate that *Salmonella* serogroup B and serogroup C are widely distributed in chickens in this area. Chickens probably play an important role as a reservoir of human *Salmonella* infection in Ho Chi Minh City.

In this study, 19 serovars of *Salmonella* were identified from 116 *Salmonella* isolates. The most common serovars were *S.* Emek, *S.* Haardt, *S.* Typhimurium and *S.*

Derby. In Japan, the predominant *Salmonella* serotype of broiler chicken was *S.* Blockey, *S.* Hadar, and *S.* Bredeney (Akiba *et al.*, 1996). In Thailand, the most common serotypes were *S.* Enteritidis, *S.* Muenchen, *S.* Blockley and *S.* Montevideo from retail chicken meat and *S.* Enteritidis was detected in 73% of one day-old chicken (Boonmar *et al.*, 1998). In Malaysia, the predominant serovars were *S.* Enteritidis, *S.* Muenchen, and *S.* Kentucky (Rusul *et al.*, 1990). In Australia among 1153 *Salmonella* isolates, the most- frequently isolated serovars from poultry was *S.* Sofia (36.6%), S. Virchow (11.3%), *S.* Infantis (10.9%), and *S.* Typhimurium PT64 (3.4%), *S.* Typhimurium PT108 (3.2%) (Sumner *et al*., 2003). In a survey done in Vietnam some years ago from slaughtered chickens, farm chickens, and retail meat in Mekong Delta, the predominant serovars were *S.* Emek, *S.* Typhimurium, *S.* Dessau, and *S.* Derby (Tran *et al*., 2005).

*S.* Enteritidis has become the predominant serovar worldwide (Popoff *et al.*, 2000). However, *S.* Enteritidis was not isolated in chicken carcasses from abattoirs in Ho Chi Minh City in the present study. This result is in accordance to a study of Tran, *et al*. (2004). According to the present results, also chicken meat is not a source of *S.* Enteritidis infections in Ho Chi Minh City.

*Salmonella* Typhimurium was the most common cause for salmonellosis in England and Wales and United States from 1991 to 1995 (Wray, and Wray 2000). In this study, the percentage of *S.* Typhimurium (7.76%) was comparably low.

The good understanding of the epidemiology of *Salmonella* in animals can be used to a effective prevention and control practices that can reduce this zoonotic pathogen in animals and humans. Such data are necessary for further studies about salmonellosis to find out relationships between human and animal sources in Vietnam.

## 5.2 *Campylobacter*

The percentage of *Campylobacter* in broiler carcasses in the present study was lower than that in previous studies (35.11%). Stern and Line (1992) detected *Campylobacter spp* in 98% of retail- packaged broiler samples from grocery stores. The prevalence of *Campylobacter* spp. in poultry and poultry meat products in Germany (Atanassova and Ring, 1999) from poultry flocks was 41,1% *Campylobacter*-positive, whereas from broiler carcasses 45.9% of samples were *Campylobacter*-positive. *Campylobacter jejuni* has frequently been isolated from migratory waterfowl, with a rate ranging from 35% to 75% (Fallacara *et al*., 2001; Savill, 2003). Various studies carried out in slaughterhouses have shown that the main source of the spread of *C. jejuni* on poultry carcasses came from their intestinal contents (Oosterom *et al*., 1983; Berndtson *et al*., 1992). However, the epidemiology of the bacteria it is still not yet complete.

The percentage of *Campylobacter* contamination in chicken carcasses in this study was higher than in a study done in Switzerland where *Campylobacter* was obtained in 24.37% of carcasses (Frediani- Volf, and Stephan, 2003).

In a study in Denmark, for *Campylobacter*, it is well known that lower isolation rates were found during the winter season (dryer) compared to the warm season (raining season) (Pearson *et al*., 1996; Wedderkopp *et al*., 2000). This study was carried out from November to May, (dry season), which may be one reason for a relatively low isolation rate compared to pervious reports. However, the present rate is much lower than a reported rate of 94% of feces testing positive for *Campylobacter* in other areas of the world (Stern and Robach, 2003). One of the reasons for this lower isolation rate might be due to different sampling sites. The caecum is the major colonization of *C. jejuni*, which are increase by use of enrichment or filtration method. These methods were not used, since birds are productive source of *C. jejuni*, recovery of the organisms on selective media would spring little difficulty (Achen *et al*., 1998; Fallacara *et al*., 2001; Jacobs- Reitsma *et al*., 1995).

The prevalence of positive flocks is also dependent on the flock size and type of production systems (Berndtson *et al*., 1996). The transporting crates were reused with high frequency and were often still contaminated with *Salmonella* and *Campylobacter*. Trucks, pellets, crates and catchers were identified as potential sources of *C. jejuni* for broilers (Ramabu *et al*., 2004).

In a study in Quebec, (Canada), macrorestriction profiles showed that approximately 20% of human *Campylobacter* isolated were genetically related to genotypes found in poultry. There was a high prevalence *C. jejuni* biotypes I and II in poultry (Nadeau *et al.*, 2002). In a study done in Hanoi with strains from hospitals, the diarrhoeal rate caused by *Campylobacter* spp. was 9% among total diarrhoeal illness (Phung and Nguyen, 2001). In the present study, the percentage (35.11%) of *Campylobacter* in broiler carcasses could be a potential source of hazard for public health in Ho Chi Minh City.

## 6. CONCLUSIONS

This study was done to assess the prevalence of *Salmonella* and *Campylobacter* spp. in chicken carcasses in 15 abattoirs (large and small) in a southern region in Vietnam. From November 2004 to May 2005, 319 chicken carcass- rinse samples were collected and examined for the presence of *Salmonella* and *Campylobacter*. The samples were obtained from the final product after the inside–outside shower stage of the processing line.

### 6.1 *Salmonella*

The prevalence of *Salmonella* was higher in the small abattoirs than in large abattoirs, the contamination among all abattoirs depended on slaughter equipment and conditions in each abattoir. The data indicate that the hygiene conditions of each abattoir contribute to the contamination of *Salmonella* in chicken carcasses.

The prevalence of *Salmonella* in chicken carcasses from abattoirs using chlorine was lower than in the abattoirs where chlorine in water to wash the chicken carcasses was not used. The hygiene of equipment and the hand contact with the carcasses was also important for the *Salmonella* presence. The prevalence of *Salmonella*- positive chicken carcasses was lower in abattoirs with good hygiene measures before and after slaughter. These data show that hygiene measures contribute to the contamination rate of *Salmonella* in carcasses at the abattoir. Therefore, it is strongly recommended that effective hygienic standards along the poultry slaughter line be implemented.

Futures studies should be set for the hygienic standard for the abattoir and should be performed to clarify the main factors of contamination in poultry processing.



Salmonella isolates belonging to the group B, C, and E. 19 serotypes were obtained *S.* Emek, *S.* Haardt, *S.* Typhimurium, *S.* Derby, and *S.* London were the most dominant serotypes. *S.* Typhimurium was found from five abattoirs.

**6.2 *Campylobacter***

The rate of *Campylobacter* spp. was a little higher in the group of large abattoirs than in the group of small abattoirs. The percentage of *Campylobacter*-positive carcasses from backyard farms was a little higher in than in intensive farms. Intensive chicken farms were came in different provinces of the South of Vietnam. The occurrence of *Campylobacter* in carcass samples was different. Therefore, the flocks have to be recognized as reservoir of *Campylobacter*.

The prevalence of *Campylobacter*-positive chicken carcasses was lower in abattoirs cleaning the floor during slaughtering at least more than the abattoirs cleaning the floor only once during slaughtering.

Overall, the proportion of both *Salmonella* and *Campylobacter* in 319 chicken carcasses was 17.87% (nearly one fifth).

Summarising, the presence of *Salmonella* and *Campylobacter* spp. in chicken carcasses poses a potential for foodborne hazards to humans. Therefore, based on these findings, effective hygienic standards along the poultry slaughter line should be implemented. In addition, further studies should be designed to establish the specific critical points in whole poultry production chain from farm to table.

## APPENDIX A

Appendix 1: Prevalence of *Salmonella* and *Campylobacter* in chicken carcasses: two types of chicken and use of chlorine in washing water in abattoirs, Ho Chi Minh City, Vietnam

| | Lager abattoir | | | | | | Small abattoir | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Abattoir No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Total |
| No. of samples examined | 39 | 45 | 39 | 18 | 20 | 20 | 21 | 12 | 15 | 10 | 10 | 12 | 22 | 16 | 20 | 319 |
| Type of chicken | | | | | | | | | | | | | | | | |
| Intensive farm | 28 | 30 | - | 15 | 20 | 20 | 21 | 12 | 15 | 10 | 10 | 12 | 19 | 16 | 20 | 248 |
| Backyard farm | 11 | 15 | 39 | 3 | - | - | - | - | - | - | - | - | 3 | - | - | 71 |
| Type of water wash | | | | | | | | | | | | | | | | |
| Using chemical | - | 45 | 39 | - | - | - | - | - | - | - | - | - | - | - | 20 | 104 |
| Not using chemical | 39 | - | - | 18 | 20 | 20 | 21 | 12 | 15 | 10 | 10 | 12 | 22 | 16 | | 215 |
| No. of *Salmonella* Positive | 17 | 21 | 4 | 7 | 12 | 7 | 14 | 10 | 10 | 10 | 4 | 6 | 7 | 7 | 0 | 136 |
| *Salmonella* prevalence (%) | 43.6 | 46.7 | 10.3 | 38.9 | 60 | 35.0 | 66.7 | 83.3 | 66.7 | 100 | 40 | 50 | 38.81 | 43.75 | 0.0 | 42.63 |
| No. of *Campylobacte* Pos. | 10 | 20 | 15 | 9 | 9 | 3 | 8 | 3 | 6 | 5 | 0 | 6 | 8 | 6 | 4 | 112 |
| Prevalence (%) | 25.64 | 44.44 | 38.46 | 50.0 | 45 | 15.0 | 38.09 | 25.0 | 40.0 | 50.0 | 0.0 | 50 | 36.7 | 37.5 | 20. | 35.11 |
| Combine *Sal.* and *Cam.* | 5 | 12 | 1 | 4 | 6 | 2 | 4 | 3 | 6 | 5 | 0.0 | 5.0 | 2 | 2 | 0.0 | 57.0 |
| Combined *Sa*l.and *Cam* prevalence (%) | 12.82 | 26.67 | 2.56 | 22.22 | 30. | 10.0 | 19.05 | 25.0 | 40.0 | 50.0 | 0.0 | 41.7 | 9.09 | 12.5 | 0.0 | 17.9 |

Appendix 2: Prevalence of *Salmonella* and *Campylobacter* in chicken carcasses from different provinces in the southern of Vietnam

| | Large abattoir | | | | | | | Small abattoir | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Abattoir No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Total |
| No. of sample examined | 39 | 45 | 39 | 18 | 20 | 20 | 21 | 12 | 15 | 10 | 10 | 12 | 22 | 16 | 20 | 319 |
| Province 1 | 8 | 8 | 10 | - | - | 10 | 10 | - | - | - | - | - | - | - | - | 45 |
| 2 | 22 | 18 | 3 | 7 | 10 | 10 | 11 | - | 7 | - | - | 12 | - | 16 | - | 116 |
| 3 | 9 | 19 | 6 | 11 | - | - | - | 12 | 8 | - | - | - | 10 | - | - | 75 |
| 4 | - | - | 16 | - | - | - | - | - | - | - | - | - | - | - | - | 16 |
| 5 | - | - | - | - | - | - | - | - | - | 10 | - | - | - | - | - | 10 |
| 6 | - | - | - | - | - | - | - | - | - | - | 10 | - | - | - | - | 10 |
| 7 | - | - | - | - | 10 | - | - | - | - | - | - | - | - | - | 20 | 30 |
| 8 | - | - | 4 | - | - | - | - | - | - | - | - | - | - | - | - | 4 |
| 9 | - | - | - | - | - | - | - | - | - | - | - | - | 12 | - | - | 12 |
| No. of *Sal.* Positive | 17 | 21 | 4 | 7 | 12 | 7 | 14 | 10 | 10 | 10 | 4 | 6 | 7 | 7 | 0 | 136 |
| *Sal.* prevalence (%) | 43.58 | 46.66 | 10.25 | 38.88 | 60.0 | 35.0 | 66.66 | 83.33 | 66.66 | 100 | 40.0 | 50.0 | 38.81 | 43.75 | 0.00 | 42.63 |
| No. of *Cam.* Pos. | 10 | 20 | 15 | 9 | 9 | 3 | 8 | 3 | 6 | 5 | 0 | 6 | 8 | 6 | 4 | 112 |
| Cam. prevalence (%) | 25.64 | 44.44 | 38.46 | 50.0 | 45.0 | 15.0 | 38.09 | 25.0 | 40.0 | 50.0 | 0.0 | 50.0 | 36.36 | 37.5 | 20.0 | 35.11 |
| Combine *Sal. and Cam.* | 5 | 12 | 1 | 4 | 6 | 2 | 4 | 3 | 6 | 5 | 0.0 | 5.0 | 2 | 2 | 0.0 | 57.0 |
| Combined prevalence (%) | 12.82 | 26.67 | 2.56 | 22.22 | 30.0 | 10.0 | 19.05 | 25.0 | 40.0 | 50.0 | 0.0 | 4167 | 9.09 | 12.5 | 0.0 | 17.9 |

Appendix 3: Distribution of variables in abattoir, Ho Chi Minh City, Vietnam

| Variable | Abattoir | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cleaning and disinfection the of crate | Yes | x | x | | x | x | x | x | x | x | x | x | x | x | x | x | 14 |
| | No | | | x | | | | | | | | | | | | | 1 |
| Stunning | Electricity | x | x | | | | | | | | | | | | | | 2 |
| | Knife | | | x | x | x | x | x | x | x | x | x | x | x | x | x | 13 |
| Scalding | Controlled | x | x | | | | | | | | | | | | | | 2 |
| | Uncontrolled | | | x | x | x | x | x | x | x | x | x | x | x | x | x | 13 |
| Evisceration | On- line | x | x | | | | | | | | | | | | | | 2 |
| | On table | | | x | x | x | x | x | x | x | x | x | x | x | x | x | 13 |
| Washing water using chemicals | Yes | | x | x | | | | | | | | | | | | x | 3 |
| | No | x | | | x | x | x | x | x | x | x | x | x | x | x | | 12 |
| Workers using protective- clothing | Yes | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | 15 |
| Cleaning before and after working | Yes | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | 15 |
| Cleaning during working time | At least twice | x | x | x | x | | | x | | | | x | | x | | x | 8 |
| | Twice | | | | | x | x | | x | x | x | | x | | x | | 7 |
| Disinfection | Once | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | 15 |
| | Twice | | | | | | | | | | | | | | | | 0 |

Appendix 4: **Questionnaire for the survey in abattoirs**

Name of abattoir ……. …… Date:……..

Abattoir location: …….Abattoir ID……..

**I General information**

| | |
|---|---|
| 1. Abattoir type | ☐ Large abattoir<br>☐ Small abattoir |
| 2. The province where the chickens are from (chicken origin) | ☐ Ho Chi Minh City<br>☐ ……..Province |
| 3. Type of farm | ☐ Intensive farm<br>☐ Backyard farm |
| 4. Duration of transportation | ……Hours |
| 5. No. of chickens per crate | **…..** |
| 6. Cleaning and disinfection of the crate prior and after transport | ☐ yes<br>☐ no |
| 7. Time of live chicken review | **……..**hours |

**II Slaughter method in abattoirs**

| | |
|---|---|
| 7. Stunning | ☐ Electricity<br>☐ Knife |
| 8. Scalding temperature | ☐ Controlled<br>☐ Uncontrolled |
| 9. Evisceration | ☐ Evisceration- online<br>☐ Evisceration on table |
| 10. Washing water | ☐ Using chemical<br>☐ No using chemical |
| 11. Chemical used | ☐ Sodium chlorine<br>☐ Acid acetic<br>------ concentration |
| 12. Protective- clothing for worker | ☐ Yes<br>☐ No |
| 13. Cleaning the floor Before and after working | ☐ Yes<br>☐ No |
| 14 Cleaning during working time | ☐ At least twice<br>☐ Twice |
| 15. Disinfection | ☐ One<br>☐ Twice |
| 16. Chemical for disinfection | ……..<br>……concentration |

**APPENDIX B**

**Equipment and material**

- Balance with a 2000 g-weight capacity and a sensitivity of 0.01gram
- Incubator, $37^0$C, $42^0$C
- $CO_2$ incubator $42^0$C
- Laboratory refrigerator, - $1^0$C to $8^0$C
- Laboratory refrigerator, - $70^0$C
- Autoclave
- Dry cabinet
- Water bath
- Vortex mixer
- Sterile culture glass dishes 15*100mm
- Sterile glass tube with cab, 100*10mm, 150*20mm
- Sterile 500, 1000 and 2000ml Erlenmeyer flasks
- Aerobic cabinet
- Sterile pipettes
- Plastic bags

**Medium and reagent**

***Campylobacter* Agar Base (Karmali)**

Code: CM935

A blood free selective medium for the isolation of *Campylobacter* jejuni and *Campylobacter* coli when incubated at $42^0$C

Formula gm/litre

| | |
|---|---|
| Columbia Agar Base | 39.0 |
| Activated charcoal | 4.0 |
| Haematin | 0.032 |
| Final pH | 7.4 + 0.2 |

***Campylobacter* Selective Supplement (Karmali)**

Code: SR167

Vial contents:

| | |
|---|---|
| Sodium pyruvate | 50.mg (equivalent to 100mg/l) |
| Cefoperazone | 16.mg (equivalent to 32mg/l) |
| Vancomycin | 10.mg (equivalent to 20mg/l) |
| Cycloheximide | 50.mg (equivalent to 100mg/l) |

Directions

Add 21.5 grams of *Campylobacter* Agar Base (Karmali) CM935 to 500ml of distilled water and bring to the boil to dissolve. Sterilise by autoclaving at $121^0$C for 15 minutes. Cool to $50^0$C. Aseptically add 1 vial of Campylobacter Selective Supplement (Karmali)

SR167 reconstituted with 2ml of sterile distilled water. Mix well and pour into sterile petri dishes.

**Campylobacter Agar Base,** Code: CM689

Formula gm/litre

| | |
|---|---|
| `Lab-Lemco' powder | 10.0 |

| | |
|---|---|
| Peptone | 10.0 |
| Sodium chloride | 5.0 |
| Agar | 12.0 |
| pH | 7.5 + 0.2 |

***Campylobacter* Selective Supplement (Preston)**

Code: SR117

Vial contents (each vial is sufficient for 500ml of medium)

| | |
|---|---|
| Polymyxin B | 2,500IU |
| Rifampicin | 5mg |
| Trimethoprim | 5mg |
| Cycloheximide | 50mg |

Directions (to prepare Preston Campylobacter Selective Agar)

Suspend 18.5g of *Campylobacter* Agar Base (CM689) in 475ml of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at $121^0$C for 15 minutes. Cool to $50^0$C. Aseptically add 25ml of Lysed Horse Blood SR48, and 1 vial of Preston

Campylobacter Selective Supplement SR117 reconstituted with 2ml of 50/50 Acetone/sterile distilled water. Mix well and pour into sterile petri dishes. Directions (to prepare Preston Campylobacter Selective Enrichment Broth)

**Brucella Medium Base**

Code: CM169

Formula gm/litre

| | |
|---|---|
| Peptone | 10.0 |
| `Lab-Lemco' powder | 5.0 |
| Glucose | 10.0 |
| Sodium chloride | 5.0 |
| Agar | 15.0 |
| pH | 7.5 + 0.2 |

Directions

Suspend 45g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121$^0$C for 15 minutes. Cool to 50$^0$C and add 5% of inactivated Horse Serum (i.e. serum held at 56 $^0$C for 30 minutes). Mix well before pouring

**Brilliant Green Agar (Modified)**

Code: CM329

| | |
|---|---|
| `Lab-Lemco' powder | 5.0 |
| Peptone | 10.0 |
| Yeast extract | 3.0 |
| Disodium hydrogen phosphate | 1.0 |
| Sodium dihydrogen phosphate | 0.6 |
| Lactose | 10.0 |
| Sucrose | 10.0 |
| Phenol red | 0.09 |
| Brilliant green | 0.0047 |
| Agar | 12.0 |
| pH | 6.9 + 0.2 |

Directions

Suspend 52 grams in 1 litre of distilled water. Heat gently with occasional agitation and bring just to the boil to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 50$^0$C, mix well and pour plates.

**Buffered Peptone Water**

Code: CM509

A pre-enrichment medium to be used prior to selective enrichment for the isolation of Salmonella species from foods.

Formula gm/litre

| | |
|---|---|
| Peptone | 10.0 |
| Sodium chloride | 5.0 |
| Disodium phosphate | 3.5 |
| Potassium dihydrogen phosphate | 1.5 |

| | |
|---|---|
| pH | 7.2 + 0.2 |

Directions

Add 20g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121$^0$C for 15 minutes. It is extremely important that the distilled water used is of a high quality with a low mineral content/conductivity.

**Rappaport-Vassiliadis (RV) Enrichment Broth**

**Code: CM669**

A selective enrichment broth for the isolation of salmonellae.

| Formula (Classical) | gm/litre |
|---|---|
| Soya peptone | 5.0 |
| Sodium chloride | 8.0 |
| Potassium dihydrogen phosphate | 1.6 |
| Magnesium chloride 6H2O | 40.0 |
| Malachite green | 0.04 |
| pH | 5.2 + 0.2 |

Directions

Add 30g (the equivalent weight of dehydrated medium per litre) to 1 litre of distilled water. Heat gently until dissolved completely. Dispense 10ml volumes into screw-capped bottles or tubes and sterilise by autoclaving at 115$^0$C for 15 minutes.

**Triple Sugar Iron Agar**

Code: CM277

| Formula | gm/litre |
|---|---|
| `Lab-Lemco' powder | 3.0 |
| Yeast extract | 3.0 |
| Peptone | 20.0 |
| Sodium chloride | 5.0 |
| Lactose | 10.0 |
| Sucrose | 10.0 |

| | |
|---|---|
| Glucose | 1.0 |
| Ferric citrate | 0.3 |
| Sodium thiosulphate | 0.3 |
| Phenol red | q.s |
| Agar | 12.0 |
| pH | 7.4 + 0.2 |

Directions

Suspend 65g in 1 litre of distilled water. Bring to the boil to dissolve completely. Mix well and distribute. Sterilise by autoclaving at $121^0$C for 15 minutes. Allow the medium to set in sloped form with a butt about 1 in. deep.

**Lysine decarboxylase broth**

**(taylor modification**)

Code: CM308 (Tablets)

To detect lysine decarboxylase production by salmonellae and some other *Enterobacteriaceae.*

| Formula | gm/litre |
|---|---|
| Yeast extract | 3.0 |
| Glucose | 1.0 |
| L-lysine | 5.0 |
| Bromocresol purple | 0.016 |
| pH | 6.1 + 0.2 |

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Directions

Add 1 tablet to 5ml of distilled water in a 1/4 oz screw-capped bottle. Sterilise by autoclaving at $121^0$C for 15 minutes. Note Uninoculated the medium should be blue/grey in colour.



**XLD Medium**

Code: CM469

A selective medium for the isolation of *Salmonella* and *Shigella* from clinical specimens and foods.

| Formula gm/litre | |
|---|---|
| Yeast extract | 3.0 |
| L-Lysine HCl | 5.0 |
| Xylose | 3.75 |
| Lactose | 7.5 |
| Sucrose | 7.5 |
| Sodium desoxycholate | 1.0 |
| Sodium chloride | 5.0 |
| Sodium thiosulphate | 6.8 |
| Ferric ammonium citrate | 0.8 |
| Phenol red | 0.08 |
| Agar | 12.5 |
| pH | 7.4 + 0.2 |

Directions

Suspend 53g in 1 litre of distilled water. Heat with frequent agitation until the medium boils. DO NOT OVERHEAT. Transfer immediately to a water bath at $50^0$C. Pour into plates as soon as the medium has cooled.

It is important to avoid preparing large volumes, which will cause prolonged heating.

**Oxidase Identification Sticks**

Code: BR64

A convenient and stable presentation of oxidase reagent for the detection of oxidase-positive bacteria. The enzyme cytochrome oxidase is produced by many organisms including Neisseria and Pseudomonas species and the `Oxidase Test' is an important and commonly used reaction for the screening and presumptive identification of microbial cultures.

Formula

The tip of each stick is impregnated with a solution of N,N-dimethyl-p-phenylenediamine oxalate, ascorbic acid and a-napthol. The other end is coloured red for identification and to ensure that the correct end is held.

In a positive reaction the enzyme cytochrome oxidase combines with N,N-dimethyl-p-phenylenediamine oxalate and a-napthol to form the dye indophenol blue.

**Blood Products**

Horse blood, haemolysed SR48
Sheep blood, defibrinated SR51
Horse serum SR35

Horse and sheep blood are the most widely used animal blood products in culture media. The choice of animal is largely traditional, with the USA and much of continental Europe preferring sheep blood, whilst the UK and Commonwealth partners prefer horse blood.

The haemolytic reactions of horse and sheep blood are not identical and blood agar media designed for horse blood may not be satisfactory with sheep blood and vice versa. See Blood Agar Base (Sheep) CM854
Section 2.

**ANAEROJAR**
Code: AG25

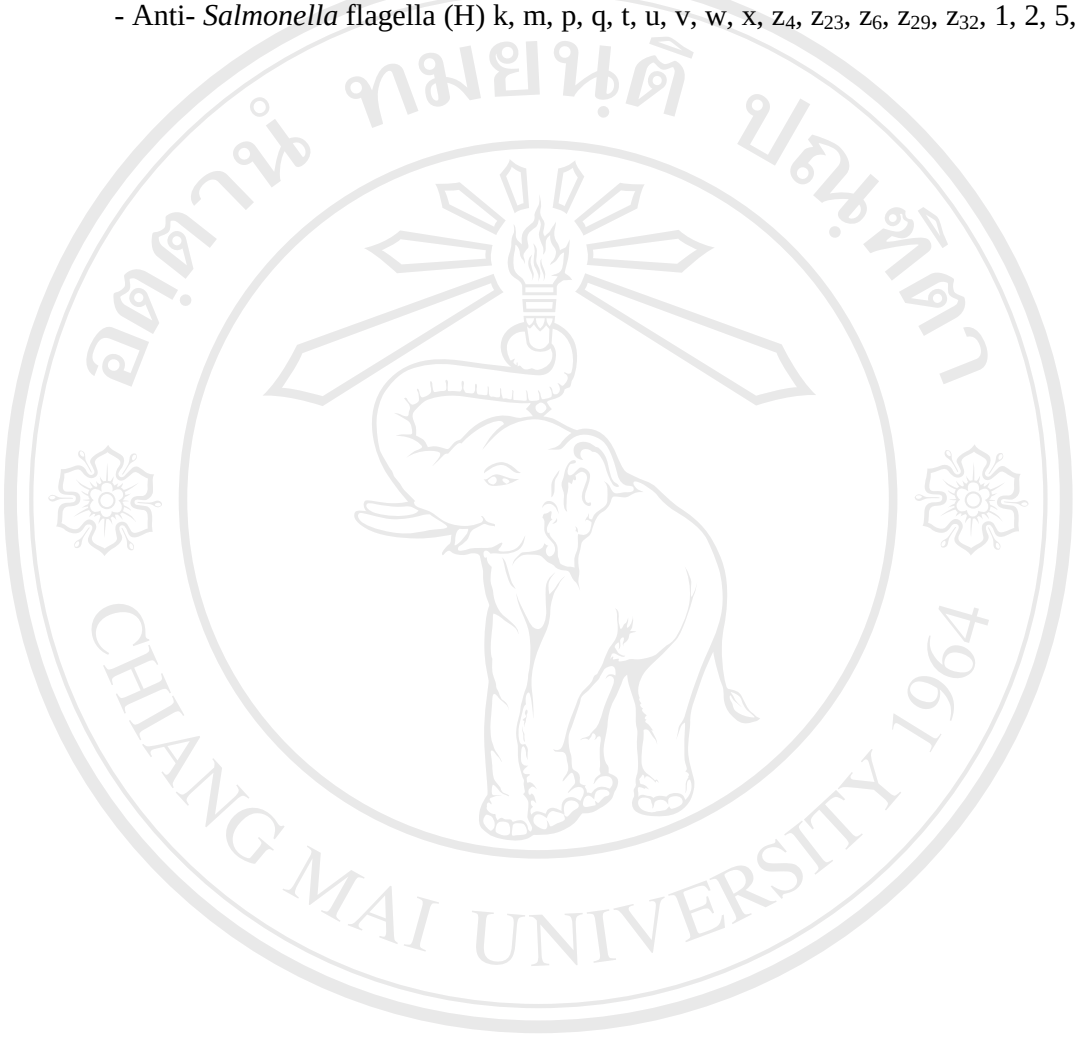
Description
The 2.5 litre Oxoid AnaeroJar is an important addition to the Oxoid range of Atmosphere Generation Products. The jar is designed for use with the 2.5 litre AnaeroGen/CampyGen sachet.



**Serum of *Salmonella***

- *Salmonella* polyvalent somatic (O) antiserum A- E
- *Salmonella* polyvalent somatic (O) antiserum F- 67
- *Salmonella* somatic (O) antiserum- *Salmonella* group B (O4, O5 , O27)
- *Salmonella* somatic (O), antiserum- *Salmonella* group C (O7, O8)

- *Salmonella* somatic (O) antiserum- *Salmonella* group D (O9, Vi)
- *Salmonella* somatic (O) antiserum - *Salmonella* group E (O3, O19)
- Anti- *Salmonella* flagella (H) k, m, p, q, t, u, v, w, x, $z_4$, $z_{23}$, $z_6$, $z_{29}$, $z_{32}$, 1, 2, 5, 6, 7



# CURRICULUM VITAE

## 1. Personal data


| | |
|---|---|
| **Name** | Mr. Vo Ngoc Bao |
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| **Second language** | English |


## 2 Education background

1991- 1996 Doctor of Veterinary Medicine at the Faculty of Animal Husbandry and Veterinary Medicine Agriculture and Forest University, Thu Duc, Ho Chi Minh City, Vietnam.

**3. Awards/ scholarship** DAAD, Germany

**Professional training**

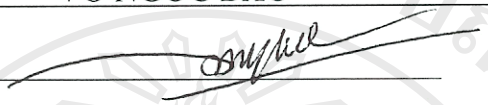
- Workshop On Veterinary Diagnostic Stage, April, 2000,

University Of Queensland And Navetco (In Vietnam)
- Dairy Cattle Production In Hot Climate Regions, May 2001, State Of Israel, Mashav And Cinadco (In Vietnam)
- Workshop On Veterinary Diagnostic Stage 2, July 2002, University Of Queensland And Navetco (In Vietnam)
- Zoonoses And Others Diseases In Dairy Cattle, November 1998, Vietnam Foremost Dairy Company (In Vietnam)
- Diagnosis Of Pasteurellosis On Cattle, Swine And Poultry, March 2003, National Institute Of Veterinary Research And Japan International Cooperation Agency (JICA, In Vietnam)

## DECLARATION

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

Name VO NGOC BAO

Signature 

Date of Submission *September* 20th, *2005*