# SALMONELLA ISOLATION FROM SLAUGHTER PIGS AND CARCASSES IN A SLAUGHTERHOUSE IN CHIANG MAI, THAILAND

WASAN CHANTONG

MASTER OF SCIENCE
IN VETERINARY PUBLIC HEALTH



CHIANG MAI UNIVERSITY AND FREIE UNIVERSITÄT BERLIN
SEPTEMBER 2005
ISBN 974-9887-80-8

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



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EXAMINING COMMITTEE

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22 September 2005

**Thesis Title** Salmonella Isolation from Slaughter Pigs and Carcasses in a Slaughterhouse in Chiang Mai, Thailand

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## ABSTRACT

This work was part of the Pork Chain Project studied in Chiang Mai. The major aim was to investigate the occurrence of *Salmonella* in the same pigs, carcasses and pork products. This particular component of the study was based on a standard slaughterhouse located in Chiang Mai. Specific objectives of this study were to determine the occurrence of *Salmonella* and to determine its serotypes in carcasses. *Salmonella* identification was conducted according to the ISO standard (ISO 6579), an Enteroclon Anti-Salmonella set for slide agglutination (SIFIN®, Germany) was used for *Salmonella* serotyping and the commercial serological test kit (SALMOTYPE® Pig LPS ELISA) was used for serological *Salmonella* investigation.

Of 181 slaughter pigs and carcasses, 723 samples of mesenteric lymph nodes, fecal content, carcass swabs before chlorinated-water spray and after overnight chilling were collected and tested for *Salmonella*. The sample proportions of *Salmonella* were as follows - 64.1% (116 *Salmonella* positive of all 181 slaughter pigs), 83.4% (151 of 181 pigs), 33.1% (60 of 181 pigs), and 13.3% (24 of 180 pigs) in mesenteric lymph nodes, fecal contents, carcass swabs before chlorinated-water spray and after overnight chilling, respectively. Of 351 *Salmonella* positive samples, 167 (47.6%) were *Salmonella* serogroup C, 117 (33.3%) serogroup B, 38 (10.8%) serogroup E, 14 (4.0%) serogroup D and 15 (4.3%) were group II (F-67). The three most identified serotypes isolated from 351 positive samples of slaughter pigs and carcasses were *S.* Rissen (161 isolates, 45.9%), *S.* Stanley (41 isolates, 11.7%), and *S.* Typhimurium (38 isolates, 10.8%). Overall, 181 meat juice samples were tested by SALMOTYPE® Pig LPS ELISA. A total of 109 samples were negative for anti-*Salmonella* antibodies (cut-off O.D. % < 40). A positive ELISA result (cut-off O.D. % ≥ 40) was obtained in 72 samples. The agreement between conventional culture and SALMOTYPE® Pig LPS ELISA at the individual pig level was slight (kappa value <0.2). The ELISA results were not the best representatives of the post-harvest *Salmonella* status but ELISA is still the test of choice in herd health monitoring.

| | |
|---|---|
| ชื่อเรื่องวิทยานิพนธ์ | การแยกเชื้อซัลโมเนลลาในสุกรและซากสุกรในโรงฆ่าสัตว์แห่งหนึ่งในจังหวัดเชียงใหม่ ประเทศไทย |
| ผู้เขียน | นายวสันต์ จันทอง |
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| คณะกรรมการที่ปรึกษาวิทยานิพนธ์ | รศ.น.สพ.ดร.เลิศรัก ศรีกิจการ ประธานกรรมการ(CMU)<br>ศ.ดร. Reinhard Fries ประธานกรรมการ(FU-Berlin)<br>รศ.ดร.สมปอง สรวมศิริ กรรมการ(CMU) |


**บทคัดย่อ**

งานนี้เป็นส่วนหนึ่งของโครงการศึกษาห่วงโซ่สุกรในจังหวัดเชียงใหม่ จุดประสงค์สำคัญเพื่อ ติดตามตรวจหาอุบัติการณ์ของเชื้อซัลโมเนลลาจากสุกรมีชีวิต ซากสุกร และผลิตภัณฑ์เนื้อสุกร การศึกษาส่วนนี้กระทำที่โรงฆ่าสัตว์มาตรฐานแห่งหนึ่งในจังหวัดเชียงใหม่ โดยมีจุดประสงค์จำเพาะของการศึกษาเพื่อหาค่าอุบัติการณ์ของซัลโมเนลลา และตรวจหาซนิดซีโรไทป์ในซากสุกร การจำแนกซัลโมเนลลาทำตามข้อกำหนดมาตรฐาน ISO 6579 การจำแนกซีโรไทป์ใช้ชุด เอ็นเทอโรคลอนแอนติซัลโมเนลลา สำหรับทดสอบสไลด์แอกกลูติเนชั่น (SIFIN®, เยอรมนี) และใช้ชุดตรวจที่มีจำหน่ายในท้องตลาด Salmotype® Pig LPS ELISA สำหรับตรวจทางเซรั่มวิทยา

จากสุกรและซากทั้งหมด 181 ตัวอย่าง ได้ทำการเก็บตัวอย่างทั้งสิ้น 723 ตัวอย่าง ประกอบด้วยตัวอย่างปุ่มน้ำเหลือง อุจจาระ จากการป้ายซาก (สวอป) ก่อนฉีดพ่นน้ำผสมคลอรีน และหลังแช่เย็นข้ามคืน นำตัวอย่างไปตรวจหาเชื้อซัลโมเนลลา สัดส่วนการตรวจพบซัลโมเนลลา ในปุ่มน้ำเหลืองเป็น 64.1% (ผลบวก 116 จาก 181 ตัว) ในอุจจาระเป็น 83.4% (151 จาก 181) ในสวอปก่อนฉีดน้ำคลอรีนเป็น 33.1% (60 จาก 181) และหลังแช่เย็นข้ามคืนเป็น 13.3% (24 จาก 180) จากตัวอย่างซัลโมเนลลา 351 ตัวอย่าง 167 (47.6%) จัดอยู่ในซีโรกรุ๊ป C 117 (33.3%) ในกรุ๊ป B 38 (10.8%) ในกรุ๊ป E 14 (4.0%) ในกรุ๊ป D และ 15 (4.3%) ในกรุ๊ป II (F-67) ซีโรไทป์ที่แยกได้สูงสุดสามอันดับแรกเป็น *S.* Rissen (161 ไอโซเลท 45. 9 %) *S.* Stanley (41 ไอโซเลท 11.7%) และ *S.* Typhimurium (38 ไอโซเลท 10.8%) ได้ตรวจน้ำจากเนื้อ 181 ตัวอย่าง ด้วยชุดตรวจ SALMOTYPE® Pig LPS ELISA พบ 109 ตัวอย่าง ให้ผลลบต่อแอนติซัลโมเนลลาแอนติบอดี้ (จุดตัดโอดี%<40) โดยมีผล ELISA บวก 72 ตัวอย่าง ผลการตรวจซัลโมเนลลาด้วยวิธีเพาะแยกเชื้อแบบดั้งเดิมและวิธีตรวจทางเซรั่มวิทยาสอดคล้องกันเล็กน้อย (ค่าแคปปา<0.2) ผลของ ELISA จึงไม่น่าใช้ได้ดีสำหรับการตรวจหลังชำแหละ แต่เป็นทางเลือกสำหรับการเฝ้าตรวจติดตามสุขภาพฝูง

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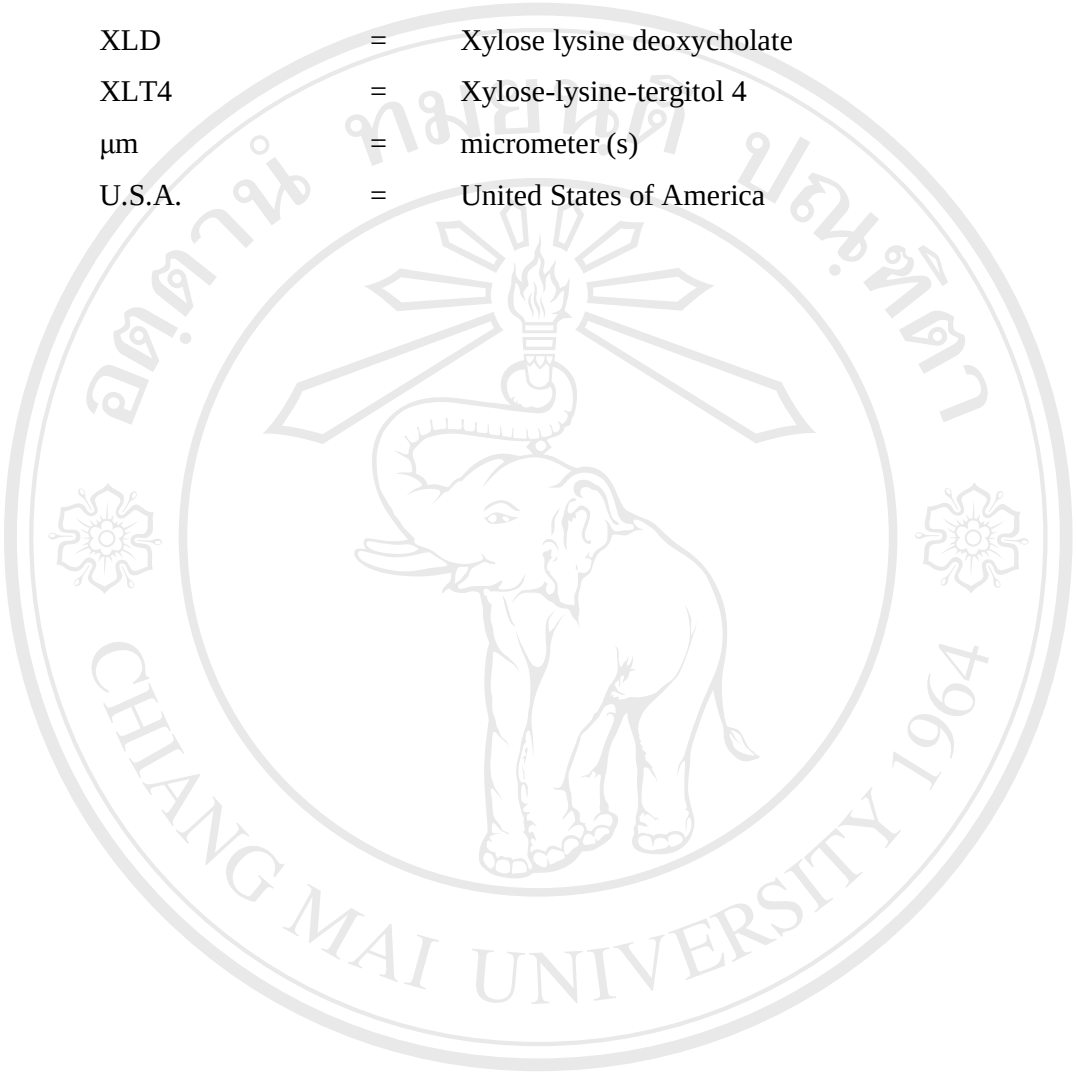
## ABBREVIATIONS AND SYMBOLS

| | | |
|---|---|---|
| + | = | positive |
| - | = | negative |
| % | = | percent |
| & | = | and |
| °C | = | degree Celsius |
| ® | = | Trademark |
| BGA | = | Brilliant Green agar |
| BPLS | = | Brilliant Green Phenol Red Lactose Sucrose agar |
| BPW | = | Buffered Peptone Water |
| CCP | = | Critical Control Point |
| CI | = | Confidence Intervals |
| CM | = | Chiang Mai (province) |
| $cm^2$ | = | Square Centimeter |
| CMU | = | Chiang Mai University (Thailand) |
| CP | = | Control Point |
| DCA | = | Desoxy Cholate Citrate agar |
| Demin. water | = | Demineralized water |
| *E. coli* | = | *Escherichia coli* |
| e.g. | = | *exempli gratia* (Latin), for example |
| ELISA | = | Enzyme Immunoabsorbent Essay |
| et al. | = | *et alii* (Latin), and others |
| FU Berlin | = | Freie Universität Berlin (Germany) |
| G, g | = | gram(s) |
| HACCP | = | Hazard Analysis and Critical Control Point |
| H antigen | = | *Hauch* (German--meaning Breath) antigen |
| $H_2S$ | = | Hydrogen Sulfide |
| Hrs | = | Hours |
| i.e. | = | *id est* (Latin), that is; in other words |

| | | |
|---|---|---|
| ISO | = | International Standard Organization |
| Lab. | = | Laboratory |
| LDC | = | Lysine decarboxylase |
| Lmt. | = | Limited |
| LP | = | Lamphun (province) |
| LPS | = | Lipopolysaccharides |
| MCLB | = | MacConkey Lactose Bile-salt agar |
| MIL | = | Motile-Indole-Lysine |
| Min. | = | Minute (s) |
| ml | = | milliliter (s) |
| MSc VPH | = | Master of Science in Veterinary Public Health |
| MSRV | = | Modified Semi-solid Rappaport Vasiliadis |
| NA | = | Nutrient agar |
| NaCl (NSS) | = | Sodium Chloride (Normal Saline) |
| No. | = | Number (s) |
| O antigen | = | *Oberfläche* (German--meaning Surface) antigen |
| O.D. | = | Optical Density |
| ONPG | = | Orthonitrophenyl-b -D-galactopyranoside |
| ppm. | = | Part per million |
| RV | = | Rappaport Vasiliadis broth |
| RVS | = | Rappaport Vasiliadis Soya broth |
| Spp. | = | species |
| ssp. | = | sub species |
| SW1 | = | Carcass swabs before chlorinated-water spray |
| SW2 | = | Carcass swabs after overnight chilling |
| TSI | = | Triple Sugar Iron agar |
| TT | = | Tetrathionate broth |
| Vi antigen | = | Virulence (Capsular) antigen |
| VP | = | Voges-Proskaur |
| WHO | = | World Health Organization |

| | | |
|---|---|---|
| XLD | = | Xylose lysine deoxycholate |
| XLT4 | = | Xylose-lysine-tergitol 4 |
| μm | = | micrometer (s) |
| U.S.A. | = | United States of America |



# 1. INTRODUCTION

Salmonellosis is an important public health problem throughout the world (Srifuengfung *et al*., 2005). In many countries, the incidences of human *Salmonella* infections or so-called salmonellosis have markedly increased in recent years, although good national or hospital-based surveillance data are frequently lacking (Guerrant *et al*., 2001). Depending on the causative *Salmonella* serotypes, the clinical symptoms of human salmonellosis correspond to either the enteric fever syndrome (typhoid fever) or to the nontyphoid-dependent gastroenteritis with possible progression to a more serious systemic infection (Lund *et al*., 2000). However, most of the *Salmonella* infectious forms in humans are due to non-typhoidal salmonellosis, which are commonly associated with the consumption of foods from animal origins and contaminated water (Vaeteewootacharn *et al*., 2005).

Non-typhoidal *Salmonella* spp., which is an important issue of Veterinary Public Health studies, has been mainly linked to some kinds of animals, for instance, poultry and poultry products. According to Hald *et al*. (2003), pigs are most frequently healthy carriers of *Salmonella* without showing any symptoms of disease. *Salmonella* infections in pigs are of concern for two major reasons: the unapparent clinical disease and high susceptibility to a broad range of *Salmonella* serotypes (Dickson *et al*., 2003). Infected pigs are considered to be the main and most important reservoirs as well as sources for the introduction and transmission of *Salmonella* on farms and/or other stages in the pork production line (Swanenburg *et al*., 2001, Hurd *et al*., 2002, Bouvet *et al*., 2003, Dickson *et al*., 2003).

Various stages in the pig and pork chain can be sources of *Salmonella* infection in slaughter pigs and contamination on pork carcasses (Wilcock and Schwartz, 1999). During transportation, feeding withdrawal, environmental contamination, and lengths of times at the lairage - all may contribute to *Salmonella* infection in slaughter pigs.

The occurrences of *Salmonella* at the slaughterhouse level are considered to be due to direct or indirect fecal contamination of slaughter pigs or carcasses (Oosterom, 1991). In addition, during the slaughtering process, carcasses may be contaminated or cross-contaminated by manual or mechanical handling. The contamination caused by, e.g. mechanical transfers, could occur in scalding tanks, de-hairing machinery and polishers. Improper handling during the early processing stages can also trigger *Salmonella* contamination in processed pork and pork products before sending to retail markets (Barends *et al*., 1997, Berends *et al*., 1998, Swanenburg *et al*., 2001, Warriner *et al*., 2002, Pearce *et al*., 2003).

Food safety and *Salmonella* is becoming an increasing concern for the global pork market today. Considering that pork is the predominant meat consumed in Thailand, an increasing demand in monitoring *Salmonella* infection throughout the pork production chain has been gradually increasing. In Chiang Mai, where pig production and pork consumption is widespread, research study or identified serotypes of this pathogenic agent is very rare. In response to the “farm to fork” food safety concept this study was conceived with aims of gaining more knowledge about *Salmonella* occurrence and serotypes particularly in the commercial pig production lines. The level at slaughtering is the main area of this study, which had the following objectives.

- To determine the occurrences of *Salmonella* spp. at the slaughterhouse and
- To determine serotypes of isolated *Salmonella*

**Significance and impact of the study:** The fundamental information obtained in this study would provide a scientific database for *Salmonella* prevalence, serotype distributions and cross contamination at this stage in the pork production chain. Furthermore, the baseline information would be used in formulating hypotheses as well as in designing long-term studies aimed at establishing monitoring trends and setting up strategic measures against *Salmonella.*

# 2. LITERATURE REVIEW

## 2.1 BIOLOGICAL AND BIOCHEMICAL ASPECTS OF *SALMONELLA*

The genus *Salmonella* belongs to the family *Enterobactericeae*. There are two species in this genus: *Salmonella enterica* and *Salmonella bongori* (Doyle *et al*., 2001). In addition, six important subspecies have been classified into *Salmonella enterica* species namely, *S. enterica* ssp. enterica, *S. enterica* ssp. salamae, *S. enterica* ssp. arizona, *S. enterica* ssp. diarizonae, *S. enterica* ssp. houtenae, and *S. enterica* ssp. indica.

Classification and detection of these bacteria are based on serology and phage susceptibility assays (Bell and Kyriakides, 2002). According to the Kaufman-White classification scheme, there are more than 2,500 serotypes isolated and more than 2,400 named serotypes, as shown in Table 1.

**Table 1:** *Salmonella* species and subspecies (Popoff *et al*., 2004)

| ***Salmonella* species and subspecies** | **Number of serotypes** |
|---|---|
| ***Salmonella enterica*** | |
| - *S. enterica* subspecies *enterica* | 1, 504 |
| - *S. enterica* subspecies *salamae* | 502 |
| - *S. enterica* subspecies *arizonae* | 95 |
| - *S. enterica* subspecies *diarizonae* | 333 |
| - *S. enterica* subspecies *houtenae* | 72 |
| - *S. enterica* subspecies *indica* | 13 |
| ***Salmonella bongori*** | 22 |
| **TOTAL** | **2,541** |

*Salmonellae* are chemo-organotrophic, with the ability to metabolize nutrients by both respiratory and fermentative pathways, the so-called **facultative anaerobic bacteria** (Doyle *et al*., 2001). Thus, they can ordinarily metabolize when oxygen is present (aerobic metabolism), but they are able to shift to anaerobic metabolism (Black, 2002). Because they are able to adjust themselves to and tolerate different environmental conditions, *Salmonellae* are widespread in natural settings, including soil and water, in which they do not usually multiply significantly but may survive for long periods (Bell and Kyriakides, 2002).

*Salmonellae* are **mesophiles** and prefer room temperature (35°C) as the optimum growth temperature. Nevertheless this group has a temperature range of 10°C minimum and 48°C maximum but grow optimally at 37°C (range between 5.2 – 46.2°C). However, most serotypes will not grow at temperatures less than 7°C (Bell and Kyriakides, 2002).

The optimum pH for growth is between 6.5 and 7.5 (Holt *et al*., 2000). *Salmonella* sometimes can grow under different pH levels (range 3.8 to 9.5) but most serotypes will not grow below 4.5 (Bell and Kyriakides, 2002). In addition, *Salmonellae* are able to catabolize D-glucose and other carbohydrates with the production of acid and gas, which can be used for biochemical identification. They are oxidase negative and catalase positive and grow on citrate as a sole carbon source. They generally produce hydrogen sulfide, decarboxylate lysine and ornithine, and do not hydrolyze urea. Many of these characteristics form the basis for the presumptive biochemical identification of *Salmonella* isolates (Table 2).

**Table 2:** Biochemical characteristics of *Salmonella* (Bell and Kyriakides, 2002)

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

+ = Positive reaction; - = negative reaction

* *S*. Typhi is negative in this test

## 2.2 MORPHOLOGICAL BASE FOR SEROTYPING

### 2.2.1 *Salmonella* morphology and basic structure

*Salmonella* are **gram-negative**, **straight**, **small** (0.7 – 1.5 X 2.0 – 5.0 μm) **rods**, which are usually motile with **peritrichous flagella** (Bell and Kyriakides, 2002). The morphology of *Salmonella* and its internal structure are shown in Figure 1.

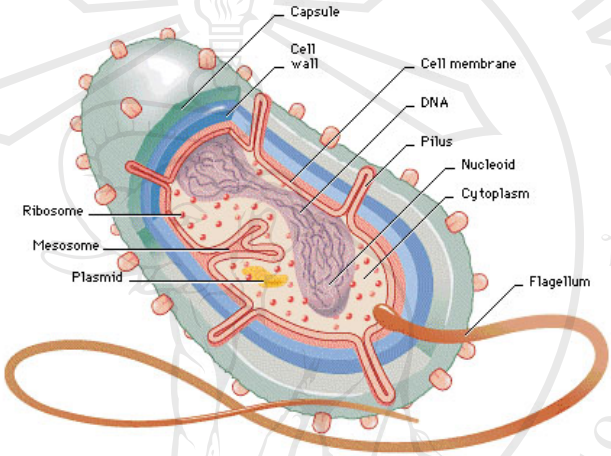



**Figure 1:** The micrographic structure of *Salmonella*
(Source: http://science.nasa.gov/.../ yeast/salmonella_sm.jpg)

### 2.2.2 Flagella and outer membrane

The term flagella (singular--flagellum), in its conventional and historical sense, suggest a helical filament extending from the cell surface. A flagellum consists of three structural parts: the filament, the hook and the basal body (Sussman *et al*., 2002a). The general structure of a typical flagellum is shown in Figure 2.

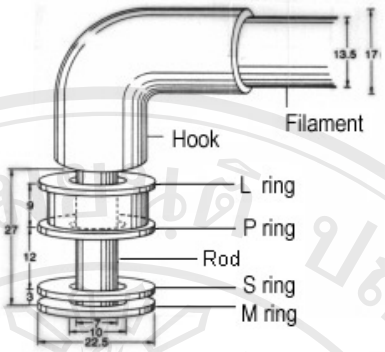



**Figure 2:** General structure of flagella
(Source: www.tnau.ac.in/.../ UGMicro/AGM151_201/theory.htm)

The genes, termed *hag* (from H antigen), were encoded at the building block of flagella filament (so-called flagellin) with the different sequences of its central region. The sequences are not only variable among different bacterial species but also among *Salmonella* serotypes. These differences impart H antigenic specificity on *Salmonella*.

Similar to those in other gram-negative bacteria, *Salmonella* has a distinguished outer membrane, which is bi-layered, forms the outmost layer of the cell wall, and is attached to the thin layer of peptidoglycan. The latter is almost a continuous layer of small lipoprotein molecules. The structural arrangement of the outer layer is shown in Figure 3.

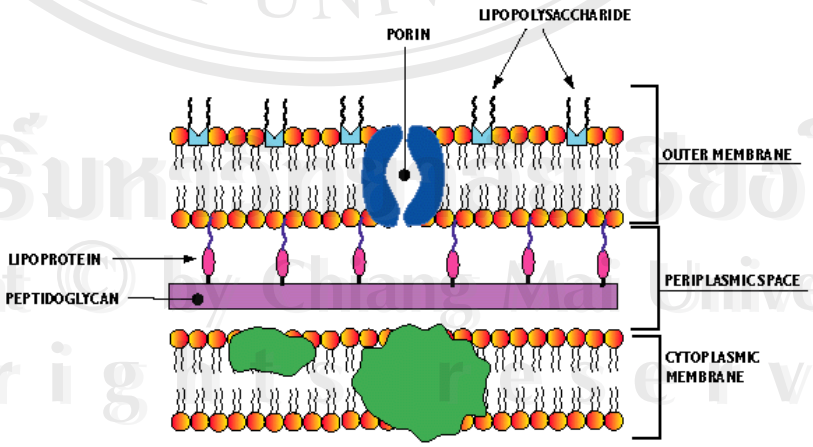



**Figure 3:** Structural arrangement of the outer layer of *Salmonella*
(Source: www.tnau.ac.in/.../ UGMicro/AGM151_201/theory.htm)

At the outer membrane lie lipopolysaccharides or endotoxins. Endotoxins in gram-negative bacteria such as those in *Salmonella*, *Shigella*, and *Escherichia*, can cause toxic and pathogenic symptoms in humans and mammals (Tamil Nadu Agricultural University, India, 2005). The components of lipopolysaccharides are shown in Figure 4.

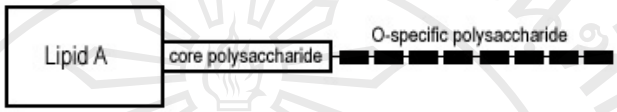



**Figure 4:** The lipopolysaccharide components
(Source: www.tnau.ac.in/.../ UGMicro/AGM151_201/theory.htm)

Lipopolysaccharides can be divided into three regions from a functional and a biosynthetic standpoint (Cary *et al*., 2000). Those components are so-called (1) Lipid A, (2) Carbohydrate core polysaccharides and (3) the O-side chains (O antigens). The latter is used to differentiate *Salmonella* serotypes.

## 2.3 SEROLOGICAL ASPECTS OF *SALMONELLA*

Serological analysis of *Salmonella* has identified three general antigens: H antigens, which are related to motility and flagella antigens, K or Vi antigens, which are present on the surface layer. These can be removed by extraction with mild solvents, such as saline or hot water. The third antigens are referred to as somatic or O antigen reference(s) (Sussman *et al*., 2002a).

### The O antigens

The O antigens are the most dominant and express their activity as endotoxin (Sussman *et al*., 2002a). The term endotoxin refers to certain common features of all lipopolysaccharide (LPS) molecules, which bind to specific receptors and elicit a broad range of host defenses, including activation of various components of the immune systems of the hosts (Sussman *et al*., 2002a). Alteration in the sugar moiety of the O antigen results in a change in the immunological specificity (Botteldoorn *et al*., 2004). The sugar found in the O antigen region can occur in a wide variety of combinations, accounting for tremendous antigenic diversity and many hundreds of chemical types or serotypes of *Salmonella* and other *Enterobateriaceae* (Moat and Foster, 1995).

### H antigens and their phase variation

*Salmonella* species have two flagellin genes, *fliC* and *fljB*, at separate locations on the chromosome (Sussman *et al*., 2002b). These can be expressed as the major flagellins, but not at the same time in any given cell. The two flagellins, H1 and H2, have significantly different antigenic specificity, resulting in two types of cells with completely different flagella antigens. This alternative expression of two different flagella with different antigenic specificities, a phenomenon known as phase variation, allows the *Salmonella* cells to escape attack by antibodies in hosts (Sussman *et al*., 2002b).

**K or Vi antigens**

Another antigen represented in *Salmonella* serotypes is the virulent **(Vi)** or capsular antigen. This occurs in *Salmonella* serotypes Typhi, Paratyphi C and Dublin (Selander *et al*., 1992, Morris *et al*., 2003). This antigen is located in an external polysaccharide microcapsule and is associated with **virulence** in particular hosts (Krieg and Holt, 1984).

Most laboratories perform agglutination reactions based on specific O antigens, designating *Salmonella* serogroups A, B, C1, C2, D and E. Examples for some *Salmonella* species are (WHO, 2001):

Serogroup A – *S*. Paratyphi A

Serogroup B – *S*. Paratyphi B, *S*. Typhimurium

Serogroup C1 – *S*. Paratyphi C, *S*. Choleraesuis, *S*. Enteritidis

Serogroup C2-C3) – *S*. Utah, *S*. Paris

Serogruop D – *S*. Typhi, *S*. Enteritidis

Serogruop E – *S*. Anatum, *S*. London

## 2.4 DISTRIBUTION OF *SALMONELLA* SEROTYPES IN THAILAND

The most common *Salmonella* serotype causing human salmonellosis found in Thailand between 1993 and 2002 was *Salmonella enterica* Weltevreden (Bangtrakulnonth *et al*., 2004). This investigation serotyped *Salmonella* from all diagnostic laboratories in Thailand, using both direct plating and enrichment broth. A total of 70,235 isolates received was confirmed as *S. enterica* and serotyped. All strains identified as *S. enterica* were serotyped according to the Kauffman-White Serotyping Scheme. *Salmonella* antisera (S and A Reagent Laboratory LMT, Bangkok, Thailand) were used in that serotyping.

A total of 118 serotypes were identified among the 44,087 isolates from humans (Table 3). The 25 prevalent serotypes accounted for 86% of the isolates, followed by 10 serotypes (64.7%), and the 5 (44.3%) most other serotypes (*S*. Weltevreden, *S*. Enteritidis, *S*. Anatum, *S*. Derby, *S*. 1,4,5,12:i) of the isolates.

The distributions of *Salmonella* serotypes in Thailand during 1993 – 2002 by different reservoirs are shown in Table 4. Samples have not been systematically taken from the different sources for *Salmonella* infections in humans. However, data from samples were available from chicken, seafood, other food products, and water for 10 years (1993-2002). Data from ducks were only available from 1998 to 2002.

**Table 3:** Common serotypes of *Salmonella* isolates from humans in 1993 to 2002, Thailand (Bangtrakulnonth *et al*., 2004).

| Serotype | Year and Numbers of Isolates (%) | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1993 | 1994 | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 | |
| 1.Weltevreden | 443 (13.5) | 574 (9.9) | 816 (12.3) | 337 (9.3) | 335 (9.7) | 485 (11.6) | 862 (18.0) | 660 (16.1) | 657 (15.9) | 322 (7.9) | 5,491 (12.5) |
| 2. Enteritidis | 471 (14.3) | 833 (14.4) | 877 (13.2) | 489 (13.4) | 365 (10.5) | 396 (9.5) | 401 (8.4) | 306 (7.5) | 357 (8.6) | 515 (12.6) | 5,010 (11.4) |
| 3. Anatum | 146 (4.4) | 397 (6.9) | 568 (8.5) | 229 (6.3) | 298 (8.6) | 320 (7.6) | 235 (4.9) | 412 (10.1) | 340 (8.2) | 318 (7.8) | 3,263 (7.4) |
| 4. Derby | 368 (11.2) | 650 (11.3) | 576 (8.7) | 277 (7.6) | 252 (7.3) | 251 (6.0) | 141 (3.0) | 156 (3.8) | 111 (2.7) | 107 (2.6) | 2,889 (6.6) |
| 5. 1, 4, 5, 12:i:-ssp.I | 193 (5.9) | 272 (4.7) | 422 (6.3) | 355 (9.8) | 212 (6.1) | 228 (5.4) | 248 (5.2) | 248 (6.1) | 336 (8.1) | 290 (7.1) | 2,804 (6.4) |
| 6. Typhimurium | 154 (4.7) | 216 (3.7) | 326 (4.9) | 238 (6.5) | 305 (8.8) | 278 (6.6) | 258 (5.4) | 205 (5.0) | 175 (4.2) | 167 (4.1) | 2,322 (5.3) |
| 7. Rissen | 54 (1.6) | 162 (2.8) | 222 (3.3) | 143 (3.9) | 295 (8.5) | 246 (5.9) | 317 (6.6) | 287 (7.0) | 259 (6.3) | 334 (8.2) | 2,319 (5.3) |
| 8. Stanley | 64 (1.9) | 147 (2.5) | 186 (2.8) | 85 (2.3) | 99 (2.9) | 147 (3.5) | 245 (5.1) | 210 (5.1) | 242 (5.9) | 263 (6.4) | 1,688 (3.8) |
| 9. Panama | 31 (0.9) | 64 (1.1) | 9 (1.4) | 80 (2.2) | 173 (5.0) | 172 (4.1) | 264 (5.5) | 209 (5.1) | 160 (3.9) | 230 (5.6) | 1,474 (3.3) |
| 10. Agona | 118 (3.6) | 215 (3.7) | 236 (3.6) | 103 (2.8) | 102 (2.9) | 76 (1.8) | 95 (2.0) | 76 (1.9) | 75 (1.8) | 90 (2.2) | 1,096 (2.7) |
| 11. Choleraesuis | 99 (3.0) | 87 (1.5) | 139 (2.1) | 122 (3.4) | 68 (2.0) | 118 (2.8) | 92 (1.9) | 69 (1.7) | 85 (2.1) | 186 (4.5) | 1,065 (2.4) |
| 12. Hadar | 64 (1.9) | 8 (1.4) | 198 (3.0) | 67 (1.8) | 80 (2.3) | 8 (2.0) | 96 (2.0) | 106 (2.6) | 136 (3.3) | 112 (2.7) | 1,023 (2.3) |
| 13. Paratyphi A | 76 (2.3) | 107 (1.9) | 134 (2.0) | 330 (9.1) | 47 (1.4) | 157 (3.8) | 108 (2.3) | — | 15 (0.4) | 7 (1.7) | 981 (2.2) |

Continued

**Table 3:** Continued

| Serotype | Year and Numbers of Isolates (%) | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1993** | **1994** | **1995** | **1996** | **1997** | **1998** | **1999** | **2000** | **2001** | **2002** | |
| 14. Krefeld | 149 (4.5) | 129 (2.2) | 135 (2.0) | 52 (1.4) | 74 (2.1) | 67 (1.6) | 72 (1.5) | 36 (0.9) | 32 (0.8) | 39 (1.0) | 785 (1.8) |
| 15. Paratyphi B Java | 31 (0.9) | 40 (0.7) | 66 (1.0) | 46 (1.3) | 61 (1.8) | 56 (1.3) | 113 (2.4) | 120 (2.9) | 117 (2.8) | 48 (1.2) | 698 (1.6) |
| 16. Typhi | 61 (1.9) | 53 (0.9) | 41 (0.6) | 42 (1.2) | 43 (1.2) | 64 (1.5) | 68 (1.4) | — | 213 (5.2) | 82 (2.0) | 667 (1.5) |
| 17. Virchow | 52 (1.6) | 69 (1.2) | 77 (1.2) | 28 (0.7) | 35 (1.0) | 45 (1.1) | 89 (1.9) | 70 (1.7) | 102 (2.5) | 79 (1.9) | 646 (1.5) |
| 18. Lexington | 40 (1.2) | 67 (1.2) | 66 (1.0) | 35 (1.0) | 45 (1.3) | 60 (1.4) | 68 (1.4) | 56 (1.4) | 88 (2.1) | 52 (1.3) | 577 (1.3) |
| 19. Blockley | 82 (2.5) | 78 (1.4) | 53 (0.8) | 27 (0.7) | 20 (0.6) | 49 (1.2) | 45 (0.9) | 56 (1.4) | 47 (1.1) | 41 (1.0) | 498 (1.1) |
| 20. Hvittingfoss | 12 (0.4) | 94 (1.6) | 125 (1.9) | 27 (0.7) | 12 (0.3) | 16 (0.4) | 66 (1.4) | 41 (1.0) | 33 (0.8) | 35 (0.9) | 461 (1.0) |
| 21. Senftenberg | 62 (1.9) | 126 (2.2) | 64 (1.0) | 16 (0.4) | 28 (0.8) | 37 (0.9) | 29 (0.6) | 20 (0.5) | 26 (0.6) | 44 (1.1) | 452 (1.0) |
| 22.Bovismorbificans | 32 (1.0) | 54 (0.9) | 87 (1.3) | 16 (0.4) | 37 (1.1) | 42 (1.0) | 56 (1.2) | 30 (0.7) | 29 (0.7) | 56 (1.4) | 439 (1.0) |
| 23. London | 27 (0.8) | 92 (1.6) | 72 (1.1) | 45 (1.2) | 67 (1.9) | 71 (1.7) | 24 (0.5) | 15 (0.4) | 8 (0.2) | 0 (0.0) | 421 (1.0) |
| 24.Schwarzengrund | 0 (0.0) | 9 (0.2) | 3 (0.0) | 3 (0.1) | 6 (0.2) | 26 (0.6) | 76 (1.6) | 99 (2.4) | 98 (2.4) | 52 (1.3) | 372 (0.8) |
| 25. Emek | 31 (0.9) | 38 (0.7) | 56 (0.8) | 29 (0.8) | 29 (0.8) | 51 (1.2) | 30 (0.6) | 26 (0.7) | 27 (0.7) | 30 (0.7) | 347 (0.8) |
| Other | 424 (12.9) | 1,116 (19.3) | 1,011 (15.2) | 415 (11.4) | 380 (11.0) | 643 (15.4) | 679 (14.2) | 577 (14.1) | 366 (8.9) | 598 (14.6) | 6,299 (14.3) |
| **Total** | **3,284** | **5,770** | **6,647** | **3,636** | **3,468** | **4,184** | **4,777** | **4,090** | **4,134** | **4,097** | **44,087** |

**Table 4:** Distribution of the 10 most common serotypes from different sources in Thailand (Bangtrakulnonth *et al*., 2004).

| Serotype | Sources and Numbers of Isolates (%) | | | | | |
|---|---|---|---|---|---|---|
| | **Humans** | **Frozen chicken** | **Frozen seafood** | **Frozen duck** | **Other food products** | **Water** |
| 1. Weltevreden | 5,491 (12.5) | — | 265 (26.3) | 320 (12.0) | 457 (6.6) | 143 (14.5) |
| 2. Enteritidis | 5,010 (11.4) | 2,901 (19.9) | 14 (1.4) | — | 309 (4.5) | 22 (2.2) |
| 3. Anatum | 3,263 (7.4) | 423 (2.9) | 20 (2.0) | — | 1,177 (17.0) | 113 (11.5) |
| 4. Derby | 2,889 (6.6) | — | 20 (2.0) | — | 370 (5.3) | 71 (7.2) |
| 5. 1, 4, 5, 12:i:- ssp.I | 2,804 (6.4) | — | — | — | — | — |
| 6. Typhimurium | 2,322 (5.3) | — | 12 (1.2) | — | 198 (2.9) | — |
| 7. Rissen | 2,319 (5.3) | — | 21 (2.1) | — | 712 (10.3) | 93 (9.5) |
| 8. Stanley | 1,688 (3.8) | — | 20 (2.0) | 279 (10.4) | — | — |
| 9. Panama | 1,474 (3.3) | — | — | 41 (1.5) | 254 (3.7) | 47 (4.8) |
| 10. Agona | 1,096 (2.7) | 452 (3.1) | — | 80 (3.0) | 273 (3.9) | 39 (4.0) |
| 11.Paratyphi B var Java | — | 1037 (7.1) | — | — | — | — |
| 12. Hadar | — | 1,357 (9.3) | 21 (2.1) | 263 (9.9) | 439 (6.3) | — |
| 13. Virchow | — | 863 (5.9) | — | — | 249 (3.6) | 27 (2.7) |
| 14. Schwarzengrund | — | 565 (3.9) | — | — | — | — |
| 15. Emek | — | 359 (2.5) | — | — | — | — |
| 16. Blockley | — | 676 (4.6) | — | — | — | — |
| 17. Amsterdam | — | 368 (2.5) | — | 103 (3.9) | — | — |
| 18. Seftenberg | — | — | 49 (4.9) | 86 (3.2) | — | — |
| 19. Lexington | — | — | 47 (4.7) | — | — | 35 (3.6) |
| 20. Newport | — | — | — | 100 (3.7) | — | — |
| 21. Tennessee | — | — | — | 77 (2.9) | — | — |
| 22. Chester | — | — | — | 171 (6.4) | — | — |
| 23. London | — | — | — | — | — | 22 (2.2) |
| Other | 15,824 (35.9) | 5,558 (38.2) | 518 (51.4) | 1,150 (43.1) | 2,490 (35.9) | 372 (37.8) |
| **Total** | **44,087** | **14,559** | **1,007** | **2,670** | **6,928** | **984** |

## 2.5 LABORATORY IDENTIFICATION AND SEROTYPING

### 2.5.1 Conventional *Salmonella* isolation

In general, the detection of *Salmonella* consists of four successive steps, namely pre-enrichment, selective enrichment, plating out, and confirmation using media (Table 5).

**Table 5:** Principles and media for conventional culturing of *Salmonella* (modified from ISO 6579 (2002))

| Steps | Commonly used components |
|---|---|
| **1. Non-selective pre-enrichment** | - Buffered Peptone Water (BPW) |
| **2. Selective enrichment** | - Rappaport Vasiliadis broth (RV)<br>- Rappaport Vasiliadis Soya broth (RVS)<br>- Modified Semi-solid Rappaport Vasiliadis (MSRV)<br>- Selenite broth<br>- Selenite Brilliant Green broth<br>- Tetrathionate broth<br>- Tetrathionate Brilliant Green broth |
| **3. Plating on solid agars** | - Brilliant Green agar (BGA)<br>- Desoxy Cholate Citrate agar (DCA)<br>- Rambach agar<br>- Brilliant Green Phenol Red Lactose Sucrose (BPLS)<br>- Xylose Lysine Deoxycholate (XLD)<br>- Xylose-lysine-tergitol 4 (XLT4) |
| **4. Verification** | - Biochemistry |
| **5. Further identification steps** | - Serotyping |

**Non-selective pre-enrichment**

Buffered Peptone Water (BPW) is the commonly used medium for *Salmonella* pre-enrichment. It is a non-selective medium that allows for the repair of cell damage and aids in the recovery of *Salmonella*. The recommended incubation temperature for pre-enrichment is 35-37°C for 18-24 hrs.

**Selective enrichments**

Various media are used for the selective enrichments of *Salmonella* prior to isolation. The temperatures and times for incubation are different, depending on the different types of media. The incubation temperature at 42°C, for 24-48 hrs, is recommended for *Salmonella* culture in Rappaport Vasiliadis (RV) broth, whereas in selective culturing in Tetrathionate (TT) broth, the recommended conditions are 37°C for 18-24 hrs for *Salmonellae*.

**Plating solid agar**

The selection of suitable nutrients in plating solid agar allows optimal growth of *Salmonellae*. At the same time, the surfactant, Tergitol-4/Sodiumtetradecylsulfate in Xylose-lysine-tergitol 4 (XLT4) agar for instance, largely inhibits the accompanying flora, so that the *Salmonella* organisms have the ability to form a unique, pure colony.

*Salmonella* colonies are presented as the different forms or colors after culture in various types of solid agars. For instance, colony appearance on Rambach agar is pink salmon, while red and translucent colonies grow on both the Brilliant Green Phenol Red Lactose Sucrose (BPLS) agar. Appearance on Xylose Lysine Deoxycholate (XLD) and Xylose-lysine-tergitol4 (XLT4) agar is black due to $H_2S$-production or mauve-gray with a central black, "bull's eye", on MCLB agar.

### 2.5.2 Biochemical identification

Based on key biochemical characteristics of *Salmonella* (Table 2), testing is performed in order to identify the particular characteristics of *Salmonella*. All biochemical tests are recommended to incubate at 37±1 °C for 18 to 24 hrs (WHO, 2001b).

Triple Sugar Iron agar (TSI) is used as a differential medium for gram-negative bacteria, based on their fermentation of lactose, dextrose and sucrose and on the production of hydrogen sulfide. Phenol red in the agar is used as an indicator when these carbohydrates are fermented. The medium changes color due to the pH. A change from red (original color) to yellow indicates the acid pH. A constant color of red indicates alkaline pH. The hydrogen sulfide produced by *Salmonella* reacts with an iron salt to yield black iron sulfide. Agar contained in TSI is the solidifier of the medium.

From Motile-Indole-Lysine (MIL) medium, *Salmonella* can be identified by its motilile characteristics. Lysine decarboxylase and Indole reaction are performed through an overnight incubation. A purple color due to Lysine reaction indicates the positive results for *Salmonella*, while a yellow/brown color indicates the negative results. In addition, Kovacs reagent is added to the medium for the detection of Indole reaction. The formation of a red ring indicates a positive- and a yellow-brown ring indicates a negative reaction.

Voges-Proskaur (VP) reaction is tested for Acetoin produced by *Salmonella*. Four drops of creatine solution, six drops of ethanolic solution of 1-naphthol and four drops of potassium hydroxide solution are added in the VP broth after incubation. A pink/red color indicates a positive reaction and a negative reaction is indicated by a colorless reaction.

Urea agar is used as a solid agar medium for the differentiation of enteric bacilli, which differentiates between *Salmonella* and urea-positive *Proteus* species or other urea-positive members of the *Enterobacteriaceae*. *Salmonella* cannot use urea agar, hence the color of test agar remains the same (yellow/brown color). More details for biochemical interpretation of *Salmonella* are shown in Table 6.

**Table 6:** Biochemical test for *Salmonella* (WHO, 2001b)

| **Medium** | **Reactions/enzymes** | **Results** | |
|---|---|---|---|
| | | **Negative** | **Positive** |
| TSI | Acid production from glucose | Butt red | Butt yellow |
| TSI | Acid production from lactose and/or sucrose | Surface red | Surface yellow |
| TSI | Gas production | No air bubble in butt | Air bubble in butt |
| TSI | Hydrogen sulfide production | No black colour | Black colour |
| Urea agar | Urease | Yellow | Rosa pink-deep cerise |
| LDC test | Lysine decarboxylase | A yellow/brown color | A purple color & yellow/brown color |
| ONPG | β-galactosidase | Remain colourless | Yellow |
| Voges-Prokauer | Acetoin production | Remain colourless | A pink/red colour |
| Indole | Indole production | Yellow ring | Red/pink ring |

### 2.5.3 Serological testing (slide agglutination)

Besides biochemical identification, serological tests are used for *Salmonella* confirmation. Serotyping is based on the somatic (O) and flagella (H) antigens. The slide agglutination test is used for this purpose. Suspicious colonies could be roughly tested using commercial polyvalent antisera, I/II/III (Behring®) or antisera I/II (Sifin®). The test could be designed for further serotype identification, which could be performed by use of commercial antisera. A drop of the serum on the slide would be rubbed into a suspicious colony. The object would be moved by slight rotation as shown in Figure 5.

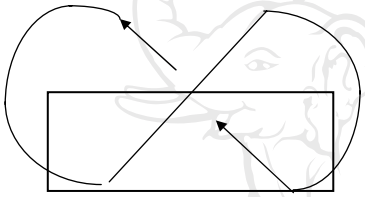


**Figure 5:** Slide movement

The test must firstly be performed with physiological NaCl-solution and material from the suspicious colony; in case of agglutination, the strain is untypable. Holding the object slide against a dark pad or a mirror could perform the result assessment. The positive result could be macroscopically detectable by the white agglutinated particles in the drop. Homogeneous, cloudy liquid indicates negative results of the agglutination (Figure 6).



Positive reaction Negative reaction

**Figure 6:** Slide agglutination: positive and negative reaction

## 2.6 *SALMONELLA* AND SALMONELLOSIS IN PIGS

The primary sources of *Salmonella* are the gastrointestinal tracts of humans and of domestic and wild animals. Consequently they are widespread in the natural environments including soil and water (Bell and Kyriakides, 2002). The ubiquitous *Salmonella* spp. can enter the pork production chain at any point, for example, via feeds at the farm production, at the slaughterhouse, in post-slaughter processing, or at the moment of food catering and preparation (Lo Fo Wong and Hald, 2000).

The epidemiology of salmonellosis in pigs must be regarded as two relatively separate problems: salmonellosis as a disease of pigs and *Salmonella* infection or contamination of pork carcasses and products.

The clinical signs of salmonellosis in pigs vary from case to case depending on serotype virulence, host resistance, and on the route and size of the infectious dose. However, the most common clinical signs may be the result of either septicemia caused by *S. choleraesuis* and/or enterocolitis mainly caused by *S. typhimurium* (Wilcock and Schwartz, 1999). Both forms of disease occur in intensively kept pigs, reared and weaned in less than five months, but may be seen occasionally in finishing pigs or adult breeding stock (Wilcock and Schwartz, 1999).

Infections in the affected adult pigs are unapparent or may be present with a wide range of severity, from mild fever to sudden death without diarrhea in case of septicemic salmonellosis. Watery diarrhea with a low mortality rate may be found in the case of enterocolitis. Most pigs recover completely but remain carriers and intermittent shedders for several months (Swanenburg *et al*., 2001, Hurd *et al*., 2002). The disease could be easily transmitted to others in the same herds via pig-to-pig contact and, most importantly, by the introduction of an infected carrier animal (Dickson *et al*., 2003).

## 2.7 DISTRIBUTION OF *SALMONELLA* IN PIGS AND PORK

Various studies have indicated that *Salmonellae* can be present either in pigs or pork at different contamination rates.

A study in the U.S.A., conducted by Morrow and Funk (2001), found out that *Salmonella* contamination was on 0-48% of pig carcasses after slaughtering. In the Netherlands, Swanenburg *et al*. (2001) revealed that 25% of carcass samples from slaughter pigs delivered from **sero-positive** herds were *Salmonella* positive, while 5% of such samples from **sero-negative** herds were positive. Based on these findings, Swanenburg *et al*. postulated that at least 5% *Salmonella* occurrence could be present during slaughter, even in those slaughter pigs that come from *Salmonella*-free herds.

Some studies performed in Italy and Belgium demonstrated different prevalence magnitudes and distributions of *Salmonella* in pigs and pork. In northern Italy, fecal material, carcass swabs, and tonsils were collected and examined for *Salmonella*. A prevalence of 36.7% was found in fecal content, 5.3% was found in tonsils, and 6.0% in carcasses. The serotypes found in that study were *S.* Derby, *S.* Bredeney, and *S.* Typhimurium (Bonardi *et al*., 2002). In Belgium, *Salmonella* was isolated from carcasses, colon contents, and mesenteric lymph nodes. The serotypes identified were *S*. Typhimurium and *S*. Derby (Botteldoorn *et al*., 2004)

In Chiang Mai, Thailand, the prevalence of *Salmonella* in pre-slaughter pigs increased from 69.5% at the farm level to 82.5% at two local slaughterhouses. This increased *Salmonella* contamination rate was considered to be due to stress before slaughtering and the hygienic aspects of slaughtering (Patchanee *et al*., 2002). The stress precipitates *Salmonella* shedding by pig carriers, which in turn increases the probability of contamination at the slaughter level.

## 2.8 SLAUGHTERING PROCESS AND *SALMONELLA* CONTAMINATION

Pig slaughtering is an open process with many opportunities for contamination with *Salmonella* and other potentially pathogenic bacteria, e.g. *Aeromonas*, *Campylobacter*, *Listeria*, *Staphylococcus* and *Yersinia* (Borch *et al*., 1996). Major risk factors for contamination during the slaughter process are feces, tonsil or cross-contamination from tools, machinery, workers or other slaughterhouse environments.

During slaughter, *Salmonella* may spread from infected to non-infected pigs. Scalding would be carried out either by hanging the pigs or in vats using stream or circular water. The scalding and dehairing procedures take 2-3 minutes and water temperature ranges from 61- 62 °C. That temperature can eliminate *Salmonella*, but not completely. In general, there are two forms of dehairing, combining with scalding in vats or separate scalding and then, dehairing. Whatever the scalding forms are, all could lead to *Salmonella* contamination on the carcasses, in which fecal material can easily spread on the surface. Flaming/singeing is usually conducted after dehairing but it is not sufficient to eliminate the bacterial contamination on the carcass surface. However, it has a significant effect in reducing the contamination level (Borch *et al*., 1996).

The workers or machinery normally perform further scalding and polishing. Both of them can contribute to the spread of bacteria that survive the previous procedures. Because of the difficulty of cleaning these machines during the slaughtering day, *Salmonella* may become established on scalding vats and the surfaces of the scrapers that may be sources of contamination.



When the intestines are removed, there is a risk of spilling their contents so that fecal matter is spread over the carcass. The tongue and the tonsils are removed along with the pluck set. Spread of pathogenic bacteria from the tonsils and pharynx to the carcass and the pluck must be expected.

Splitting of the carcasses is done using splitting saws. There is a risk that the machines will come into contact with the intestinal content or head, which can cause spread of pathogenic bacteria. Knives, cutters and other tools used are likely to become contaminated by *Salmonella* and other pathogenic bacteria that will subsequently be transferred to the carcasses.

During the operation following slaughtering, e.g. cutting, de-boning, and further processing, a further spread of pathogenic bacteria might be extensive.

According to the HACCP system, Borch *et al*. (1996) had specified the microbiological hazards in pig slaughterhouses at each step together with Control Points (CP) and Critical Control Points (CCP), hygienic aspects and preventive actions (Table 7).

**Table 7:** Hygienic aspects and preventive actions (Borch *et al*., 1996)

| Process step | Hygienic aspect | Preventive action | CP/CCP |
|---|---|---|---|
| Lairage | Contamination between animals | Cleaning and disinfection | CP |
| Stunning | | | |
| Bleeding (killing) | Contamination from tools | Cleaning and disinfection | CP |
| Scalding | Reduction of bacterial levels | Time/Temperature | CP |
| Dehairing | Contamination from machine | Cleaning and disinfection | CP |
| Singeing/ flaming | Reduction of bacterial levels | Time/Temperature | CP |
| Polishing | Contamination from machine | Cleaning and disinfection | CP |
| Evisceration | Contamination from intestine, tongue, pharynx and tonsils<br>Contamination from tools | Enclosure of rectum<br>Working instruction<br>Disinfection of tools | CCP |
| Splitting | Contamination via splitter/saw | Line-speed<br>Water temperature | CP |
| Meat inspection | Contamination from inspection | Disinfection of tools | CCP |
| Chilling | Bacterial growth at improper temperature | Time/Temperature | CCP |
| Processing | Contamination from personnel and tools | Working instruction<br>Tool disinfection | CCP |

# 3. MATERIAL AND METHODS

## 3.1 STUDY DESIGN

This study was a cross-sectional study for microbiological examination of samples from animals that were being examined in a parallel study at the farm level.

### 3.1.1 Study sites

A standard slaughterhouse belonging to a pork production company in Chiang Mai was selected. The pigs slaughtered in this slaughterhouse came from over 40 contract farms located in the Chiang Mai and Lumphun provinces. The pig slaughtering and pork processing were commercially performed for local consumption at a rate of approximate 120 - 140 pigs per day.

### 3.1.2 Sample type and laboratory investigation

According to the *Salmonella* study, along the pork chain, the slaughter pigs and carcasses sampled for this study were the same ones, which were investigated in a sister study at the farm level. The slaughter pigs were re-identified by ear tattoo and spray marking at the slaughterhouse. During the slaughtering process, tag numbers used to identify the pigs were secured on the forelegs of the carcasses. From each individual pig, one fecal, one lymph node and two-carcass swab samples (before chlorinated-water spray and after overnight chilling) were collected for *Salmonella* isolation. One muscle sample was also collected for serological investigation.



*Salmonella* isolation was performed following ISO 6579 (2002) and Sifin® Enteroclon Anti-Salmonella Antigens (Slide agglutination methods) were used for *Salmonella* serotyping. For serological testing, the commercial ELISA test kit SALMOTYPE® Pig LPS ELISA was used.

## 3.2 SAMPLE SIZE DETERMINATION

The Win Episcope 2.0 was used for sample size determination. A population estimate of 30,000 pigs from all contract farms and prevalence of *Salmonella* of 69.5 % in pre-slaughter pigs in Chiang Mai Province (ranging from 50-83%) (Patchanee *et al*. 2002) was used. At a 95% level of confidence and 8% error rate, the calculated sample size for this study was 128 pigs. However, 181 pigs were actually selected from the 21 farms (17 open- and 4 closed-house raising systems). The sample sizes from these two systems were 141 and 40 pigs respectively.

## 3.3 THE SLAUGHTERING PROCESS

Upon arrival, pigs were immediately sprinkler-showered with potable water and rested for 1-2 hours. They were then transferred to the slaughter line, stunned using low voltage electrical tongs (110 - 180 Volts) for less than 15 seconds. The stunned pigs were secured to an overhead conveyor rail by a chain looped around one of the hind angles. By cutting the main blood vessels of the neck using a sharp knife, the animals were immediately bled. Combined scalding and dehairing of pigs was done for 1.30 – 3.0 min. in a scalding tank (kept at temperatures between 62 and 65°C). The final dehairing was manually performed using a sharp knife.

After dehairing, the slaughter pigs were given a pre-evisceration wash by manual hosing with potable normal water. A singeing or flaming procedure was not implemented in this slaughterhouse. Slaughtered pigs were secured to an overhead conveyor chain by hooking the hind legs. Evisceration involved three separate tasks (de-bunging, slitting the belly open and gut removal), all of which were performed by the same operative. De-bunging or detachment of the rectum was completed prior to opening the belly. The connective tissues joining the bung and viscera to the carcasses were cut. The diaphragm, heart, lungs, and some part of trachea were manually removed together as part of the pluck set, along with the digestive tract. At this stage, the head was removed.

The carcasses were manually cut along the midline from the hind to the fore using a power-splitting saw. Thereafter, the kidneys, spinal cord, and fascia were removed.

The carcasses were finally hose-washed with normal potable water and sprayed with cold (5 - 10°C), chlorinated water (50 - 100 ppm) to lower the bacterial load before shock freezing (-18 to -20°C for approximate 2 hrs) and cold storage overnight in the chilling room (≤ 4°C). Further cutting and processing were performed the next day after overnight chilling.

## 3.4 COLLECTION OF SAMPLES

### 3.4.1 Fecal samples

Immediately after evisceration, at least 25 g of intestinal contents was taken from the colon of each sampled pig using disposal gloves, kept in plastic bags and refrigerated (4°C) in an icebox. The samples were brought to laboratory within 4 hours of collection and tested for *Salmonella* with 24 hours post collection.

### 3.4.2 Meat and lymph node samples

Ten grams of diaphragmatic muscle was collected for ELISA testing. Meat juice was harvested by freezing and then thawing of the muscle samples. This was done in the laboratory.

At least 25 g of intestinal lymph nodes were collected from the same carcass. Both meat and lymph node samples were collected using sterile tools. Each sample was collected in a plastic bag and kept refrigerated at 4°C in an icebox container. Time of sample delivery and laboratory procedure was the same as described in section 3.4.1.

### 3.4.3 Carcass swabs

The carcass swabs were taken on the carcass surface at two stages: (1) prior to spraying the carcass with chlorinated-water and (2) following overnight chilling.

Based on the Commission Decision of the European Communities (The Commission of the European Communities, 2001), four carcass swabs were taken (Figure 7) pooled into one sample. Because the heads were removed before swab taking, the lowest part of the neck was swabbed instead of the jowl in this case. The samples were then kept in a separate icebox and transported to the laboratory within a few hours after collection (the same procedure as the other samples).

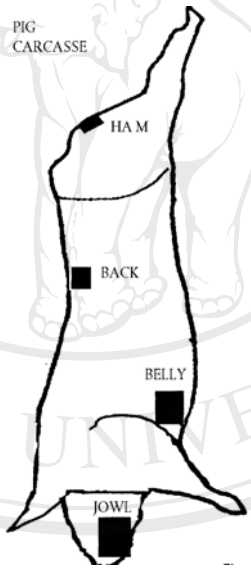



**Figure 7:** Swab sampling sites on the carcass
(Source: Official Journal of the European Community 471/2001)

Cotton swabs were moistened in sterile normal saline prior to sampling. Each of the sites was at least 100 $cm^2$. Moistened swabs were rubbed in the following motion: vertically, then horizontally, then diagonally for not less than 20 seconds across the entire surface site. The pooled samples were stored in a bottle of 50 ml Buffered Peptone Water before transportation to the laboratory.

### 3.4.4 Overall numbers of samples

Overall, 723 samples were taken for *Salmonella* investigation, i.e.181 fecal samples, 181 lymph nodes, and 181 carcass swabs taken before carcasses were washed with chlorinated-water. The rest, 180 carcass swabs, were taken after the carcass was chilled overnight. In addition to the lab samples, 181 diaphragm muscles were collected for ELISA testing.

## 3.5 LABORATORY PROCEDURES

### 3.5.1 Conventional culture: ISO 6579 (2002)

#### *3.5.1.1 Salmonella isolation*

- *Sample preparation*

Twenty-five grams of fecal samples were mixed with 225 ml Buffered Peptone Water (BPW; Merck KGaA, Germany). The lymph node samples were each put into 70% alcohol and flamed for a few seconds to eliminate superficial contamination, and thereafter cut into small pieces with a sterile scalpel. Thereafter, 10 g of the sample was transferred into a stomacher bag filled with 90 ml Buffered Peptone Water. The muscle samples were also cut into small pieces using sterile scalpels or blades and then kept separately in plastic bags and kept frozen.

- *Pre-enrichment*

A pre-enrichment medium was used to resuscitate any stressed microorganisms and enhance their growth. The medium of choice recommended for *Salmonella* resuscitation is the highly nutritional and non-selective medium, Buffer Peptone Water (BPW; Merck KGaA, Germany). After transferring 25 g of samples into a stomacher bag, 225 ml of Buffer Peptone Water was added and then stomachered for 2 minutes. All samples were incubated at 37°C for 18–24 hrs.

- *Selective enrichment*

The pre-enrichment broth was mixed and 0.1 ml was transferred to 9.9 ml pre-warmed Rappaport Vasiliadis (RV; Merck KGaA, Germany) enrichment broth, which was incubated at 42°C for 24–48 hrs. One ml of the broth was also transferred to 9.0 ml of Tetrathionate (TT; Merck KGaA, Germany) broth for the secondary selective enrichment. It was incubated at 37°C for 24 hrs.

- *Selective solid media*

A loop of material from the RV broth was transferred and spread onto the surface of a Xylose-lysine-tergitol 4 (XLT4; Merck KGaA, Germany) agar. The second agar of choice, Brilliant Green Phenol Red Lactose Sucrose (BPLS; Merck KGaA, Germany) agar, was used for growing the isolated colonies. The plates were incubated in an inverted position at 37°C for 18–24 hrs. After incubation, the plates were checked for growth of typical *Salmonella* colonies. The latter have a black center and a lightly transparent zone of reddish color on XLT4 and reddish color and a translucent colony on BPLS.

When no typical colonies were found after 24 hrs of incubation, a loop of both enrichment broths was plated out again on XLT4 and BPLS agar and then incubated for another 24 hrs at 37 °C.

- *Confirmation*

Suspected colonies were streaked on the surface of pre-dried nutrient agar plates and incubated at 37±1 °C for 24 ± 3 hrs, in a manner that allowed the isolated colonies to develop. Up to five colonies per plate were purely cultured and used for biochemical and serological confirmation. Both methods were performed and interpreted as described in subsection 5.5.1.2 and 5.5.1.3.

### *3.5.1.2 Biochemical confirmation*

The pure colonies after incubation on nutrient agar were picked up and inoculated into Triple Sugar Iron (TSI; Merck KGaA, Germany) slant, Voges-Proskaur (VP; Merck KGaA, Germany) broth, Motile-indole-lysine (MIL; Merck KGaA, Germany) broth and Urea (Urea; Merck KGaA, Germany) slant. All inoculated biochemical media were incubated at 37°C for 18-24 hrs, with the exception of VP that was incubated for 48 hrs.

The biochemical confirmation followed the results reactions. In the case of *Salmonella*, the reaction would present the following appearances.

1. Glucose : Positive (+)
2. Gas : Positive (+)
3. Lactose : Negative (-)
4. Sucrose : Negative (-)
5. $H_2S$ : Positive (+)
6. Urease : Negative (-)
7. LDC : Positive (+)
8. VPR : Negative (-)
9. Indole : Negative (-)

### *3.5.1.3 Serological confirmation*

The serological confirmation of *Salmonella* antigens was performed by slide agglutination testing, according to the commercial product (SIFIN®, Germany). All isolates from each type of sampls were tested using the following antisera:

a) *Salmonella* Polyvalent I (A-E) and II (F-67)
b) *Salmonella* Somatic (O) Group A, B, C, D and E, and
c) *Salmonella* Flagella (H) Antisera set

The sequences of the serological testing are depicted in Figure 8.

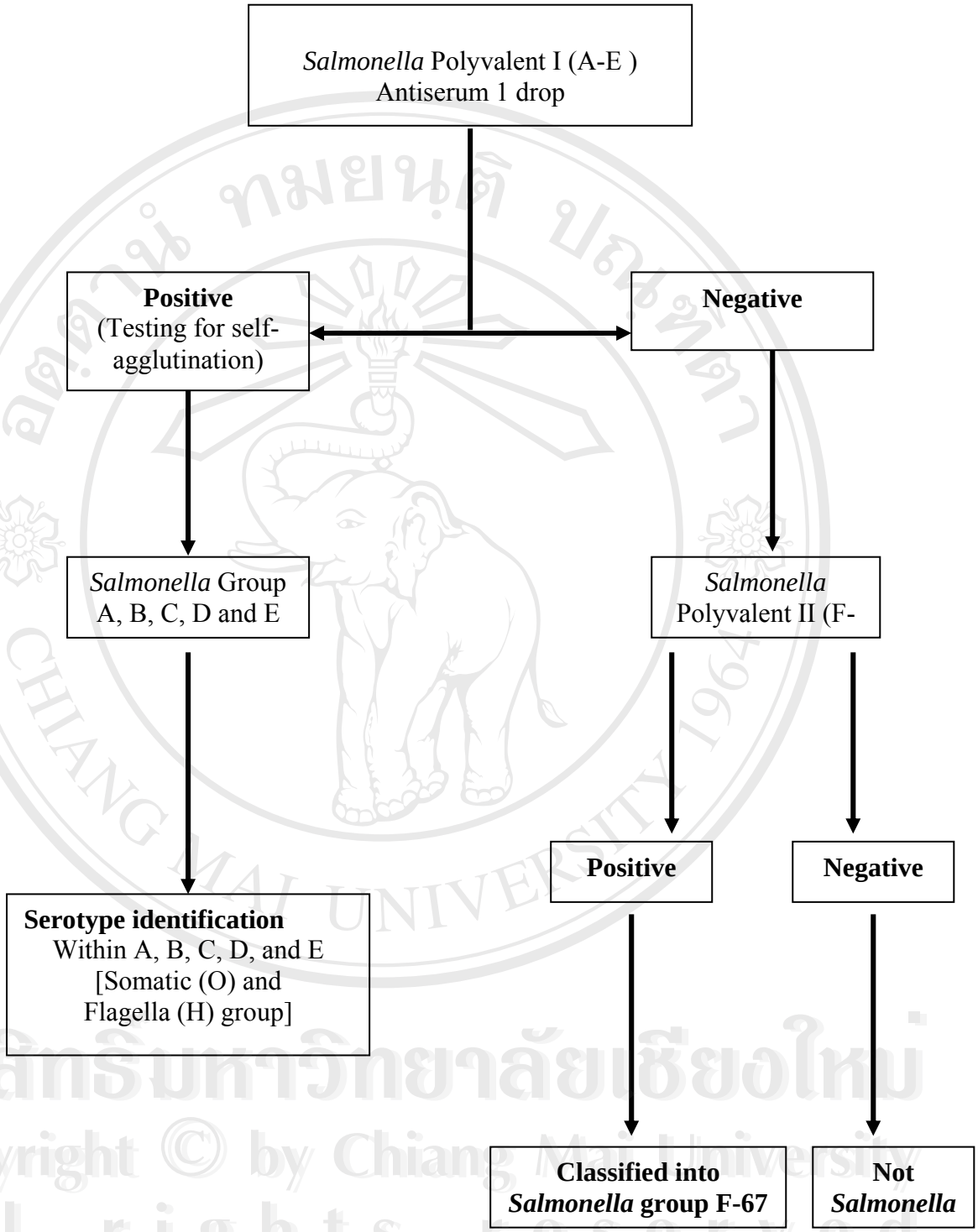





**Figure 8:** *Salmonella* Serotyping flow chat (Slide agglutination test)

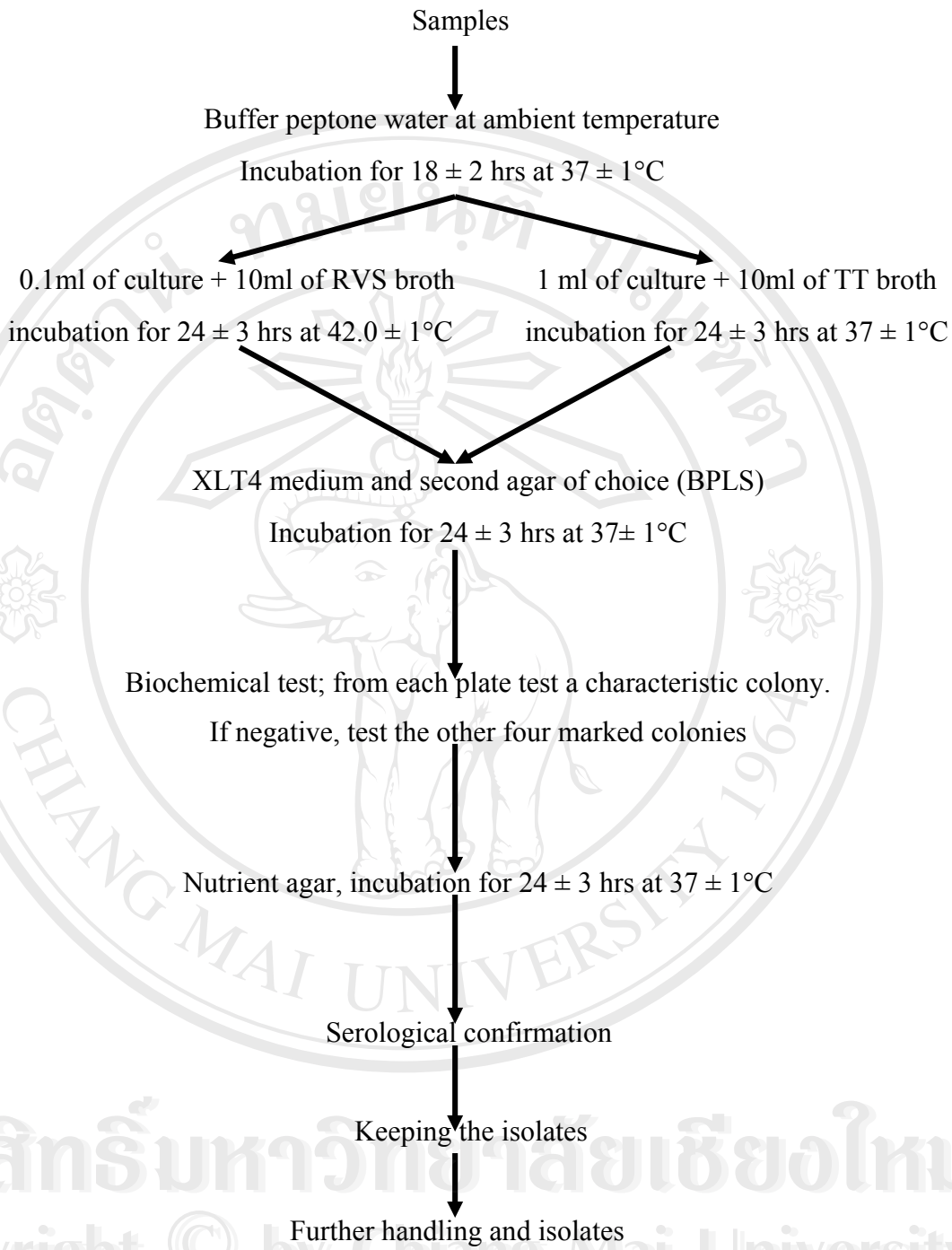



**Figure 9:** Overall summary of sample handling and *Salmonella* identification procedure

### 3.5.2 Serological testing: SALMOTYPE® Pig LPS ELISA

#### 3.5.2.1 Usage of the test

An enzyme immunoassay (ELISA) for the detection of specific antibodies against *Salmonella* in pork meat juice was used. In this study the diaphragmatic muscle was used and meat juice was harvested by thawing the frozen pork meat. The steps following were those given by the manufacturer, Labor Diagnostik Leipzig, Germany (Figure 10). The test result was the optical density produced by the sample relative to the optical density of the positive reference sample (O.D. %). Negative or positive result was interpreted following the different cut-off values of O.D. %. As recommended by the manufacturer, interpretation was as follows.

#### 3.5.2.2 Result interpretation

- Cut-Off values for samples (serum, meat juice, plasma);
  ≥ 40 OD% positive
  20 OD% - < 40 OD% weak positive
  10 OD% - < 20 OD% doubtful (positive)
  < 10 OD% negative

- Cut-Off values of samples for categorization of herds according to monitoring programs:
  ≥ 40 OD% or ≥ 20 OD% are positive depending on national regulations
  For the assay to be valid, the P/N-ratio between the Positive Control Serum 1 (P) and the Negative Control Serum (N) should be greater than 4.0.



#### 3.5.2.3 Flow chart of the steps used in SALMOTYPE® Pig LPS ELISA

The instruction of the test is presented in the Figure 10.

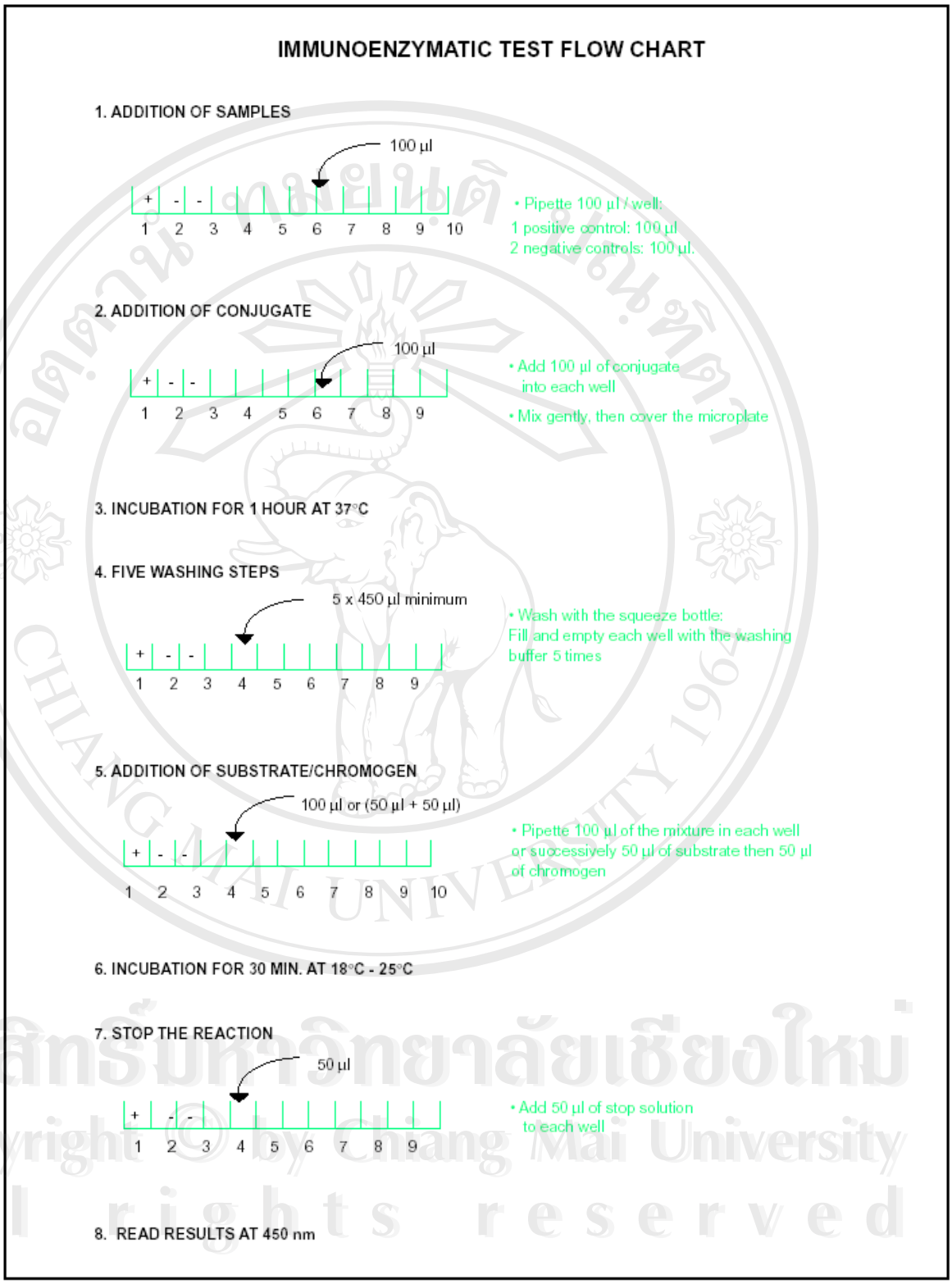





**Figure 10:** Flow chart of SALMOTYPE® Pig LPS ELISA (Labor Diagnostik, Leipzig)

## 3.6 DATA MANAGEMENT AND STATISTICAL ANALYSIS

Data from conventional *Salmonella* culture was entered into a Microsoft Excel database and error checks done. In order to reach thesis objectives, data analysis was conducted following these performances.

(1) Using the Excel calculation program, sample prevalence of *Salmonella* and 95% confidence intervals (CI 95%) was calculated.

(2) Distribution of *Salmonella* serotypes in various types of samples and from different farms of origin was conducted using the pivot table in Microsoft Excel.

(3) Win Episcope 2.0 was used for calculation of sensitivity and specificity of the ELISA test, using bacteriological culture (lymph node- and fecal culture results) as the golden standard.

(4) Kappa statistics was used for assessment of agreement between two different methods of *Salmonella* isolation, conventional culture of fecal samples and commercial ELISA test using Epi Info 2002. According to Dahoo *et al.* (2003), the criterion of kappa statistics was categorized into the following:

Kappa value <0.2: slight agreement
0.2- 0.4: fair agreement
0.4-0.6: moderate agreement
0.6-0.8: substantial agreement
>0.8: almost perfect agreement

# 4. RESULTS

## 4.1 SAMPLE PREVALENCE OF *SALMONELLA*

Of the 181 lymph node samples examined, 116 were *Salmonella* positive, (64.1 %; 95% CI: 56.6 – 72.1%), while, 151 fecal samples were positive (83.4 %; 95% CI: 77.2 – 88.5%). As for carcass swabs, before use of chlorinated-water spray, 60 were *Salmonella* positive (33.1 %; 95% CI: 26.3 – 40.5%). Out of 180 carcass swabs sampled after chilling, 24 were *Salmonella* positive (13.3 %; 95% CI: 8.7 – 19.2%). Results of sample-specific prevalence of *Salmonella* are summarized in Table 8.

**Table 8:** Sample prevalence of *Salmonella* and 95% Confidence Intervals

| Sample | Numbers of samples | *Salmonella* positive | % positive | 95% CI |
|---|---|---|---|---|
| Mesenteric lymph nodes | 181 | 116 | 64.1 | 56.6-71.1 |
| Feces | 181 | 151 | 83.4 | 77.2-88.5 |
| Swabs before spray | 181 | 60 | 33.1 | 26.3-40.5 |
| Swabs after chilling | 180 | 24 | 13.3 | 8.7-19.2 |

*CI = Confidence Intervals

## 4.2 *SALMONELLA* SEROTYPES

The distribution of *Salmonella* somatic serogroups is presented in Table 9. Of 351 *Salmonella* positive samples, 167 (47.6%) were *Salmonella* serogroup C, 117 (33.3%) serogroup B, 38 (10.8%) serogroup E, 14 (4.0%) serogroup D and 15 (4.3%) serogroup F-67.

**Table 9:** Distribution of *Salmonella* somatic serogroups

| | **Somatic serogroup (%)** | | | | | **Sample Prevalence (%)** |
|---|---|---|---|---|---|---|
| **Sample** | **B** | **C** | **D** | **E** | **F-67** | |
| Mesenteric lymph nodes | 30 (25.6%) | 65 (38.9%) | 6 (42.9%) | 12 (31.6%) | 3 (20.0%) | 116 (64.1%) |
| Feces | 52 (44.4%) | 66 (39.5%) | 6 (42.9%) | 20 (52.6%) | 7 (46.7%) | 151 (83.4%) |
| Swabs before spray | 21 (18.0%) | 26 (15.6%) | 2 (14.2%) | 6 (15.8%) | 5 (33.3%) | 60 (33.1%) |
| Swabs after chilling | 14 (12.0%) | 10 (6.0%) | - | - | - | 24 (13.3%) |
| **Total (%)** | **117 (100.0%)** | **167 (100.0%)** | **14 (100.0%)** | **38 (100.0%)** | **15 (100.0%)** | **351 (100.0%)** |

The distribution of *Salmonella* somatic serogroups isolated from lymph node samples is given in Table 10. The distribution of serogroups varied from farm to farm with the most prevalent groups being as follows: C (65%), B (30%), E (10.3%), D (5.2%), and F-67 (2.6%).

**Table 10:** *Salmonella* somatic serogroup found in lymph nodes by farms

| | **Number of *Salmonella* somatic serogroup** | | | | | **Grand** |
|---|---|---|---|---|---|---|
| **Farm** | **B** | **C** | **D** | **E** | **F-67** | **Total** |
| 1 | | 5 | | 1 | | 6 |
| 2 | 3 | 4 | | 1 | | 8 |
| 3 | 3 | 1 | 1 | 3 | | 8 |
| 4 | 2 | 5 | | | 3 | 10 |
| 5 | 1 | | 5 | | | 6 |
| 6 | 2 | 4 | | 1 | | 7 |
| 7 | | 8 | | | | 8 |
| 8 | | 3 | | | | 3 |
| 9 | 1 | 3 | | 1 | | 5 |
| 10 | 1 | 2 | | | | 3 |
| 11 | | 2 | | | | 2 |
| 12 | 6 | 2 | | | | 8 |
| 13 | 5 | | | 1 | | 6 |
| 14 | 2 | 1 | | 1 | | 4 |
| 15 | | 1 | | | | 1 |
| 16 | 1 | 4 | | | | 5 |
| 17 | | 3 | | | | 3 |
| 18 | 2 | 4 | | | | 6 |
| 19 | | 3 | | 2 | | 5 |
| 20 | | 5 | | | | 5 |
| 21 | 1 | 5 | | 1 | | 7 |
| **Grand Total** | **30** | **65** | **6** | **12** | **3** | **116** |
| **(%)** | **(25.9%)** | **(56.0%)** | **(5.2%)** | **(10.3%)** | **(2.6%)** | **(100%)** |

Fecal proportions of *Salmonella* somatic serogroups are shown in Table 11. The serogroup C was still the most prominent serogroup followed by B, E, F-67, and D.

**Table 11:** Fecal proportions of *Salmonella* somatic serogroups distributed by farms

| Farm | Number of *Salmonella* somatic serogroup | | | | | Grand Total |
|---|---|---|---|---|---|---|
| | B | C | D | E | F-67 | |
| 1 | 1 | 3 | | 2 | | 6 |
| 2 | 2 | 5 | | 2 | | 9 |
| 3 | 4 | 2 | 2 | 2 | | 10 |
| 4 | 1 | 3 | | | 6 | 10 |
| 5 | 4 | 5 | 1 | | | 10 |
| 6 | 3 | 5 | | | | 8 |
| 7 | | 8 | | | | 8 |
| 8 | 2 | 1 | | 1 | | 4 |
| 9 | 3 | 2 | | 3 | | 8 |
| 10 | 2 | 5 | | | | 7 |
| 11 | 1 | 6 | | | | 7 |
| 12 | 6 | 1 | | | | 7 |
| 13 | 5 | 1 | | 1 | | 7 |
| 14 | | 1 | 3 | 3 | 1 | 8 |
| 15 | 7 | | | 1 | | 8 |
| 16 | | 3 | | | | 3 |
| 17 | | 4 | | | | 4 |
| 18 | 3 | 3 | | | | 6 |
| 19 | 4 | | | 4 | | 8 |
| 20 | 4 | 2 | | | | 6 |
| 21 | | 6 | | 1 | | 7 |
| **Grand Total** | **52** | **66** | **6** | **20** | **7** | **151** |
| **(%)** | **(34.4%)** | **(43.7%)** | **(4.0%)** | **(13.2%)** | **(4.6%)** | **(100%)** |

*Salmonella* of all somatic serogroups were found in carcass swabs prior to chlorinated-water spray (Table 12). The serogroup C was the prominent one (43.3%) among the carcass swab-1 samples. This was followed by serogroup B (35.0%), E (10.0%), F-67 (8.3%), and D (3.3%).

**Table 12**: Distribution of swab-1 prevalence of *Salmonella* somatic serogroups before use of chlorinated-water spray by farms

| | **Number of *Salmonella* somatic serogroup** | | | | | **Grand** |
|---|---|---|---|---|---|---|
| **Farm** | **B** | **C** | **D** | **E** | **F-67** | **Total** |
| 1 | | 3 | | 3 | | 6 |
| 2 | | 2 | 1 | | | 3 |
| 3 | 1 | | | 1 | | 2 |
| 4 | | 3 | | | 5 | 8 |
| 5 | 2 | 2 | 1 | | | 5 |
| 6 | 3 | | | | | 3 |
| 7 | | 2 | | | | 2 |
| 8 | | 3 | | | | 3 |
| 9 | | 1 | | 1 | | 2 |
| 10 | | 1 | | | | 1 |
| 12 | 6 | 1 | | | | 7 |
| 13 | 1 | | | | | 1 |
| 14 | 1 | | | | | 1 |
| 15 | 5 | | | | | 5 |
| 16 | 1 | | | | | 1 |
| 17 | | 1 | | | | 1 |
| 18 | 1 | | | | | 1 |
| 19 | | 1 | | | | 1 |
| 20 | | 5 | | | | 5 |
| 21 | | 1 | | 1 | | 2 |
| **Grand Total** | **21** | **26** | **2** | **6** | **5** | **60** |
| **(%)** | **(35.0%)** | **(43.3%)** | **(3.3%)** | **(10.0%)** | **(8.3%)** | **(100%)** |

Table 13 presents the sample prevalences of *Salmonella* somatic serogroups isolated from carcasses after an overnight chilling. Only two serogroups (B and C) were found. From 24 *Salmonella* positive samples, 14 (58.3%) isolates were serogroup B and 10 (41.7%) were serogroup C.

**Table 13:** Distribution of *Salmonella* serogroups isolated from carcasses after overnight chilling classified by farms

| Farm | Number of *Salmonella* somatic serogroup | | Grand Total |
|---|---|---|---|
| | B | C | |
| 3 | | 2 | 2 |
| 4 | | 1 | 1 |
| 5 | | 1 | 1 |
| 6 | 1 | | 1 |
| 9 | 2 | | 2 |
| 12 | 3 | | 3 |
| 14 | 2 | | 2 |
| 15 | 3 | | 3 |
| 17 | 1 | | 1 |
| 18 | | 3 | 3 |
| 19 | | 1 | 1 |
| 20 | 1 | 2 | 3 |
| 21 | 1 | | 1 |
| **Grand Total (%)** | **14 (58.3%)** | **10 (41.7%)** | **24 (100%)** |

From all 21 farms, one farm had only one *Salmonella* serogroup and another had all *Salmonella* serogroups, B, C, D, E, and F-67. The other farms had groups in different frequencies. The graphical proportion of all serogroups found in 21 farms is shown in Figure 11.

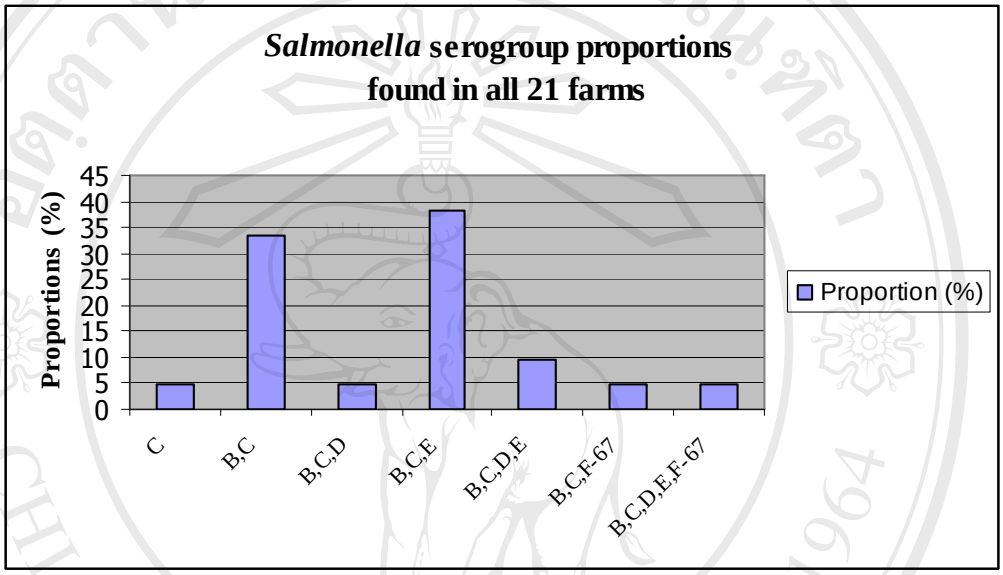



**Figure 11:** *Salmonella* serogroup proportions found in all 21 farms

## 4.3 DISTRIBUTION OF *SALMONELLA* SEROTYPES

In all the 723 samples, 351 (48.5%) were *Salmonella*-positive. Definitive *Salmonella* serotyping was conducted from these 351 samples. The three most prevalent serotypes were *S.* Rissen (161 isolates), *S.* Stanley (41 isolates), and *S.* Typhimurium (38 isolates), which were 45.9%, 11.7%, and 10.8%, respectively. Other *Salmonella* serotypes were also found in this study. Overall, the summary distributions of all serotypes obtained are given in Table 14.

**Table 14:** *Salmonella* serotypes isolated from the slaughter pigs and carcasses

| Serotype | No. of identified isolates (%) | Main somatic group (O) | Somatic Sub-group O | Flagella Phase 1 | Flagella Phase 2 |
|---|---|---|---|---|---|
| 1. Rissen | 161 (45.9%) | C | O6, 7 | f, g | - |
| 2. Stanley | 41 (11.7%) | B | O4, [5], 12 | d | 1, 2 |
| 3. Typhimurium | 38 (10.8%) | B | O4, [5], 12 | i | 1, 2 |
| 4. Glaucester | 17 (4.8%) | B | O4, [5], 12 | i | l, w |
| 5. Anatum | 16 (4.6%) | E | O3, 10 | e, h | 1,6 |
| 6. Panama | 14 (4.0%) | D | O9, 12 | l, v | 1, 5 |
| 7. Krefeld | 12 (3.4%) | E | O1, 3, 19 | y | l, w |
| 8. Weltevredren | 10 (2.8%) | E | O3, 10 [15] | r | z6 |

Continued

**Table 14:** Continued

| Serotype | No. of identified isolates (%) | Main somatic group (O) | Somatic Sub-group O | Flagella Phase 1 | Flagella Phase 2 |
|---|---|---|---|---|---|
| 9. Lagos | 9 (2.6%) | B | O4, [5], 12 | i | 1, 5 |
| 10. Tsevie | 8 (2.3%) | B | O4, [5], 12 | i | e, n, z15 |
| 11. Saintpoul | 1 (0.3%) | B | O4, [5], 12 | e,h | 1, 2 |
| 12. Eppendof | 1 (0.3%) | B | O4, [5], 12 | d | 1, 5 |
| 13. Group II (F-67) | 15 (4.3%) | | | | |
| **Other*** | **8 (2.3%)** | | | | |
| **Total (%)** | **351 (100.0%)** | | | | |

* Other =Self-agglutination (6 isolates) and unidentified (2 isolates)

The *Salmonella* serotypes identified in various samples are given in Tables 15. The most frequent serotype found in this study was *S*. Rissen, which was mainly found in mesenteric lymph nodes (63 of 116, 54.3%) and feces (63 of 116, 41.7%). *S*. Stanley, Typhimurium, and other strains were generally distributed in all the samples.

**Table 15:** Distribution of *Salmonella* serotypes identified from various types of samples

| Serotype | Distribution of serotypes by samples | | | | All isolated serotypes (%) |
|---|---|---|---|---|---|
| | MLN* (%) | Feces (%) | SW1** (%) | SW2*** (%) | |
| 1. Rissen | 63 (54.3%) | 63 (41.7%) | 26 (43.3%) | 9 (37.5%) | 161 (45.9%) |
| 2. Stanley | 10 (8.6%) | 25 (16.6%) | 3 (5.0%) | 3 (12.5%) | 41 (11.7%) |
| 3. Typimurium | 11 (9.5%) | 14 (9.3%) | 9 (15.0%) | 4 (16.7%) | 38 (10.8%) |
| 4. Glaucester | 6 (5.2%) | 5 (3.3%) | 5 (8.3%) | 1 (4.2%) | 17 (4.8%) |
| 5. Anatum | 7 (6.0%) | 8 (5.3%) | 1 (1.7%) | 0 | 16 (4.6%) |
| 6. Panama | 6 (5.2%) | 6 (4.0%) | 2 (3.3%) | 0 | 14 (4.0%) |
| 7. Krefeld | 2 (1.7%) | 5 (3.3%) | 5 (8.3%) | 0 | 12 (3.4%) |
| 8. Weltevreden | 3 (2.6%) | 7 (4.6%) | 0 | 0 | 10 (2.8%) |
| 9. Lagos | 2 (1.7%) | 3 (2.0%) | 2 (3.3%) | 2 (8.3%) | 9 (2.6%) |
| 10. Tsevie | 0 | 3 (2.0%) | 2 (3.3%) | 3 (12.5%) | 8 (2.3%) |

Continued

**Table 15:** Continued

| Serotype | Distribution of serotypes by samples | | | | All isolated serotypes (%) |
|---|---|---|---|---|---|
| | MLN* (%) | Feces (%) | SW1** (%) | SW2*** (%) | |
| 11. Saintpoul | 1 (0.7%) | 0 | 0 | 0 | 1 (0.3%) |
| 12. Eppendof | 0 | 1 (0.7%) | 0 | 0 | 1 (0.3%) |
| 13. Group F-67 | 3 (2.6%) | 7 (4.6%) | 5 (8.3%) | 0 | 15 (4.3%) |
| **Other** | 2 (1.7%) | 4 (2.6%) | 0 | 2 (8.3%) | 8 (2.3%) |
| **Total (%)** | **116 (100.0%)** | **151 (100.0%)** | **60 (100.0%)** | **24 (100.0%)** | **351 (100.0%)** |

- * MLN = Mesenteric Lymph Nodes
- ** SW1 = Carcass swabs before chlorinated-water spray
- ** SW2 = carcass swabs after overnight chilling

From 21 farms of origin, *Salmonella* serotypes were classified as presented in Table 16. *Salmonella* serotype Rissen was generally distributed in all farms, while *Salmonella* Typhimurium was mostly found in farm 12 and *Salmonella* group II (F-76) mostly found in farm 4. Other serotypes were commonly dispersed. The meaning of serotype abbreviations is described as followed;

Stnly = Stanley TM = Typhimurium Glcstr = Glaucester
Anat = Anatum Krfld = Krefeld Wetvd = Weltevedren
St.pl = Saintpoul Eppdf. = Eppendorf Gr.II = Group II (F-67)

**Table 16:** *Salmonella* serotypes classified by farms of origin

| | Numbers of *Salmonella* serotypes | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Farm** | **Rissen** | **Stnly** | **TM** | **Glcstr** | **Anat** | **Panama** | **Krfld** | **Wetvd** | **Lagos** | **Tsevie** | **St.pl** | **Eppdf** | **Gr. II** | **Other** | **Total** |
| 1 | 11 | 1 | | | | | 6 | | | | | | | | 18 (5.1% |
| 2 | 11 | 1 | 1 | 2 | 5 | 1 | | 3 | 1 | | | | | | 25 (7.1% |
| 3 | 5 | 2 | 5 | 1 | | 3 | 1 | | | | | | | | 17 (4.8% |
| 4 | 12 | | 1 | 1 | | | | | 1 | | | | 14 | | 29 (8.3% |
| 5 | 8 | 2 | 2 | | | 7 | | | 1 | 1 | 1 | | | | 22 (6.3% |
| 6 | 9 | 4 | 2 | | | | | 1 | 1 | 2 | | | | | 19 (5.4% |
| 7 | 18 | | | | | | | | | | | | | | 18 (5.1% |
| 8 | 7 | 1 | 1 | | 1 | | | | | | | | | 2 | 12 (3.4% |
| 9 | 6 | 1 | | 2 | | | 5 | | | | | 1 | | | 15 (4.3% |
| 10 | 8 | | 3 | | | | | | | | | | | | 11 (3.1% |
| 11 | 8 | 1 | | | | | | | | | | | | | 9 (2.6% |
| 12 | 4 | 2 | 19 | | | | | | | | | | | | 25 (7.1% |
| 13 | 1 | 10 | 1 | | 1 | | | 1 | | | | | | | 14 (4.0% |
| 14 | 2 | 3 | 1 | 1 | 2 | 3 | | 2 | | | | | 1 | | 15 (4.3% |
| 15 | 1 | 1 | 1 | 7 | | | | 1 | 3 | 3 | | | | | 17 (4.8% |
| 16 | 7 | | | 2 | | | | | | | | | | | 9 (2.6% |
| 17 | 5 | | | | | | | | 1 | | | | | 3 | 9 (2.6% |
| 18 | 9 | 5 | 1 | | | | | | | | | | | 1 | 16 (4.6% |
| 19 | 4 | 2 | | 1 | 6 | | | | 1 | | | | | 1 | 15 (4.3% |
| 20 | 13 | 3 | | | | | | | | 2 | | | | | 18 (5.1% |
| 21 | 12 | 2 | | | 1 | | | 2 | | | | | | 1 | 18 (5.1% |
| | 161 | 41 | 38 | 17 | 16 | 14 | 12 | 10 | 9 | 8 | 1 | 1 | 15 | 8 | 351 |
| **Total** | (45.9%) | (11.7%) | (10.8%) | (4.8%) | (4.6%) | (4.0%) | (3.4%) | (2.8%) | (2.6%) | (2.3%) | (0.3%) | (0.3%) | (4.3%) | (2.3%) | (100%) |

## 4.4 SEROLOGICAL RESULTS

One hundred and eighty one meat juice samples tested by SALMOTYPE® Pig LPS ELISA are presented in Table 17. Of those, one sample was negative for anti-*Salmonella* antibodies (cut-off O.D. % < 10), 49 samples were doubtful (cut-off O.D. % 10 - < 20), and 59 samples were weakly positive (cut-off O.D. % 20 - < 40). Positive ELISA results (cut-off O.D. % ≥ 40) were found in 72 samples (39.8%).

**Table 17:** Summary of serological results of all 181 meat juice samples

| | **Cut-off O.D. %*** | | | |
|---|---|---|---|---|
| **Meat juice samples (%)** | **< 10 (%)** | **10 - < 20 (%)** | **20 - < 40 (%)** | **≥ 40 (%)** |
| 181 (100%) | 1 (5.5%) | 49 (27.1%) | 59 (32.6%) | 72 (39.8%) |

* Cut-Off values for meat juice samples (recommended by manufacturer's instructions);

| | |
|---|---|
| ≥ 40 OD% | positive |
| 20 OD% - < 40 OD% | weak positive |
| 10 OD% - < 20 OD% | doubtful (positive) |
| < 10 OD% | negative |

At the individual pig level, serological ELISA results were compared with the results of conventional *Salmonella* culture (positive lymph node- and fecal culture, Tables 18 and 19). Using the lymph node culture results as the gold standard, sensitivity and specificity of the test were calculated using Win Episcope 2.0. Kappa statistics (Epi Info, version 2.0) was used to assess an agreement between those two different methods. Calculated kappa values were 0.122 and 0.057 compared to lymph node culture and fecal culture results, respectively.

**Table 18:** Comparison of lymph node culture and the ELISA results of *Salmonella* at the individual pig level

| **ELISA** | **Lymph node culture** | | |
|---|---|---|---|
| **Result*** | **+** | **-** | **Grand Total** |
| + | 52 | 20 | 72 |
| - | 64 | 45 | 109 |
| **Grand Total** | 116 | 65 | 181 |

*ELISA Result (OD%≥ 40 = positive, OD%< 40 =negative)

| | % | Lower limit | Upper limit |
|---|---|---|---|
| Sensitivity of ELISA test | 44.8 | 35.8 | 53.9 |
| Specificity of ELISA test | 69.2 | 58.0 | 80.5 |

Kappa statistics** (Epi Info 2002)

Observed proportion of agreement = 0.536

Expected proportion of agreement = 0.471

Observed minus chance agreement = 0.065

Max possible agreement beyond chance = 0.529

Kappa = 0.122

95% Confidence Intervals = -0.050 – 0.295

**Kappa value (Dahoo *et al*., 2003);

| | |
|---|---|
| <0.2: | slight agreement |
| 0.2- 0.4: | fair agreement |
| 0.4-0.6: | moderate agreement |
| 0.6-0.8: | substantial agreement |
| >0.8: | almost perfect agreement |

**Table 19:** Comparison of fecal culture and the ELISA results of *Salmonella* at the individual pig level

| **ELISA** | **Fecal culture** | | |
|---|---|---|---|
| **Result*** | **+** | **-** | **Grand Total** |
| + | 63 | 9 | 72 |
| - | 88 | 21 | 109 |
| **Grand Total** | 151 | 30 | 181 |

*ELISA Result (OD%≥ 40 = positive, OD%< 40 =negative)

| | % | Lower limit | Upper limit |
|---|---|---|---|
| Sensitivity of ELISA test | 41.7 | 33.9 | 49.6 |
| Specificity of ELISA test | 70.0 | 53.6 | 86.4 |

Kappa statistics (Epi Info 2002)

Observed proportion of agreement = 0.364

Expected proportion of agreement = 0.464

Observed minus chance agreement = 0.032

Max possible agreement beyond chance = 0.568

Kappa = 0.057

95% Confidence Intervals = -0.036 – 0.150

Based on the results of kappa statistics and its 95% Confidence Intervals, the agreement between ELISA test results and bacteriological assays (lymph node- and fecal culture results) was slight (both Kappa values < 0.2). Thus, it was neither statistically significant between both different methods nor better than an expected proportion of agreement (expected proportion = 0.471 and 0.464) due to chance.

# 5. DISCUSSIONS AND CONLUSIONS

## 5.1 Prevalence of *Salmonella* in the slaughter pigs

### 5.1.1 Fecal prevalence

*Salmonella* prevalences of the finisher pork herds, followed through by this investigation from production to consumption, at farm level 1 to 2 days prior to slaughter, were 62.9% and 64.4%, by investigation of fecal and serum samples of the same animals, respectively (Dorn-in, 2005):

At slaughter, *Salmonella* were isolated in 83.4% from feces of the same pigs investigated before at farm level. The overall infection rate from farm via transport and lairage until at slaughter had increased by 20.1 percent.

So far, only one previous prevalence study investigated *Salmonella* in slaughter pigs and carcasses in Chiang Mai, Thailand (Patchanee *et al.*, 2002). That study revealed a farm prevalence estimate of 69.5%, which increased to 80.5%, just prior to the slaughtering of the pigs. Prevalences at source farms and their significant increase with slaughter of that study are therefore almost in total agreement with the results of this study. It can be postulated that this increase in prevalence of *Salmonella* was due to the new infection and/or cross-contamination during transportation and during the waiting phase at the slaughterhouse lairage. Transportation of pigs from farm to slaughterhouse took up to ½ day, the waiting period after unloading at the slaughterhouse typically lasted from the morning to the afternoon.

### 5.1.2 Mesenteric lymph node prevalence

The prevalence of *Salmonella* in the mesenteric lymph nodes of the study pigs was 64.1%; this percentage is significantly lower than that from the fecal samples, which were concurrently collected with the lymph node samples, but it is almost identical with the infection prevalence at farm level. Obviously isolations of *Salmonella* from lymph nodes immediately at slaughter almost perfectly reflect infection rates of finisher herds at farm level.

### 5.1.3 Danish Mix ELISA results of meat juice

Major disadvantages of *Salmonella* isolation by culture are the relatively low sensitivity of bacteriology and the complicated and time-consuming culture processes. Serological testing is inexpensive; a large number of samples can be rapid and at relatively low cost analyses. ELISA tests using muscle fluid samples from pigs taken at slaughter can be used as a practical alternative to serum to detect antibodies to *Salmonella* polysaccharide. The SALMOTYPE® Pig LPS ELISA (Labor Diagnostik, Leipzig, Germany) was used in this study. According to the manufacturer's instructions, the assay detects antibodies to the O-antigens 1, 4, 5, 6, 7 and 12, representing more than 90% of the most common *Salmonella* serotypes isolated from pigs in Europe. This assay is designed to measure the quantity of antibodies to *Salmonella* in pork meat juice or in pig serum. However, demonstrated serum- or meat juice antibodies do reflect only previous exposure rather than current infection with *Salmonella*.

Using the lymph node culture results as the gold standard, a sensitivity of 44.8% and a specificity of 69.2% were determined for the ELISA test. Respective test properties from a comparison of results with those of the fecal cultures were 41.7% and 70%, respectively.

At the individual pigs' level, results of the ELISA test did not demonstrate a strong agreement (kappa value = 0.057) between the *Salmonella* status in the meat juice of slaughter pigs and definite diagnosis from bacteriology. Test agreement between ELISA and lymph node culture results (kappa value = 0.122) was slightly better but still low. The 95% confidence intervals for the kappa values further indicate that these estimates did carry a large degree of uncertainty.

According to Lo Fo Wong *et al*. (2003), results from bacteriological and serological tests cannot be easily compared because of the different characteristics of both methods, such as their sensitivities and specificities on the one hand and on different sampling methods, such as different sample-sizes, -frequencies and –locations on the other hand.

A major complicating factor in bacteriology for detecting *Salmonella* organisms in individual pigs is the occurrence of apparently healthy carriers, which shed the organism intermittently in the feces, and silent carriers, which do not shed, but harbor the organism in mesenteric lymph nodes or in the mucosa of the cecum and colon. The difficulty varies according to *Salmonella* genotype. Serological tests like ELISA on the other hand are restricted to the herd level. Both tests aim at different study units (individual animals or herds) and at different stages or location of infection (carrier of organisms primarily in the intestines or systemic infection).

Considering these limitations, investigations of pooled serum or meat juice samples by ELISA are suitable, fast and cheap for screening for the presence of infection with *Salmonella* on a herd basis.

## 5. 2 *Salmonella* prevalence of the pig carcasses

### 5.2.1 Prevalence in carcass swabs before chlorinated-water spray

The prevalence of *Salmonella* obtained from the surfaces of carcasses of pigs after evisceration indicates contamination or cross-contamination by fecal contents, infected tissues, and by the slaughterhouse environment (Oosterom, 1991). Slaughter pigs themselves are believed to be the main sources of contamination of carcasses, with improper slaughtering processes or unhygienic technical handling adding to such surface contamination during slaughter. Carcass swabbing is used to assess *Salmonella* carcass contamination/cross-contamination, summarized under slaughtering hygiene.

*Salmonella* were detected in carcass swabs of 33.1% of pig carcass' surfaces before the carcasses were washed with chlorinated water. With about 1/3$^{rd}$ of the carcasses being contaminated, the high level of obviously poor slaughtering hygiene is indicated. Borch *et al.* (1996) mentioned that in a slaughtering line, evisceration is the most important stage for hygienic awareness. Enclosure of the rectum and continuous disinfection of handling tools are major preventive measures, which have to be applied at this slaughter stage. *Salmonella* isolations from carcass swabs consequently point to the need to review the slaughter process and to take corrective actions. Practical standards such as Hazard Analysis Critical Control Points (HACCP) should be strictly applied.



### 5.2.2 Prevalence in carcass swabs after overnight chilling

Carcasses before chilling were washed and sprayed with 50-100 ppm chlorinated-water to reduce carcass surface contamination. Carcasses then were shock-frozen for 2 hours at –18 to 20°C, followed by storage and overnight cooling at 4°C. The remaining 13.3% *Salmonella* prevalence of carcasses after chilling indicates that chlorinated water spraying did reduce carcass contamination by about 20%, from

33.1% prior to spraying to 13.3 % after overnight chilling, but in combination with chilling was by far insufficient to reduce carcass contamination to truly low levels. About 1 out of 8 pigs remained to be *Salmonella*-infected and did enter the subsequent processing line.

Reasons for decreases or increases in the amounts of contaminating microorganisms on carcass surfaces are manifold. Gill and Bryant (1992) observed a reduction in the levels of gram-negative bacteria during chilling. In contrast, Bolton *et al.* (2002) found that final washing did increase bacterial counts, and chilling led to a small but statistically significant increase in total viable cell counts. Such observations may lead to the conclusion that the observed decrease of *Salmonella* prevalence, apart from the use of chlorinated water spray, may be due to further factors. Effective chilling may be a particular point to consider because it should prevent the proliferation of bacteria on warm carcass surfaces.

### 5.3 Pre-slaughter factors effecting *Salmonella* prevalence

Many studies corroborate on the effects of factors on the increase of *Salmonella* prevalence at slaughterhouse level. Hald *et al.* (2003) indicated that infected pigs are mostly unapparently infected; these clinically normal carrier pigs are considered to be the main source of *Salmonella* shedding. Dickson *et al.* (2003) summarize that shedding of *Salmonella* may be exacerbated by a long list of stressors, including noise, unfamiliar smells, vibration, changes in temperature, breakdown of social groupings or food deprivation. It is important to consider that stresses principally may affect the hosts' immune system. However, no conclusive report so far exists which demonstrates a direct association between stress or immune status and increased shedding or susceptibility to *Salmonella* infection in pigs (Dickson *et al.*, 2003).



Moreover, Stärk *et al.* (2002) stressed that experts from different countries failed to come to total agreement on probable sources of *Salmonella* introduction in slaughter pigs. Consensus though existed that typically between 21 and 33% of pigs coming from a chronically infected farm would be infected with *Salmonella*, but only

one-third of these infected pigs would be shedders. Differences in opinion regarding *Salmonella* dynamics could be due to either true differences in risks as a consequence of distinct management and transport practices in variable sites or to a difference in perception. Hence, further research and studies concerning the actual causes of *Salmonella* occurrence in slaughter pigs are required.

## 5.4 *Salmonella* serotype distribution in the slaughter pigs and carcasses

The most frequent serotype identified in this study was *S.* Rissen (45.8%), in which there were 54.3% obtained in mesenteric lymph nodes and 41.7 % in feces, similar to the 45.4% obtained from total samples and 53.7% from feces of the finisher pigs at farm level (Dorn-in, 2005). The next most prevalent serotypes found in this study were *S.* Stanley (11.7%) and *S.* Typhimurium (10.8%). Of those, 16.6% of *S.* Stanley was obtained from feces and 8.6% from mesenteric lymph nodes, while 9.3% of *S.* Typhimurium was found in mesenteric lymph nodes and 9.5% in feces. Serotypes found in those samples were closely related to 15.7% of *S.* Stanley and 9.9% of *S.* Typhimurium obtained in feces of finisher pigs at farm level by Dorn-in (2005).

Based on various proportions of serotypes found in this study, most of them gradually decreased in magnitudes on finished carcasses, but still existed with low proportions in the final carcasses. Only some serotypes, e.g. *S.* Anatum, Panama, Krefeld, Weltevreden, and *Salmonella* serogroup II (F-67), disappeared on the final carcass surfaces after they were sprayed with chlorinated-water and were chilled overnight. *Salmonella* spp. Weltevreden, Saintpoul, and Eppendorf, which were already found in the lymph nodes and faeces, had non cross-contaminated pork carcasses either before use of chlorinated water or after chilling. No emerging serotype was found on the final carcasses, reflecting absence of additional contamination on carcasses from handling in this slaughterhouse.

According to the study of *Salmonella* serogroups in Chiang Mai slaughterhouse by Pachanee *at el.* (2002), the most frequent serogroup was C. This group was also the most prevalent in this study.

Occurrences of *Salmonella* serotypes for Thailand have been summarized by Bangtrakulnonth *et al*. (2004). Their report only includes serotype distribution from human food-borne gastrointestinal infections and from different foods, but does not include pork. Nevertheless, the most five common serotypes found during the past 10 years (1993-2002) according to that report were *S.* Weltevreden, Enteritidis, Anatum, Derby, and 1, 4, 4, 12:i:-sspI.

## 5.5 Conclusions

The incidence of salmonellosis in man has increased in recent years and animals, particularly pigs, are incriminated as the principal reservoir. *Salmonella* monitoring in many countries is a prerequisite to enter global pork markets today. For Chiang Mai, Thailand, where pig production and pork consumption are predominant and widespread, essentially no baseline data concerning *Salmonella* occurrence through the pork production chain were available prior to this study.

This work is part of the first *Salmonella* investigation conducted along the entire pork production chain in Chiang Mai, Thailand. The particular focus of this study was on establishing *Salmonella* prevalences in slaughter pigs. Bacteriological laboratory investigation for *Salmonella* infections of individual slaughter pigs did follow international standard methods (ISO 6579). A commercial meat juice ELISA test additionally was used for serological screening of *Salmonella* infection at herd (slaughter batch) level.

The study revealed high levels of *Salmonella* in pigs during slaughter and on their carcasses with variable serotype distributions. At the end of the slaughtering process, *Salmonella* contamination still was present despite disinfection with chlorinated-water spray and chilling.

No particular and practical suggestions are made to remedy the situation in light of the actual management of the slaughter process. Considering the high infection levels, a comprehensive program seems the only viable option to reduce *Salmonella.* Such a program will have to contain multiple components of individual good hygienic practices and standard measures in the slaughtering system or, even better, involve the establishment of a HACCP system throughout the slaughter process.

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# APPENDICES

## APPENDIX A

## LIST OF CHEMICALS AND REAGENTS USED FOR *SALMONELLA* CULTURE IN THIS STUDY

1. Culture Media
   - Buffered Peptone water (BPW)
   - Rappaport Vasiliadis broth (RV)
   - Tetrationate (TT broth)
   - Brilliant Green Phenol Red Lactose Sucrose agar (BPLS)
   - Xylose-lysine-tergitol 4 agar (XLT4)
   - Nutrient agar (NA)

2. Biochemical Media
   - Triple Sugar Iron agar slants (TSI)
   - Urea slant
   - Voges-Proskaur broth (VP)
   - Motile-indole-lysine broth (MIL)

3. Media for Serogroup Typing and Flagella Phase
   - Nutrient agar (NA) + 65% Nutrient agar (NA)

4. Media for Storage of *Salmonella* Isolates
   - 50% Nutrient agar (Half-NA)

5. Other Chemicals and Reagents
   - Kovac's reagent
   - creatine solution
   - ethanolic solution of 1-naphthol
   - Potassium hydroxide solution

# APPENDIX B

## MEDIA PREPARATION

**Buffered peptone water (BPW; Merck KGaA, Germany)**

Preparation: Suspend **25.5 g in 1 liter** of demineralized water; if desired dispense into smaller vessels; autoclave **(15 min. at 121 °C)**.

**pH: 7.0 ± 0.2 at 25 °C**

**Rappaport Visiliadis broth (RV; Merck KGaA, Germany)**

Preparation: Suspend **42.5 g in 1 liter** of demin. water; dispense into tubes or flasks; autoclave (15 min. at 121 °C).

**pH: 5.2 ± 0.2 at 25 °C**

**Tetrathionate broth (TT; Merck KGaA, Germany)**

Preparation: Suspend **82 g in 1 liter** of demineralrized water, heat briefly to boiling. **Do not autoclave!** After cooling, add 20 ml/l iodine potassium iodine solution and 10-ml/l 0.1% brilliant green solution (brilliant green, Cat. No.1.01310.). Dispense any eventual precipitate.

**Brilliant Green Phenol Red Lactose Sucrose Agar (BPLS; Merck KGaA, Germany)**

Preparation: Suspend **51 g in 1 liter** of demin. water by heating in a boiling water bath or in a current of stream; autoclave (15 min. at 121 °C); pour to plates.

**pH: 6.9 ± 0.2 at 25 °C**

**Xylose-lysine-tergitol 4 (XLT4; Merck KGaA, Germany)**

Preparation: Suspend **59 g in 1 liter** of demin. water, add 4.6 ml XLT4 Agar Supplement solution and heat the medium in a boiling water-batch (*not* on a heating-plate!).Cool to approx. 50 °C and pour plates. **Do not overheat, do not autoclave!**

**pH: 7.4 ± 0.2 at 25 °C**

**Triple Sugar Iron Agar Slants (TSI; Merck KGaA, Germany)**

Preparation: Suspend **65 g in 1 liter** of demin. water by heating in a boiling water bath or in a current of stream; dispense into tubes; autoclave (15 min. at 121 ℃). Allow solidifying to give agar slants.

**pH: 7.4 ± 0.2 at 25 °C**

**Urea Agar (Urea; Merck KGaA, Germany)**

1. Suspend 29 g of medium in 100 ml of demineralized water.
2. Mix thoroughly and sterilize by filtration.
3. Dissolve 15 g of agar in 900 ml of demineralized water.
4. Sterilize by autoclaving at 121℃ for 15 minutes.
5. Cool to 45-50℃ and aseptically add the sterile Urea Agar Base.
6. Mix thoroughly and dispense into sterile tubes.
7. Cool in a slanted position so that deep butts are formed.

**Nutrient Agar (NA; Merck KGaA, Germany)**

Preparation: Suspend **20 g in 1 liter** of demineralized water by heating in a boiling water bath or in a current of stream; autoclave (15 min. at 121 ℃). Pour to plates.

**pH: 7.0 ± 0.2 at 25 °C**

# APPENDIX C

## HISTORY OF SUBJECTS (THE SLAUGHTER PIGS)

**Table 1:** Sources (districts and provinces) of slaughter pigs (LP = Lumphun Province, CM = Chiang Mai Province)

| Slaughterhouse visit | Date collected | Numbers of studied pigs | Location of farms of origin | Farm type (closed/open) |
|---|---|---|---|---|
| 1st | Dec.20, 2004 | 8 | Mae-tha (LP) | Open |
| 2nd | Jan.7, 2005 | 10 | Ban-thi (LP) | Open |
| 3rd | Jan.14, 2005 | 10 | San-sai (CM) | Closed |
| 4th | Jan.25, 2005 | 10 | San-sai (CM) | Open |
| 5th | Feb.4, 2005 | 10 | San-sai (CM) | Open |
| 6th | Mar.4, 2005 | 8 | Pa-sang (LP) | Open |
| 7th | Mar.8, 2005 | 8 | Mae-tang (CM) | Open |
| 8th | Mar.10, 2005 | 8 | Mae-rim (CM) | Open |
| 9th | Mar.16, 2005 | 8 | Pa-sang (LP) | Open |
| 10th | Mar.18, 2005 | 8 | Mae-tang (CM) | Open |
| 11th | Mar.25, 2005 | 7 | Mae-wang (CM) | Open |
| 12th | Mar.29, 2005 | 10 | San-kam-phang (CM) | Closed |
| 13th | Apr.2, 2005 | 8 | Mae-tha (LP) | Open |
| 14th | Apr.5, 2005 | 10 | San-kam-phang(CM) | Closed |
| 15th | Apr.20, 2005 | 10 | San-kam-phang (CM) | Closed |
| 16th | Apr.24, 2005 | 8 | Mae-on (CM) | Open |
| 17th | Apr.27, 2005 | 8 | San-kam-phang(CM) | Open |
| 18th | Apr.30, 2005 | 8 | Mae-taeng (CM) | Open |
| 19th | May4, 2005 | 8 | Mae-tha (LP) | Open |
| 20th | May6, 2005 | 8 | Mae-tha (LP) | Open |
| 21st | May9, 2005 | 8 | Mae-tha (LP) | Open |
| **Total** | **21 visits** | **181 pigs** | **2 provinces** | **2 types** |

# CURRICULUM VITAE

## 1. PERSONAL DATA:

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**Place of birth** Nan, Thailand

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**1993 – 1995** High School Certificate, Sa School, Nan

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|---|---|---|
| Lecturer | Chiang Rai Rajabhat University | April17, 2002 – present |

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- Royal Thai Government Scholarship granted for MSc study in Veterinary Public Health, the joint MSc VPH program of the Faculties of Veterinary Medicine, CMU and FU Berlin, Germany, October 2003 - September 2005.

# DECLARATION

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

**Name:** Wasan Chantong

**Signature:** Wasan Chantong

**Date of Submission:** 2 September 2005