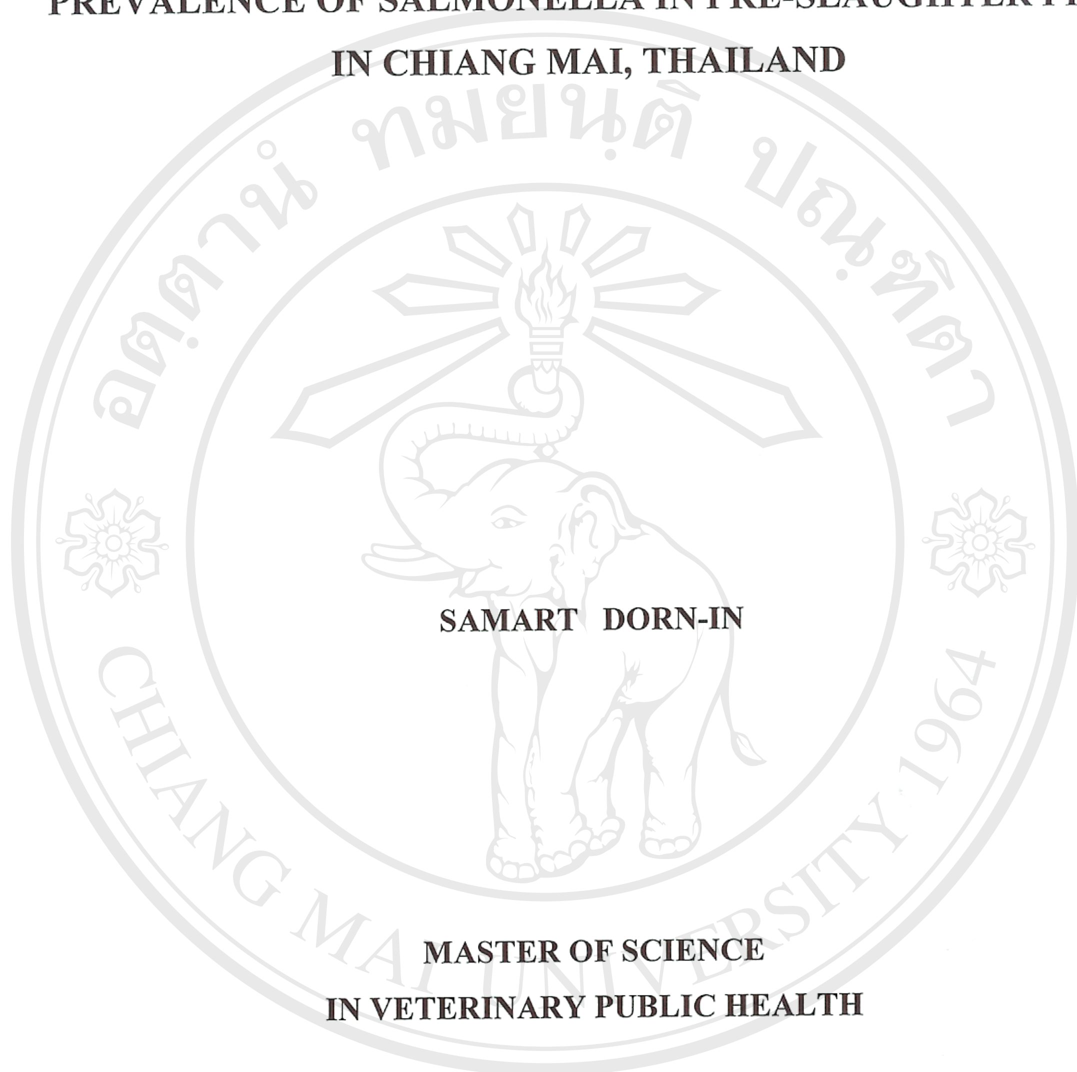
PREVALENCE OF SALMONELLA IN PRE-SLAUGHTER PIGS IN CHIANG MAI, THAILAND



SAMART DORN-IN

MASTER OF SCIENCE
IN VETERINARY PUBLIC HEALTH

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PREVALENCE OF SALMONELLA IN PRE-SLAUGHTER PIGS IN CHIANG MAI, THAILAND

SAMART DORN-IN

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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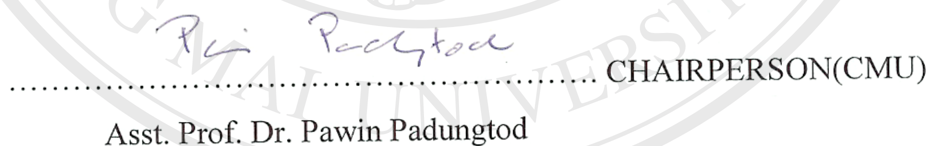
EXAMINING COMMITTEE



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Thesis title Prevalence of Salmonella in Pre–Slaughter Pigs in Chiang Mai, Thailand

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ABSTRACT

Fattening pigs are sources of *Salmonella* contamination in pork. There are control measures for reducing the contamination at slaughterhouses. In order to facilitate control of *Salmonella*, the *Salmonella* infection status of herds and the farm intervention methods to reduce the risk of infection should be evaluated. This study was conducted in order to investigate the prevalence of *Salmonella* in pre-slaughter pigs for a particular slaughterhouse in Chiang Mai province, Thailand, to identify the *Salmonella* serotypes and to determine the relationship between farm management characteristics and the prevalence of *Salmonella.* This was a cross-sectional study. A total of 22 pig farms were included in this study. A total of 427 serum samples, 194 faecal samples, 195 floor swab samples and 22 samples for each type of water were collected. The isolation procedure followed the ISO 6579 (2000) and serotyping identification followed the instructions from the manufacturer (Sifin, Germany). The result from that *Salmonella* sero-prevalence was 64.4%, while the prevalence in faecal isolation was 62.9%. The percentage of contamination in environmental samples was 94.8% in floor swab samples and 95.5% in waste water samples. The serotypes most frequently found were *S.* Rissen (45.4%) followed by *S.* Typhimurium (18.6%), *S.* Stanley (11.2%), *S.* Weltevreden (3.7%), *S.* Krefeld (3.1%) and *S.* Anatum (2.4%). From the results of logistic regression of multivariable analysis, herds of (i) less than 800 pigs (ii) raised in a closed house system had a significant lower risk of getting *Salmonella* ($p<0.05$) both in serological and faecal isolation results. Farms which

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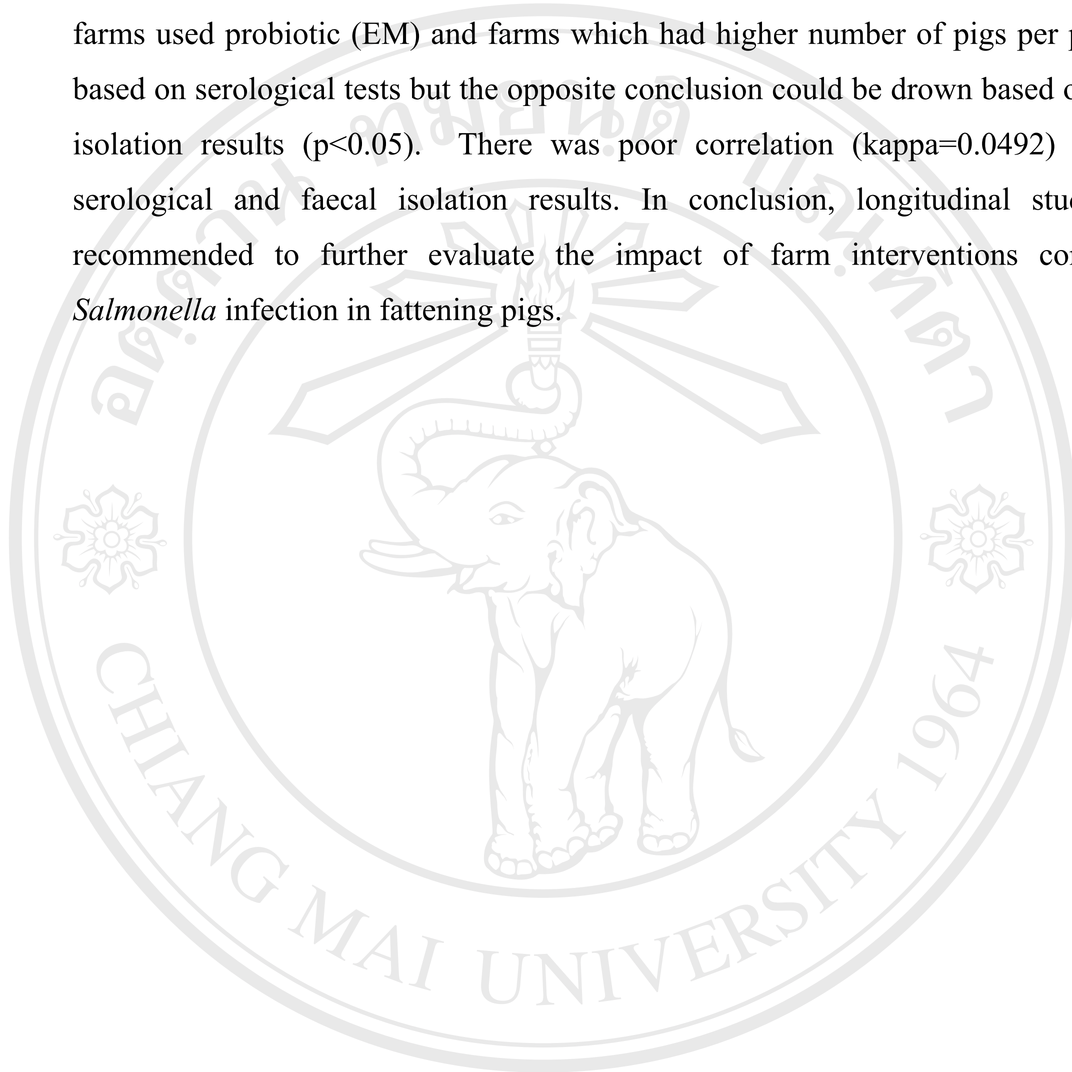
used (i) probiotic and (ii) those which had lower numbers of pigs per pen appeared to have significantly ($p<0.05$) lower chance of getting *Salmonella* infection compared to farms used probiotic (EM) and farms which had higher number of pigs per pen; this based on serological tests but the opposite conclusion could be drown based on faecal isolation results ($p<0.05$). There was poor correlation (kappa=0.0492) between serological and faecal isolation results. In conclusion, longitudinal studies are recommended to further evaluate the impact of farm interventions combat of *Salmonella* infection in fattening pigs.



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ชื่อเรื่องวิทยานิพนธ์	ความชุกของเชื้อซัลโมเนลลาในสุกรขุน ในจังหวัดเชียงใหม่ ประเทศไทย
ผู้เขียน	นายสามารถ ดรอินทร์
ปริญญา	วิทยาศาสตร์มหาบัณฑิต (สัตวแพทย์สาธารณสุข)
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บทคัดย่อ

สุกรขุนเป็นแหล่งของเชื้อซัลโมเนลลาที่ปนเปื้อนในเนื้อสุกรสด การควบคุมการปนเปื้อนเชื้อซัลโมเนลลาในฟาร์มสุกรจะต้องมีการประเมินวิธีการลดความเสี่ยงวิธีต่างๆด้วย การศึกษานี้มีวัตถุประสงค์เพื่อประเมินความชุกของเชื้อซัลโมเนลลาในสุกรขุนที่เลี้ยงโดยกลุ่มฟาร์มที่ส่งสุกรเข้าฆ่าที่โรงฆ่าแห่งหนึ่งในจังหวัดเชียงใหม่ และศึกษาความสัมพันธ์ระหว่างการจัดการฟาร์มกับความชุกของการพบเชื้อซัลโมเนลลา จากการศึกษาฟาร์ม

สุกรขุน 22 ฟาร์ม โดยเก็บตัวอย่าง ซีรั่ม 427 ตัวอย่าง อุจจาระ 194 ตัวอย่าง สิ่งแวดล้อม 195 ตัวอย่าง และน้ำ 22 ตัวอย่าง ตรวจหาเชื้อซัลโมเนลลาโดยวิธีมาตรฐาน และจำแนกชนิดของเชื้อด้วยวิธีตกตะกอนกับแอนติบอดี้ ผลของการศึกษาพบว่า ความชุกของภูมิต้านทานต่อซัลโมเนลลาในซีรั่ม เท่ากับร้อยละ 164.4 ส่วนความชุกของเชื้อซัลโมเนลลาในอุจจาระ เท่ากับร้อยละ 62.9 สัดส่วนตัวอย่างจากสิ่งแวดล้อม และน้ำที่ปนเปื้อนในเชื้อซัลโมเนลลา เท่ากับร้อยละ 94.8 และร้อยละ 95.5 ตามลำดับ เชื้อซัลโมเนลลาที่พบมากที่สุด ได้แก่ S.Vissen (45.4%) รองลงมาได้แก่ S.Typhinuriun (18.6%) S.Stariley (11.2%) S.Welfevreder (3.7%) S.Kvefeld (3.1%) และ S.Anatum (2.4%) การวิเคราะห์ปัจจัยเสี่ยงพบว่า ฝูงสุกรขนาดน้อยกว่า 800ตัว ที่มีโรงเรือนปิดมีโอกาสปนเปื้อนเชื้อซัลโมเนลลาน้อยกว่า ($p<0.05$) ฟาร์มที่ใช้โปรไบโอติก และมีจำนวนสุกรต่อคอกน้อยกว่าจะมีภูมิต้านทานต่อเชื้อซัลโมเนลลาน้อยกว่า ($p<0.05$) แต่จะพบเชื้อในอุจจาระมากกว่า ($p<0.05$) ผลการตรวจการปนเปื้อนเชื้อซัลโมเนลลาในอุจจาระกับการตรวจภูมิต้านทานมีความสอดคล้องกันต่ำ ($K=0.049$) ควรทำการศึกษาแบบติดตามเพื่อศึกษาผลของมาตรการควบคุมการปนเปื้อนของเชื้อซัลโมเนลลาในสุกรขุน

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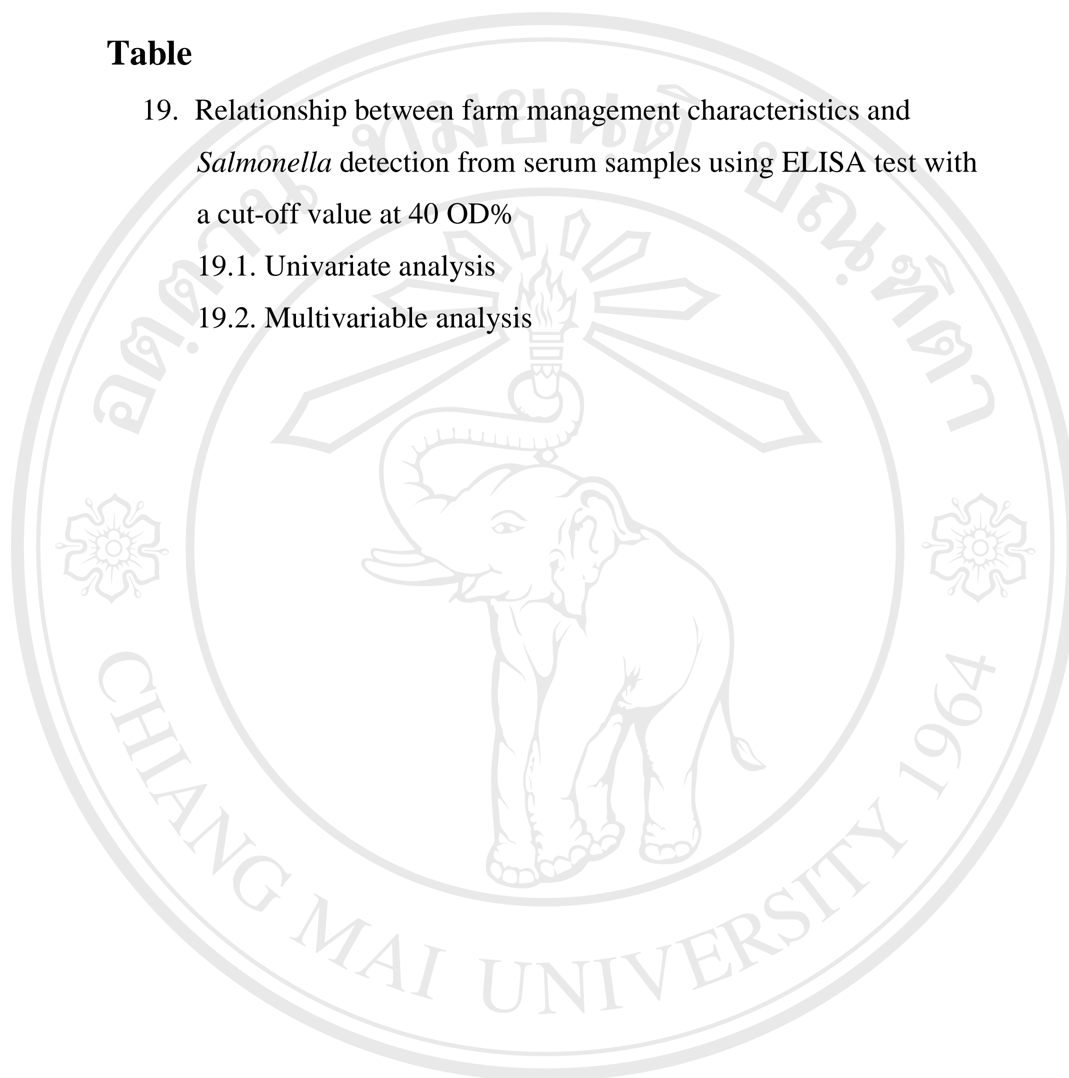

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ABBREVIATION AND SYMBOLS

µm	micrometer
a_w	Water activity
^{o}C	^{o} Celsius
CFU	Colony forming unit
CI	Confidence Interval
DLD	Department of livestock development
DNA	Deoxyribonucleic acid
EC	European Commission
EM	Effective Microorganisms
ELISA	Enzyme-linked immunosorbent assay
et al.	et alii
EU	European Union
Evap	Evaporative cooling system
FMD	Foot and mouth disease
g	gram
h	hour
ISO	International Standardization Organization
kg	kilogram
ml	milliliter
No.	Number
OIE	Office International des Epizooties
S.	*Salmonella*
WHO	World Health Organization

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1. INTRODUCTION AND OBJECTIVES

1.1. Introduction

Among the various important pathogenic bacteria that are known to cause mass food-poisoning, belongs to the genus *Salmonella* (Krieg and Holt, 1984). The ingestion of these organisms in contaminated food or water may lead to salmonellosis, a serious bacterial toxin-infection syndrome associated with gastroenteritis, typhoid and non-typhoid (Jay, 1996). Although most people survive a *Salmonella* infection, it can be life-threatening for infants and elderly and for persons already weakened by other serious diseases. The accidental contamination of *Salmonella* in raw and processed foods is a major problem for the food and feed industries worldwide due to the following reasons: (i) their strong pathogenic characteristics, (ii) their frequent presence in raw products, (iii) their rapid development in foods that are not kept properly after preparation, (iv) their responsibility for highly-publicized toxin-infection which may discredit a manufacturer or a type of food product (Axelsson and Sorin, 1997).

The wide spread of *Salmonella* in the natural environment, coupled with the intensive husbandry practices used in the meat, fish, and shell fish industries and the recycling of offal and inedible raw materials into animal feeds, have favored the continued prominence of this human bacterial pathogen in the global food chain. Poultry meat and eggs are a predominant reservoir of *Salmonella*, and pork is generally recognized as the second important source of human salmonelloses (D' Aoust *et al*., 2001, Hanes, 2003, Jay, 1996). A study in Great Britain during 1999-2000 found, that the carriage rate of *Salmonella* in prime slaughter cattle and sheep was very low compared with pigs. This suggested that future control measures should be focused on reducing *Salmonella* infection on pigs and minimizing contamination of carcass at slaughter (Davies *et al*., 2004).

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Thailand is a primary chicken-meat exporting country. Importing countries such as Japan or the European Union are going to require a zero tolerance for *Salmonella* because of its pathogenicity for humans (Regulation EC No 2160/2003). The quality assurance programs and regulations for controlling *Salmonella* infection in the poultry production chain are presented in Thailand, and are rather effective. In the case of pork, however, we can not export fresh pork because of FMD (OIE, list A) and the regulations to control the safety and quality of pork and pork products have not attracted much attention. However, since the avian influenza outbreak in Thailand, the demand for pork and pork products within the country has increased. Recently, Thai government has included pork as a "price control" product. As a probable subsequence, the pork production business will expand and be better controlled. The DLD (Department of Livestock Development) of Thailand encourages farmers to improve the standard production system and the bio-security of the farm. If any farm meets the standard set by the DLD, it will be certified as a 'Standard Pig Farm'. This is the primary step to guarantee that the important diseases are under control. However, control measures, specific to *Salmonella*, are still far from the attention of most Thai pig farmers.

This project was to determine the prevalence and the risk factors associated with *Salmonella* contamination in fattening-pigs at the pre-slaughter stage. Fattening-pigs carrying *Salmonella enterica* are implicated as a main source of carcass and pork contamination at the later stages (Beloeil *et al.*, 2004). *Salmonella* control programs in the pork production chains should start from the farm, then embrace the slaughterhouse and finally the market.

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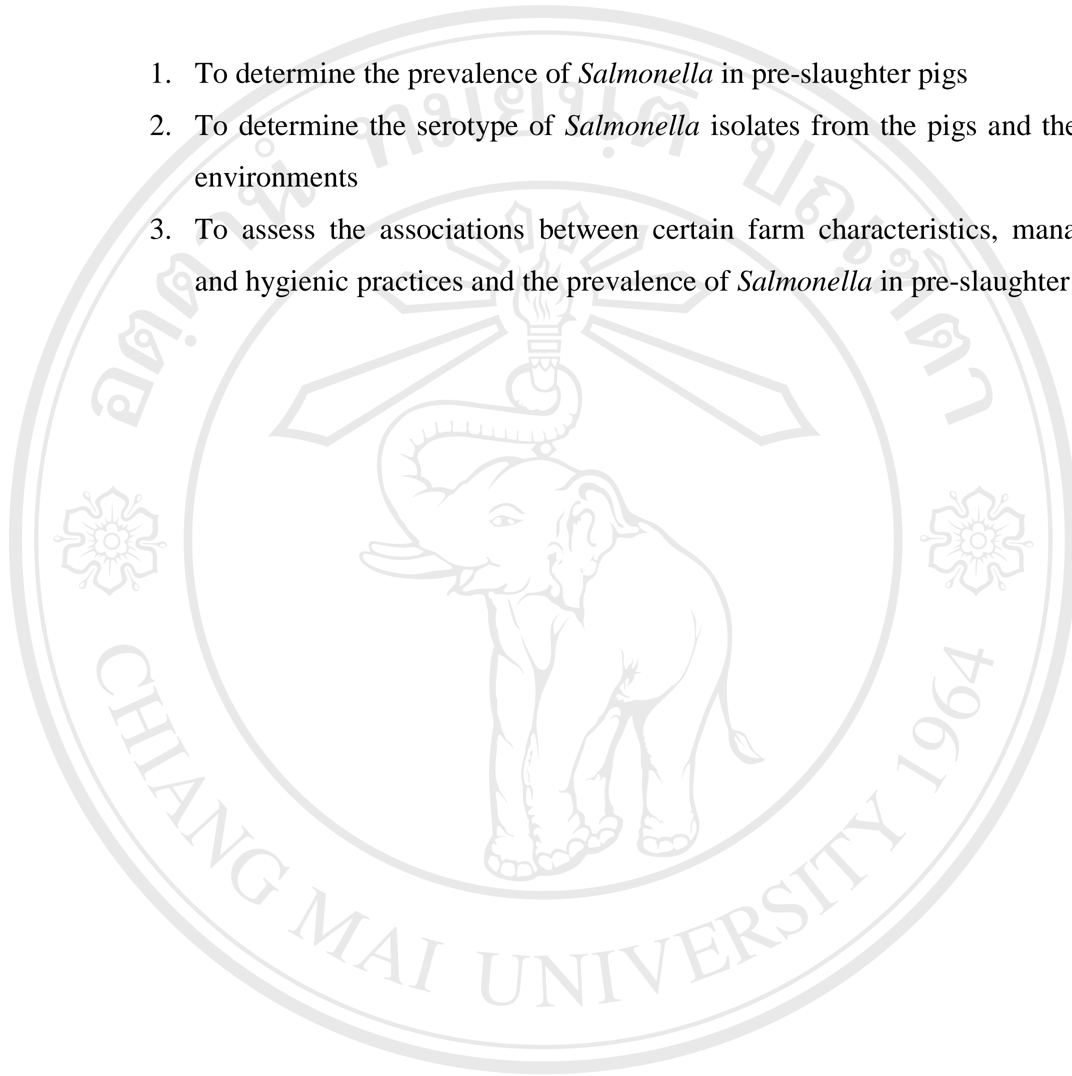
1.2. Objectives

1. To determine the prevalence of *Salmonella* in pre-slaughter pigs
2. To determine the serotype of *Salmonella* isolates from the pigs and the farm environments
3. To assess the associations between certain farm characteristics, managerial and hygienic practices and the prevalence of *Salmonella* in pre-slaughter pigs



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2. LITERATURE REVIEW

2.1. *Salmonella*

2.1.1. Microbiology

Salmonellae are gram-negative bacteria belonging to the genus *Salmonella* of the family *Enterobacteriaceae*. They are straight rods of 0.7-1.5x2-5 µm that have the capacity to grow under either aerobic or anaerobic conditions (Krieg and Holt, 1984). They are non-encapsulated and non-sporular bacteria. The bacteria grow optimally at 37 ^{o}C on ordinary culture media, where they develop small colonies of 2 to 4 mm in diameter which are smooth, shiny and homogenous in color (Krieg and Holt, 1984). Metabolic characteristics of *Salmonella* usually include the utilization of citrate as a sole carbon source and the production of gas from glucose. Lactose is generally not fermented by salmonellae, except for some strains of *S. diarizonae* (Table 1, Holt *et al.*, 2000, Hanes, 2003). Like most bacteria, their optimum pH for growth is neutral (pH 6.5-7.5), although growth may still occur in a wide pH range (4.5 to 9.5) depending on the surrounding conditions. The lowest temperature at which *Salmonella* has been found to grow is 2 ^{o}C and the highest is 54 ^{o}C (for *S.* Typhimurium). *Salmonella* require water activity (a_w) above 0.94 (Hanes, 2003) and growth inhibition has been reported at a_w below 0.93 (D' Aoust *et al*., 2001). A salt content of 3-4% generally inhibits the growth of *Salmonella*, but increasing the temperature increases salt tolerance in the range of 10 to 30 ^{o}C (D' Aoust *et al*., 2001). However, a salt content above 8% is bactericidal for salmonellae (Jay, 1996).

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Table 1: Biochemical profile of *Salmonella*

Test or substrate	*Salmonella* result[a]	Indicating agent	Media colour
Glucose	+	Phenol red	Yellow butt
Lysine decarboxylase	+	Bromocresol purple	Purple butt
H2S	+	-	Blackening
Urease	-	Phenol red	No color change
Lysine decarboxylase broth	+	Bromocresol purple	Purple color
Phenol red dulcitol broth	+[b]	Phenol red	Yellow color and/or gas
KCN broth	-	-	No growth
Malonate broth	-[c]	Bromothymol blue	No color change
Indole test	-	Kovac's reagent	Yellow color at surface
Phenol red lactose broth	-[c]	Phenol red	No gas, no color change
Phenol red sucrose broth	-	Phenol red	No gas, no color change
Voges-Proskauer test	-	Alphanaphthol, Ethylalcohol, KOH	No color change
Methyl red test	+	Methyl red	Diffuse red color
Simmons citrate	v	Bromothylmol brue	Growth, blue color Or no growth, no color change

[a] +, 90% or more positive in 1 or 2 days; -, 90% or more negative in 1 or 2 days; v, variable

[b] Majority of *S. arizonae* cultures are negative

[c] Majority of *S. arizonae* cultures are positive

Source: Hanes (2003), Quinn *et al*. (1999)

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The vast majority of salmonellae is motile and propelled by peritrichous flagella with the exception of rare non-motile *Salmonella* serotypes such as *S.* Gallinarum and *S.* Pullorum (Krieg and Holt, 1984, D' Aoust *et al*., 2001). The movement is linear most of the time, but may be interrupted by a brief moment of 'tumbling' (Krieg and Holt, 1984). Like other flagellated cells, the motile salmonellae may lose their ability to develop flagella under the effect of sub-lethal 'stress', caused by external physicochemical influence such as refrigeration or high temperatures (Krieg and Holt, 1984, D' Aoust *et al*., 2001).

2.1.2. Taxonomy

In recent years, there has been a change in the taxonomy of *Salmonella*. In the early development of taxonomic schemes, each *Salmonella* serotype was treated as a species. However, according to the new taxonomic scheme based on DNA-hybridization and enzyme electrophoretic characterizations, all salmonellae have been placed into two species, *S. enterica* and *S. bongori*. *S. enterica* is divided further into six subspecies or groups (Table 2), the main one being *Salmonella enterica* subspecies *enterica*, which represents nearly 99% of the salmonellae isolated in medical practice. It should be noted that the old way of naming serotypes is no longer valid. For example, *Salmonella typhimurium* should be *S. enterica* serotype Typhimurium, or simply *Salmonella* Typhimurium (note that 'typhimurium' is capitalized and not italicized).

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Table 2: *Salmonella* species and subspecies

Salmonella species and subspecies	No. of serotypes
Salmonella enterica	2,443
S. enterica subspecies *enterica*	1,454
S. enterica subspecies *salamae*	489
S. enterica subspecies *arizonae*	94
S. enterica subspecies *diarizonae*	324
S. enterica subspecies *houtenae*	70
S. enterica subspecies *indica*	12
Salmonella bongori	20
TOTAL	2463

Source: D' Aoust *et al*. (2001)

2.1.3. Serotypes

According to the Kaufman-White classification scheme, there are 2,463 serotypes (serovars) of *Salmonella*, defined by the WHO Collaborating Centre for Reference and Research on *Salmonella* at the Pasteur Institute in Paris, France in the year 2000 (Table 2) (D' Aoust *et al*., 2001). All serotypes in subspecies *enterica* are named whereas serotypes in other subspecies (except for some in subspecies *salamae* and *houtenae*) and *S. bongori* are not named but designated by antigenic formulae.

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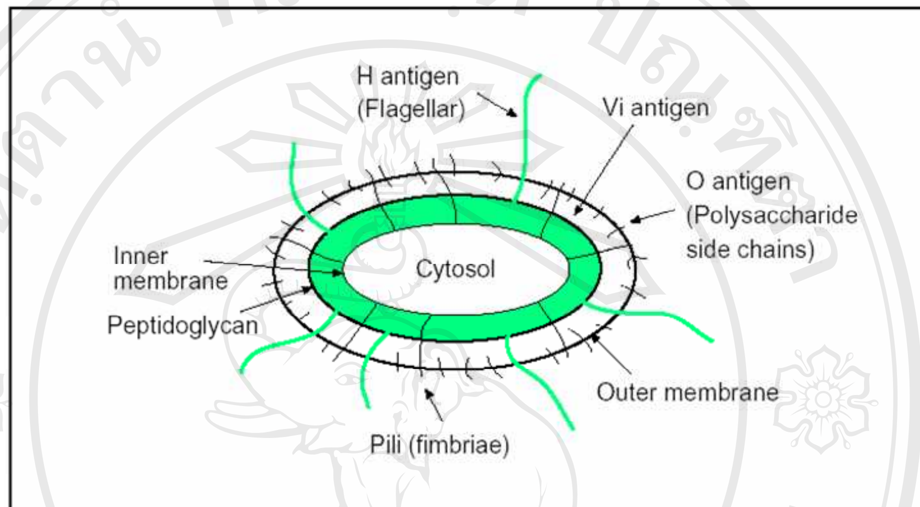
The serologic typing of salmonellae has led to the identification of a large number of strains. According to the Kaufman-White scheme, organisms are represented by the numbers and letters given to the different somatic (O) lipopolysaccharides (LPS) on the external surface of the bacterial outer membrane, to flagella (H) antigens associated with the peritrichous flagella, and to capsular (Vi) antigen appearing in *Salmonella* serotypes Typhi, Paratyphi C and Dublin. The Vi antigen is located in an external polysaccharide microcapsule and is associated with virulence for particular hosts (Figure 1) (Krieg and Holt, 1984, D' Aoust *et al.*, 2001).

Figure 1: Schematic representation of the antigen structure of *Salmonella* Typhi showing the relative locations of O, H and Vi antigens



Source: Axelsson and Sorin (1997)

These antigens are heterogeneous structures, and antigenic specificity is determined by the composition and linkage of the O group lipopolysaccharides. Mutations that affect the lipopolysaccharides may lead to new O antigens. In many serotypes the flagellar H antigens can switch between two types, called phase 1 and phase 2. This switching results in two alternative sets of H antigens. Because H antigens are less heterogenous than the carbohydrate side chains, considerable fewer H antigenic serotypes exist. Presently, *Salmonella* serotypes are placed into 67 serogroups (A to 67) designated with letter or numbers according to similarities in content of one or more O antigens (e.g. *S.* Typhi, *S.* Enteritidis, *S.* Gallinarum are serogroup D because all have the same somatic O antigen 9 and 12) (Krieg and Holt, 1984). The antigenic formulae for some salmonellae are shown in Table 3.

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Table 3: Examples of antigenic structure formulae for some common salmonellae

Group	Species/Serotypes	O antigen	H Antigens	
			Phase 1	Phase 2
A	*S*. Paratyphi A	1, 2, 12	a	[1,5]
B	*S*. Typhimurium	1, 4, [5], 12	i	1, 2
C1	*S*. Choleraesuis	6, 7	[c]	1, 5
	S. Paratyphi C	6, 7, [Vi]	c	1, 5
D	*S*. Typhi	9, 12, [Vi]	d	-
	S. Enteritidis	1, 9, 12	g, m	[1, 7]
	S. Gallinarum	1, 9, 12	-	-
E1	*S*. Anatum	3, 10	e, h	1, 6

Symbols: [ ], may be absent; ( ) not well developed (weakly agglutination). The underlined antigens are associated with phage conversion

Source: Krieg and Holt (1984)

2.2. Distribution of *Salmonella* in pigs

The primary habitat of *Salmonella* is the intestinal tract of animals such as birds, reptiles, farm animals, humans, and occasionally insects (Jay, 1992, Hanes, 2003). Although their primary habitat is the intestinal tract, they may be found in other parts of the body (Jay, 1992, Hanes, 2003). As intestinal forms, the organisms are excreted in faeces from which they may be transmitted by insects and other living creatures to many places such as to water, soils and building surfaces. In pig production, the two important factors of introducing *Salmonella* into the herds are the feeds and new animals (Lo Fo Wong and Hald, 2000).

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The contribution of management to the prevalence of *Salmonella* in farms has been illustrated in various studies. For example, increasing herd sizes would increase the within-herd seroprevalence of *S. enterica* (Mousing *et al.*, 1997). However, this depends on the type of management, feeding system, cleaning and disinfection and bio-security systems (Christensen and Rudemo, 1998). Van der Wolf *et al.* (2001) have indicated that small to moderate herd sizes (<800 finishers) were associated with a higher *Salmonella* seroprevalence than herds that were larger because the larger farms are more hygiene-conscious than the smaller farms. Beloeil *et al.* (2004) and van der Wolf *et al.* (2001) found that the risk for *Salmonella* shedding at the end of the fattening period was increased when dry feed (versus wet feed) was provided. The trough feeding was also associated with a higher *Salmonella* infection level compared to the other type of feeding systems (van der Wolf *et al.*, 1999). In cases where the herds were infected by other diseases such as *Lawsonia intracellularis* and/or PRRS (Porcine Reproductive and Respiratory Syndrome), the prevalence of *Salmonella* in those herds was higher because *Lawsonia intracellularis* disturbs the ecology of the intestine and gut flora, while PRRS induces immunosuppression (Beloeil *et al.,* 2004).

Table 4 shows the prevalence of *Salmonella* in pork, beef and chicken meat in different countries. However, the sensitivity of the test used, sample size and the distribution of the proportions of infected animals within herds have influence on the results (Steinbach *et al*., 2002). Thus, the real number of *Salmonella* carriers might be much higher than shown by bacteriological and serological examination (Steinbach *et al*., 2002).

The distribution of *Salmonella* serotypes shows in Table 5. In Denmark, Canada, the United States and Japan, the most frequently serotypes found in pigs were *S.* Typhimurium and *S.* Derby. In Thailand, there was no report of serotypes isolated from pigs. The serotypes isolated from human cases in Thailand show in Table 5, that *S*. Weltevreden was the serotype most frequency isolated, followed by *S*. Enteritidis and *S*. Anatum.

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Table 4: Prevalence of *Salmonella* in raw meats or products

Product	Country	Number of Samples	
		Tested	Percent Positive
Beef	Denmark, 1995[a]	2,559	1.3
	Germany, 1991[b]	18,242	5.1
	United States, 1993[b]	2,112	2.7
Pork	Canada, 1985[b]	448	10.0
	Mexico, 1994[a]	50	76.0
	Portugal, 1987[b]	405	5.4
	Thailand, 1986[a]	130	21.5
Chicken	Cuba, 1990[b]	200	62.5
	Denmark, 1995[b]	4,099	45.7
	France, 1994[a]	616	19.8
	Germany, 1994[b]	630	28.6
	United States, 1995	1,297	20.0
	Mexico, 1993[a]	70	68.6

[a] Retail samples

[b] Post slaughter carcasses

Source: D' Aoust (2001)

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Table 5: *Salmonella* serotypes isolated in the different countries

Country	Origin	Serotype	Percentage	Reference
Denmark	Pigs	*S.* Typhimurium *S.* Derby *S.* Altona	75 6 4	Sorensen *et al.* (2004)
Japan	Diarrhea pigs	*S.* Typhimurium O 4, 12: d:- *S.* Derby	91.9 13.1 7.1	Asai *et al.* (2002b)
United States (North Carolina)	Pigs	*S.* Derby *S.* Typhimurium *S.* Schwarzengrund *S.* Heidelberg	6.3 5.7 3.7 3.2	Davies *et al.* (1997)
United States (North Carolina)	Pigs	*S.* Typhimurium *S.* Derby	47.7 7.8	Funk *et al.* (2005)
Canada (Alberta)	Pigs	*S.* Typhimurium *S.* Derby *S.* Infantis *S.* Califonia *S.* Enteritidis	24.1 22.0 14.6 7.5 5.0	Rajic *et al.* (2005)
Thailand	human cases	*S.* Weltevreden *S.* Enteritidis *S.* Anatum *S.* Derby *S.* Typhimurium *S.* Rissen *S.* Stanley	12.5 11.4 7.4 6.6 5.3 5.3 3.8	Bangtrakulnonth *et al.* (2004)

2.3. Foodborne Salmonellosis

Eggs, poultry and raw meat products are the most important food vehicles of *Salmonella* infection in humans, with *S.* Typhimurium and *S.* Enteritidis being the most commonly isolated food-borne serotypes (Krieg and Holt, 1984, Jay, 1996). In Thailand, the most common serotypes isolated from humans were *S.* Weltevreden and *S.* Enteritidis: these serotypes are increasingly isolated from humans and other reservoirs, e.g. chicken, seafood and ducks (Bangtrakulnonth *et al.*, 2004). Symptoms of *Salmonella* usually develop 12 to 14 hours after exposure, although shorter or

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longer incubation times have been reported. Symptoms consist of nausea, vomiting, abdominal pain (not as severe as staphylococcal food poisoning), headache, chills and diarrhea. These symptoms are usually accompanied by prostration, muscular weakness, faintness, moderate fever, restlessness and drowsiness. Symptoms usually persist for 2 to 3 days. *Salmonella* generally disappear rapidly from the intestinal tract after recovery from the disease. However, up to 5% of patients may become carriers upon recovery from the disease (Jay, 1996). The pathogenesis of salmonellosis may involve two toxins – an enterotoxin and a cytotoxin. Numbers of cells in the order of 10^7-10^9/g are generally necessary for salmonellosis (Krieg and Holt, 1984). But from one salmonellae outbreak, numbers of cells as few as 100 cells/100 grams of food (*S.* Eastbourne in chocolate) have been reported to make people sick (Jay, 1996).

Determinant factors of salmonellosis are not limited to the immunological heterogeneity within human populations and to the virulence of infecting strains; they may include the chemical composition of incriminated food vehicles. A common determinant of the foods associated with low infectious doses is the high fat content in chocolate (cocoa butter), cheese (milk fat), and meat (animal fat). Suggestively, entrapment of salmonellae within hydrophobic lipid micelles would provide protection against the bactericidal action of lipid moieties in the duodenum, the viable salmonellae would resume their infectious course in search of suitable points of attachment in the lower portion of the small intestine (colonization) (D' Aoust *et al.,* 2001). And commensal *Salmonella* may be found in healthy carriers who are in a state of convalescence, but there are also permanent carriers who contribute to the spread of the illness. However, the true incidence of *Salmonella* infection is difficult to determine. Reported cases represent only a small proportion of the actual number. Normally only large outbreaks are investigated and documented; sporadic cases are underreported, mainly because only patients with protracted diarrhea report to a health care provider for microbiological evaluation (Hanes, 2003).

A study by Hanes (2003) showed a close relationship between the *Salmonella* serotypes most often responsible for human infection and those isolated from animals

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in any one geography. These similarities document the importance of nonhuman reservoirs of *Salmonella* in epidemiology of infection in human.

2.4. *Salmonella* Detection

The 2 most used diagnostic methods for detection of *Salmonella* infections in pigs are the microbiological examination of faeces, faecal contents, swab samples of lymph nodes and the serological examination of blood samples or meat juices (Lo Fo Wong and Hald, 2000, Sorensen *et al.*, 2000). Examination of faeces is a useful tool for determining the current infection level in a pig herd. A positive isolation of *Salmonella* will leave little doubt of the presence of the bacteria in the animal or in the samples. Therefore, this method is often defined as the 'gold standard' when comparing results with those obtained from alternative tests (Lo Fo Wong and Hald, 2000). However, present culturing methods are time consuming and laborious, requiring pre-enrichment, selective enrichment, indicative plating and bio/serotyping. Therefore, there is a need for *Salmonella* tests that provide results more rapidly with a similar sensitivity to, or greater than, the conventional methods. These tests should be simple and reproducible and have a specificity that minimizes false-positive results (Axelsson and Sorin, 1997).

Thus, immuno-serological tests have been developed for the detection of *Salmonella*. These can be broadly divided into those based on enzyme-labeled antibodies (ELISA), fluorescent antibody staining, radio immunoassay and other methods. The most popular test for routine use is ELISA (Enzyme-Linked Immunosorbent Assay) technology. This technique takes only about 2 hours to perform. ELISA has the disadvantage that we can not be sure that the infection is still present at the farm at the moment of positive testing. Furthermore, it will not detect infections that occurred shortly (1-2 weeks) before sampling (van der Wolf *et al.*, 2001).

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Some studies show the correlation between conventional culture methods and serology in individual pigs. In general most *Salmonella* infections are silent in pigs, they nevertheless undergo an infectious process resulting in an immune response. Thus, serological and bacteriological results generally have a poor correlation (Davies *et al.*, 2003). While Sorensen *et al.* (2004) found that there was a strong association between herd serology and the prevalence of *Salmonella* bacteria measured at tree sampling sites: faecal-content, pharynx and carcass surface. For these sites, the odds for being culture-positive for *Salmonella* varied from 1.3 to 1.5 for each increase of 10% in herd serology. In a study of Asai *et al.* (2002a), *Salmonella* was isolated from 26 (28.9%) of 90 antibody-positive pigs and 21 (11.9%) of 117 antibody-negative pigs at 4 months of age. The authors found that sero-conversion generally occurred during the last third of the fattening phase from 140 days of age to slaughter (Asai *et al.*, 2002a, Beloeil *et al.*, 2003), while shedding was considerable in the first half of the fattening period (Beloeil *et al.*, 2003), particularly in pigs between 4 to 5 months of age (Asai *et al.*, 2002a). According to the above studies, if the intention is to monitor *Salmonella* pre-harvest, measures of herd serology or faecal content are appropriate (Sorensen *et al.* 2004). For more precise results, the prevalence in fattening pigs should be investigated in the late stage of the fattening period or before slaughtering. If the transmissions within the herd are to be studied, it should be done during the first half of fattening period.

Sensitivity regarding bacteriological detection will be relatively high where the animals examined suffer from an acute infection and harbor a high number of microorganisms, and it will be low if only a small number of microorganisms remain in the animal body. Regarding serological diagnosis, there may be differences in sensitivity depending on the intensity of the infection process among the herd and the time lag between infection and examination. The specificity of serological detection of *Salmonella* may become reduced by microorganisms not belonging to *Salmonella*, but inducing antibodies which react with the *Salmonella* antigen (Steinbach *et al.*, 2002). Malorny, *et al.* (2003), found that the inter-laboratory diagnostic accuracy, (i.e. diagnostic specificity and sensitivity) was shown to be 97.5% when detecting

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Salmonella by the PCR based method. This was conducted in 5 laboratories, one in Spain, one in France and three in Germany.

2.5. Control of *Salmonella* in pigs

For safety reasons, European Regulations concerning food products stipulate a *Salmonella* contamination rate of less than 1 bacterium per 25 grams. This means that in practice a total absence of the organism is intended. It is important to note that all types of *Salmonella*, whatever their serotype, are considered undesirable and they are tested for. To fulfill this purpose and to respond to the consumers' and society's expectations about food safety, most countries with developed pork production, especially in countries that export pork, have in slightly different ways developed standards for swine production that are run by producer associations (e.g. the Canadian Pork Quality Assurance system, and the PQA system of the U.S. National Pork Producer Council), or by industry associations (e.g. the Quality Assurance System of the UK meat and Livestock Council, or the Dutch Produktschapt voor Vee and Vlees with the renowed IKB-program = Integrate Keten Beheersing), or with laws or ordinances issued by governments that set the basic standards (as in the European Union with the "Zoonosis Directive" or in Germany with the "Schweinehaltungshygiene-Verordnung" or in Denmark with the "National *Salmonella* Control Program in the Danish Pork Industry").

Several studies have shown that the implementation of preventive measures could reduce the prevalence of contamination. Berends *et al.* (1998) reported the implementation of GMP codes from farm to cutting/retail could reduce the current levels of *Salmonella*-positive pigs and pork by 50-60%. If pigs were bred according to the rather costly 'specific pathogen free' (SPF) concept, the prevalence of contaminated carcasses and pork could in total be reduced by 95%. Berends *et al.* (1998) believe that the current EU Regulation, in relying on hazard analysis of critical control points (HACCP)-inspired production in cutting plants, will not be effective in reducing the prevalence of *Salmonella* in pork. This is because there is currently an

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almost steady stream of *Salmonella* positive carcasses that enter slaughter and the cutting process and when contaminated carcasses are being processed, further cross contamination during working hours is unavoidable. No steps in the carcass-cutting process are intentionally designed to effectively reduce the risks of the consequences of cross contamination of cuts and retail-ready products (Berends *et al.,* 1998). However, from the study by D' Aoust (2001) in the United States, the preliminary results indicate that after implementation of HACCP in pig and poultry plants, *Salmonella* prevalence in broiler carcasses dropped from 20% to 10.4% and for swine carcasses, the prevalence dropped from 8.7 to 5.5%. Although these are preliminary data, they suggest that HACCP programs can reduce salmonellae in the food supply to a certain animal.

However, controlling *Salmonella* in pork needs a lot of investment. From a study by van der Gaag *et al*. (2004), seven stages can be distinguished in a pork supply chain: breeding and multiplying, finishing, transportation, lairage, slaughtering, processing and retailing, and household. Van der Gaag *et al*. (2004) concluded that the most cost-effective strategy for the pork supply chain is to implement interventions firstly in the slaughterhouse; especially at the lairage stage, secondly in the finishing farms. An additional result from this study is that the reduction of *Salmonella* in the pork chain to a level where the average prevalence, plus standard deviation, is below 2%, can be achieved when at least 4.5 Euro per pig is invested. This is relatively expensive, but it has to be stated that almost all interventions in order to reduce *Salmonella* in the pork chain are also effective in reducing other pathogens. In other words, the direct benefits are outside the pork supply chain, i.e. for society. An indirect benefit is the increased trust of the consumers, the improved image of pork and the strengthened position on the global market for pork (van der Gaag *et al*., 2004).

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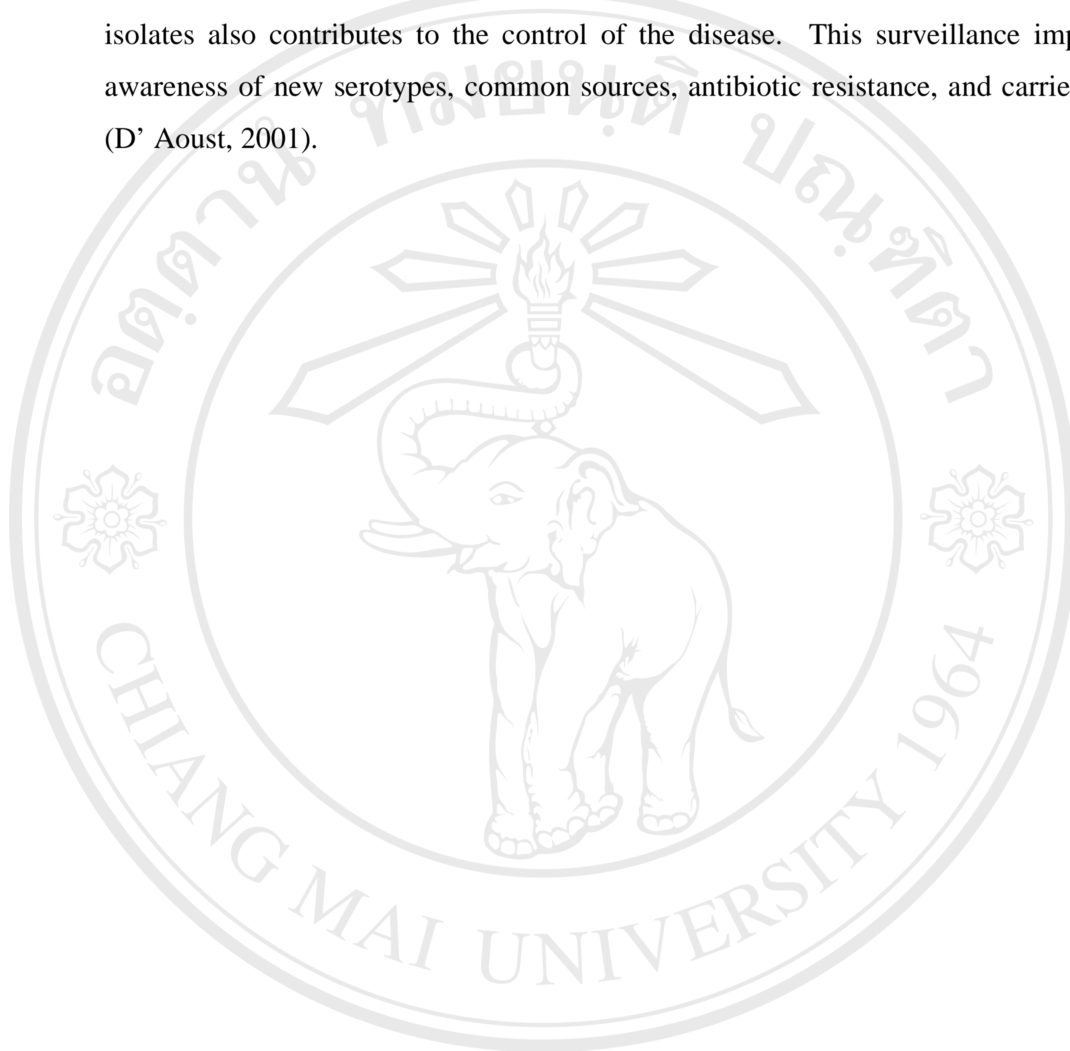
Up to now the pre-harvest stages of the pork supply chain cannot ensure a zero prevalence of contaminated carcasses. Therefore, the next stages (processing, storage at retail and storage and preparing the pork by the consumer) are also important. For instance, the consumer can reduce the risk of food-born salmonellosis by cool storage

and through heating the pork and by avoiding cross-contamination in the kitchen (van der Gaag *et al.*, 2004). Continuous surveillance and careful reporting of *Salmonella* isolates also contributes to the control of the disease. This surveillance improves awareness of new serotypes, common sources, antibiotic resistance, and carrier state (D' Aoust, 2001).



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3. MATERIAL AND METHODS

3.1. Study Design

A cross-sectional study design was used. Samples were collected and questionnaires were administered to each farm during December 2004 to May 2005.

3.2. Sample Size and Sample Selection

3.2.1. Sample Size Determination

In order to estimate the prevalence of *Salmonella* infection in pre-slaughter pigs in the Chiang Mai province, using the prevalence of 69.5% (Patchanee *et al.*, 2002) on a pig level with a maximum allowable error of 8% and 95% confidence level, 420 fattening pigs (about 1-3 days before slaughter, 90-100 kg live weight) were selected conveniently for individual blood sampling and 194 pigs were selected for faecal sampling (Daniel, 1987). Questionnaires were used to collect the management information of those herds. Environmental samples related to the risk of introducing *Salmonella* into the herd, including house floor and water supply, were collected and tested for the presence of *Salmonella.* A convenient sample of 22 pig herds was observed in this study.

3.2.2. Farm and Pig Selection

A total of 22 farms was selected from 2 groups. The first group had open house (17 farms), the second group was environment-controlled farms (5 farms). For each farm, twenty pigs were selected for blood sampling, and 10 of these 20 pigs were selected for faecal sampling.

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3.2.3. Environmental Sample Selection

Two types of environmental samples, a water sample and a floor swab sample were collected. Water samples included (i) water used for cleaning and disinfection, (ii) drinking water and (iii) waste water. Seven pens in each farm were selected for floor swabbing.

3.3. Collection of Samples

3.3.1. Serum Samples

Blood samples, each 10 milliliter, were taken at slaughter during bleeding and collected in test tubes individually. Each tube was labeled with each pig's unique identification number and centrifuged to separate serums and platelets. Then, the serum was removed from each blood sample and stored at -20 ^{o}C until tested.

3.3.2. Faecal Samples

Faecal samples were used to indicate the current infected proportion in the respective pig herds. Individual faecal samples (25-30 g) were collected by hand per rectum, using new disposal gloves. The faecal samples were submitted to the laboratory for examination within 4 hours after collection and processed on the same day of collection or kept at 4 ^{o}C and processed within 24 hours.

3.3.3. Pen Swab Samples

Pen swabs were collected on the same day as faecal samples and tested for *Salmonella* presentation simultaneously. A sterile pair of gauze socks was used. The pair of socks consisted of an elastic cotton tube, each sock was sized approximately 15x20 centimeters. The socks were pulled over the investigator's boots. The investigator walked through the entire pen (approximately 30 steps) and turned the

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socks during sampling to allow all parts of the socks to be exposed and to absorb faecal material. A soiled pair of socks was placed in a sterile plastic bag with 225 ml of peptone water. The labeled bags were kept in an icebox and put in the incubator at 37 ^{o}C within 3-5 hours after collection. This sampling technique has been used to evaluate bacterial (*Salmonella*) contamination in the chicken house (Skov *et al*., 1999) and the fattening pig house (Beloeil *et al*., 2004).

3.3.4. Water Samples

Each water sample comprised 1,000 ml in a sterile bottle. Samples were kept at 4 ^{o}C and sent to the laboratory for testing within 3-4 hours after collection.

3.4. Laboratory Procedures

3.4.1. Serology; ELISA

The commercial test kit SALMOTYPE® Pig LPS ELISA (Labor Diagnostik Leipzig, Germany) was used.

The kit is an enzyme immunoassay for the detection of antibodies specific to *Salmonella* in pork meat juice or pork serum, it detects antibodies to the O-antigens 1, 4, 5, 6, 7 and 12. The SALMOTYPE® Pig LPS ELISA detects more than 90% of the most common *Salmonella* serotypes in the Western European area.

This assay is designed to measure the quantity of antibodies to *Salmonella* in pork meat juice or in pig serum. The *Salmonella* antigen is coated on 96-well plates. Upon incubation of the test sample in the coated well, antibodies specific to *Salmonella* form a complex with the coated *Salmonella* antigen. Unbound material is washed away and a conjugate is added which binds to any bound pork antibody in the wells. After washing away unbound conjugate from the wells, enzyme substrate is added. Subsequent colour development from the conjugate-bound enzyme is directly

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related to the amount of antibodies to the *Salmonella* present in the test sample (Figure 2).

The ratio of the OD values of the controls and their concentrations give a linear regression line. The linear regression line is calculated by plotting the OD values of control on the X-axis versus the measured OD-values on the Y-axis. The antibody concentration of the samples has to be calculated by use of the straight-line formula.

- Cut-off values for samples (serum, meat juice, plasma):

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

- Cut-off values of samples for categorization of stocks according to monitoring programs:

  ≥ 40 OD% or ≥ 20 OD% are positive depending on national regulations

For the assay to be valid, the P/N-quotient between the Positive Control Serum 1 (P) and the Negative Control Serum (N) should be greater than 4.0.

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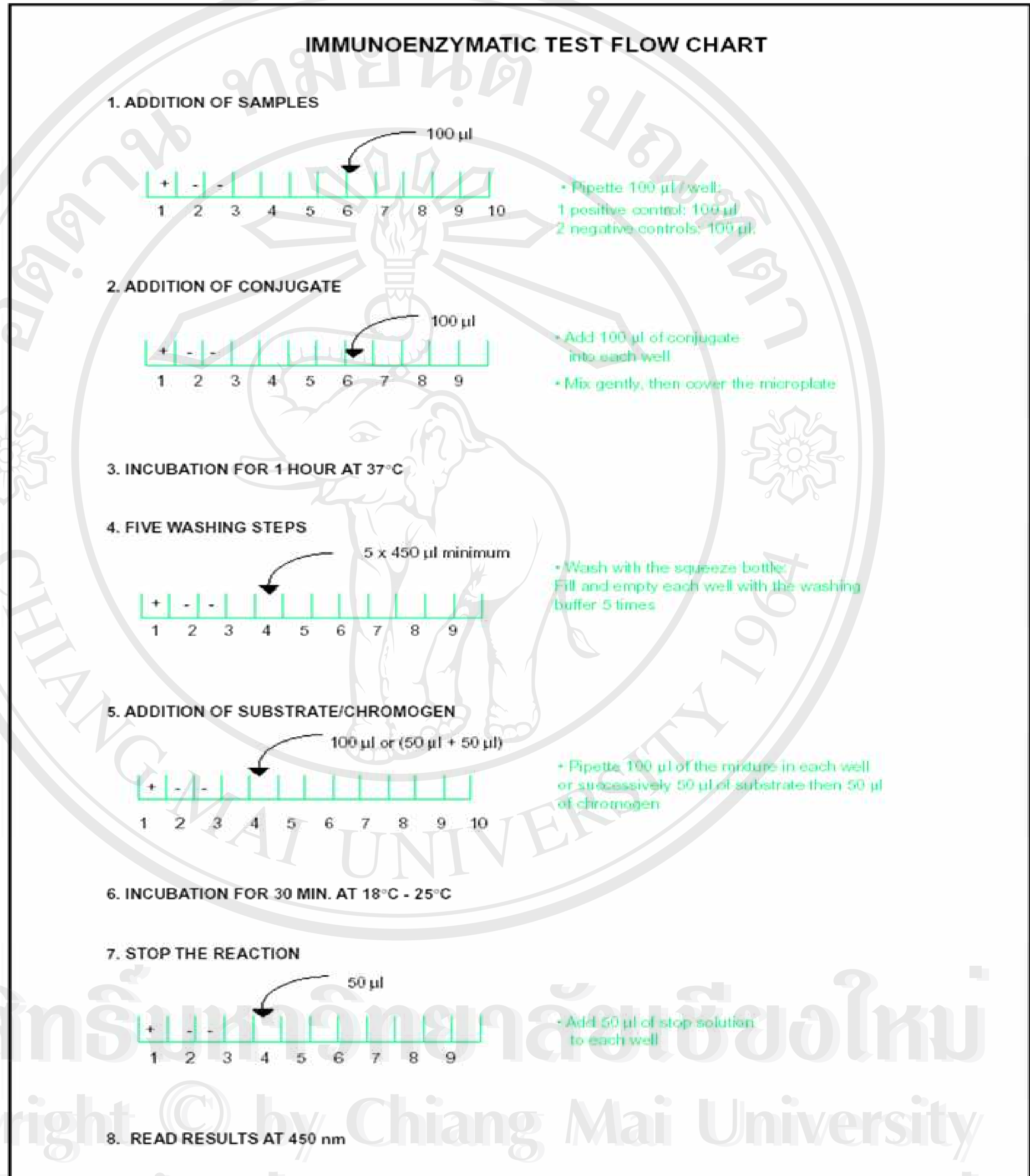
Figure 2: ELISA test flow chart



Source: Axelsson and Sorin (1997)

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3.4.2. Conventional Culture Method

The conventional culture methods used were slightly modified from ISO 6579 (2002); Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. The protocol generally has four distinct phases or steps (Figure 3).

Step 1. Non-selective pre-enrichment: The sample was blended in a nonselective medium and incubated at 37 °C for 18-24 hours to allow resuscitation of any stressed organism and growth of all organisms as well.

Step 2. Selective enrichment step: To allow growth of the organism under investigation, while reducing the numbers of accompanying organisms in the broth. Two types of selective enrichment media were used in this study. The first media used was Tetrathionate broth (Merck® Ltd.), another media used was the Rappaport-Vassiliadis medium (Merck® Ltd.).

Step 3. Isolation step: Selective enrichment media were streaked on selective solid agars containing one or more agents that inhibit non-salmonella organisms. There were 2 selective solid agars used in this study, the first one was BPLS (Brilliant-Phenolred-bile-Lactose-Saccharose Agar, Merck® Ltd.) and the second one was XLT4 (Xylose lysine tergitol 4 agar**,** Merck® Ltd). XLT4 is a highly selective plating medium used for the isolation of salmonellae from food, environmental and clinical samples. The properties of *Salmonella* colonies are described in Table 5.

Step 4. Confirmation step: Characteristic colonies on the plates were submitted for biochemical testing and seroagglutination testing to confirm that the isolates were members of the species *S. enterica*. Biochemical properties of *Salmonella* are shown in Table 6.

Completing all the steps involved in this method required at least 4-7 days, in order to obtain a definite diagnosis of *Salmonella.*

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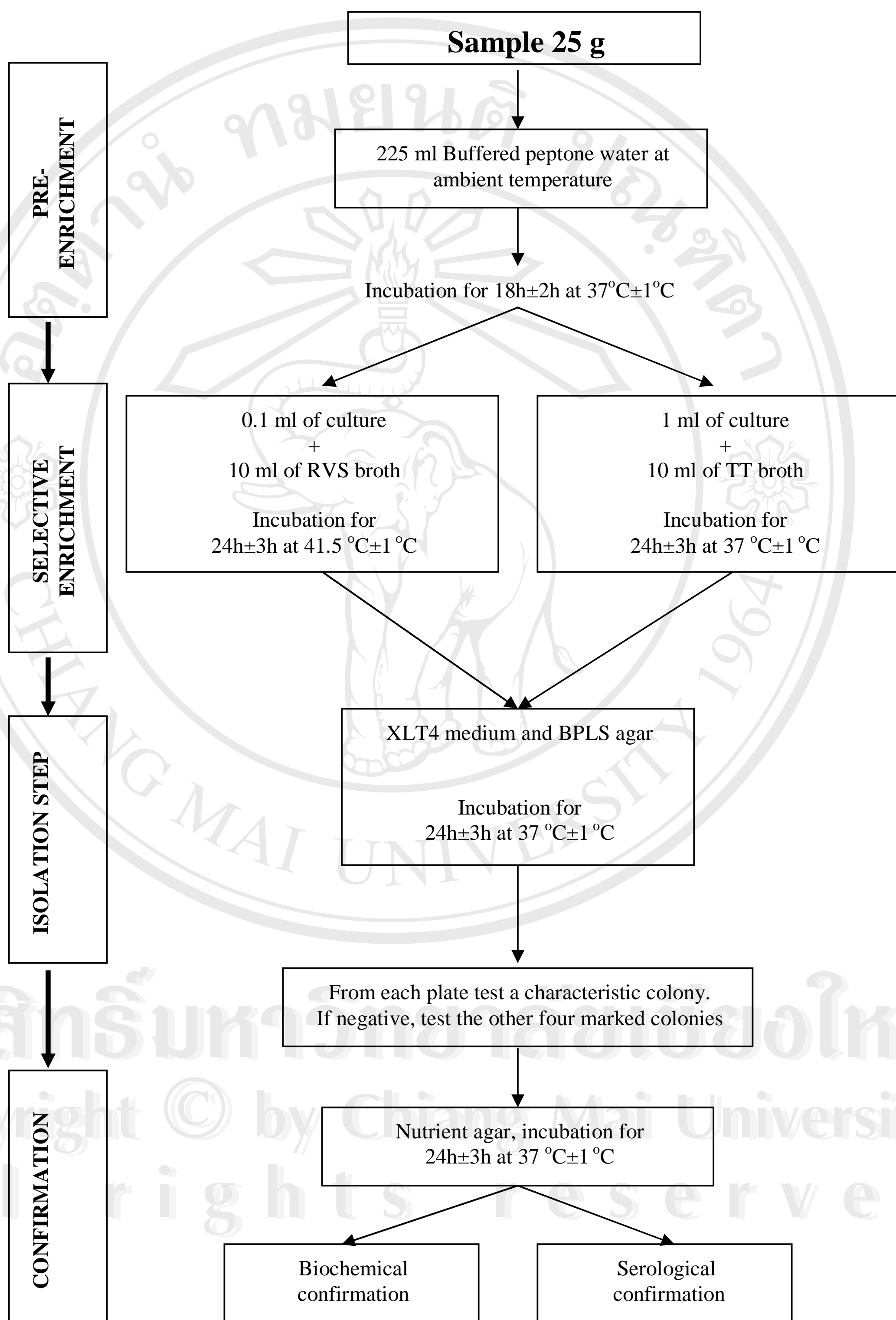
Figure 3: Flow chart of *Salmonella* conventional culture methods



Source: Adapted from ISO 6579 (2002)

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Table 6: Typical growth of *Salmonella* colonies on selective and differential media

Media	Colony appearance
BPLS	Pink colonies surrounded by red zone
XLT4	Black centered red colonies with H_2S producer, red colonies with non-producer

Table 7: Biochemical testing results of *Salmonella*

Biochemical test	Bergy's Manual Result	Official collection Result
Glucose from TSI	+ (> 90%)	+ (100 %)
Gas from TSI	+ (> 90%)	+ (91.9 %)
Lactose from TSI	- (> 90%)	- (99.2 %)
H_2S from TSI	+ (> 90%)	+ (91.6 %)
Urease	- (> 90%)	- (100 %)
Lysine decarboxylation	+ (> 90%)	+ (94.6 %)
Voges-Proskauer reaction	- (> 90%)	- (100 %)
Indole	- (> 90%)	- (98.9 %)

Source: Holt *et al.*, 2000, Institute of Meat Hygiene and Technology, Faculty of Veterinary Medicine, FU Berlin, Germany)

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3.4.3. Serotyping

All isolates were serotyped by agglutination according to the Kauffmann-White scheme using *Salmonella* Polyvalent I (A-E) and *Salmonella* Polyvalent II (F-67) (Sifin, Germany) and *Salmonella* antiserum specific to the individual group by the following process (Figure 4).

1. Test the selected colonies with *Salmonella* polyvalent I (A-E), if the result was positive (+), the selected colonies possessed the antigen to this group, colonies were regarded as a member of *Salmonella* group A-E.

2. Test negative (-) result colonies (from the first step) with *Salmonella* polyvalent II (F-67), if the result was positive (+), those colonies possessed the antigen to this group; colonies were regarded as a member of *Salmonella* Group F-67.

3. Serotyping of Somatic (O) antigens to determination *Salmonella* main groups (A (O 2), B (O 4,5,27), C (O 6,7,8,20), D (O 9,27,46,Vi), E (O 3,10,15,19,34)) by using a sequence of somatic antigen sera (Procedure based on manufacturer Sifin, Germany). Sequence of testing based on information of the occurrence in Thailand and South East Asia.

4. Determination of flagella antigens, this step was done after transfer of the isolate to the motility agar. Performing agglutination for flagella antigen phase 1 and phase 2. If phase 2 did not appear, the serotype might be in the first phase only or vice versa. Then proceeding with the challenge test, where the antigens were to be blocked by the particular H antiserum to force the strain to develop the other phase (procedure based on manufacturer Sifin, Germany).

5. Diagnosis of the serotype of *Salmonella*.

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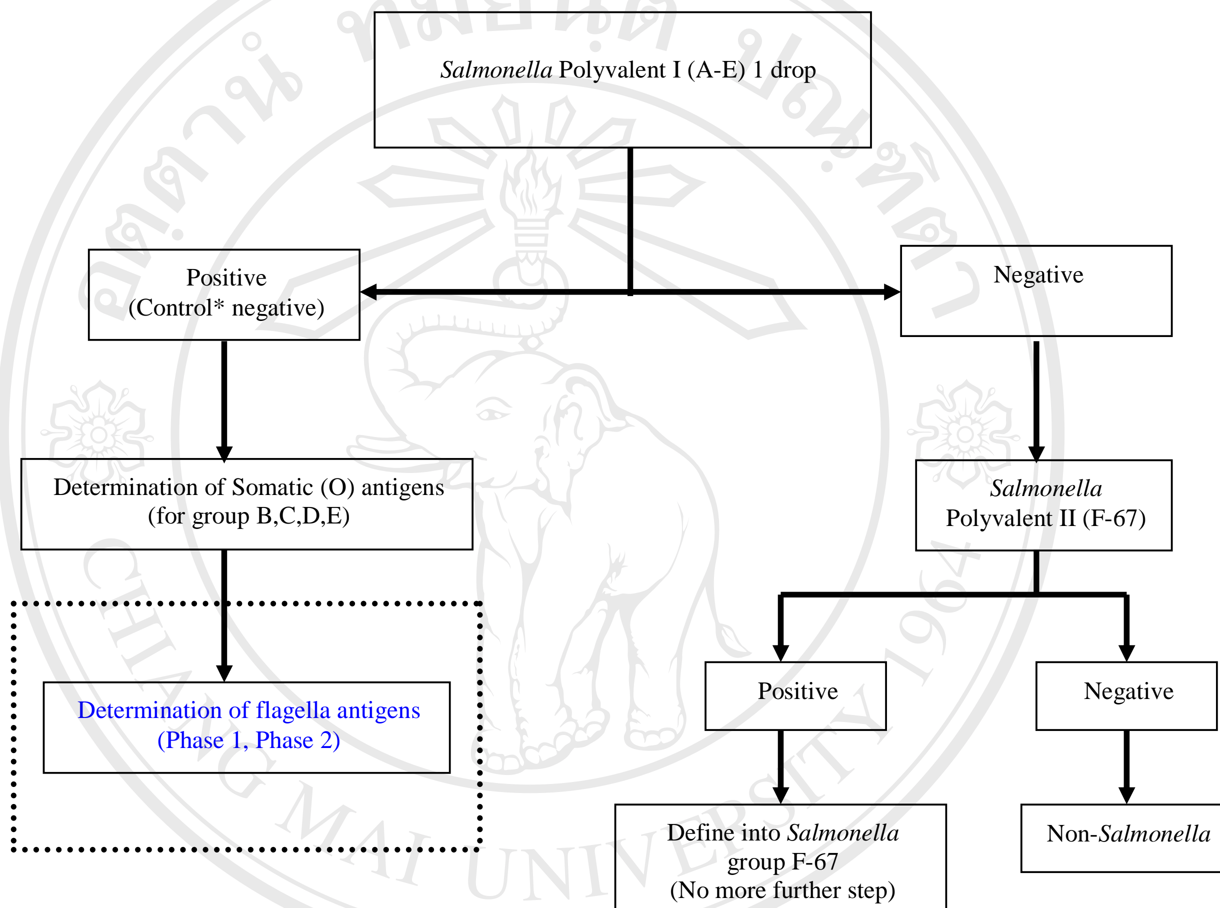
Figure 4 : *Salmonella* serotyping flow chat



* The negative control used was NaCl solution

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3.5. Questionnaires Survey

A specific questionnaire was administered to each farmer by the author. Data concerning the general characteristics of the farm and the premises, biosecurity procedures, type of feeding and the rearing characteristics of the batch during finishing periods were collected. In addition, the on-farm technical documents were examined for this purpose too.

The questionnaires and check lists were used for estimation of the management in each selected farm. Factors affecting the occurrence of *Salmonella* in fattening-pigs, and which were parts of the questionnaire, are shown in Table 8.

3.6. Statistical Analysis

For descriptive analysis, herds were considered seropositive when one or more blood sample was found positive. All herds, in which *Salmonella* was cultured from one or more samples, were considered bacteriological positive. The statistical analysis in use was

1. Chi square test for univariate risk factor analysis. This was to evaluate the impacts of each factor to the prevalence of *Salmonella* in faecal isolation and in the serological test
2. Logistic regression model for multivariable analysis. All relevant factors were included in the model. This was to evaluate the impacts of particular risk factors without interaction from the other factors (David, 1994).

The statistical programs used were EpiCalc 2000, NCSS 2000, Win Episcope 2.0, Intercooled Stata 6, Epi Info 2002, SAS statistic program.

In the case of environmental samples, if at least one sample was found positive, the herd was classified as *Salmonella* contaminated (Beloeil *et al.*, 2004).

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Table 8: Summary of questionnaires and checklist

Cluster	Factors
Animals	Kind of animals, number, origin and breed.
Integrated quality control program	Whether or not, and if so, which program.
Feed and feeding system	Which antibiotic growth promoter, type of feeding and type of drinking water and watering system. Feed storage and sanitation.
Housing	Number of house/pen, total number of compartments, number of animals per compartment, type of floor, type of slurry or waste management system.
Medication and vaccination	If, when, why and what sort of medication, dose rate and duration of treatment. Type of vaccine and probiotic used.
Hygiene	All-in/all-out procedure, cleaning and disinfection procedure, chemicals used, methods of fly and rodent control, personal hygiene and number of visits by vets, isolation of sick animals
Production parameters	Average daily gain (ADG), feed conversion ratio, mortality and the percentage of loss during fattening.

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4. RESULTS

4.1. Results of *Salmonella* Isolation and Serotyping

4.1.1. Results of *Salmonella* Isolation

Table 9 shows the distribution of farm faecal prevalence of *Salmonella*, which ranged from 30-88% with an average of 62.9% (95% CI: 56%-70%). In the open farms, a prevalence ranging from 38% to 88% with an average of 65.3% (95% CI: 57%-73%) was obtained, while in the closed farms, prevalence ranged from 30% to 80% with an average of 56.0% (95% CI: 41%-70%). These two averages were not significantly (p=0.308) different.

Table 10 shows a total of 415 samples from 22 farms examined for *Salmonella*. Overall *Salmonella* was isolated in 71.3% (296/415). Specifically, *Salmonella* was isolated in 62.9% (122/194) of the faecal samples, 94.8% (147/155) of the floor swab samples and 40.9% (27/66) of the water samples. Farm 11 had the highest proportion of the isolates, the lowest number of isolates were received from farm 18. The proportion of *Salmonella* isolates from closed farms and open farms of 69.0% and 72.1% was not significantly (p = 0.643) different.

Table 11 shows the percentage of samples tested positive for *Salmonella* for the three water types. In general, the same source of water was used for drinking and cleaning the pens. However, drinking water samples were collected from the nipples, while cleaning water samples were collected from the pipe at the front or beside the pig house. The drinking water and cleaning water had similar results of 13.6% positivity while waste water had 95.5% positive result.

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Table 9: Distributions of farm faecal prevalence of *Salmonella* obtained from pigs in Chiang Mai province, Thailand

Farm	Faecal Sample	No. Positive	% Proportion (95% CI)
Open Farms			
1	10	8	80 (44-97)
2	10	7	70 (35-93)
4	10	7	70 (35-93)
5	10	7	70 (35-93)
7	8	7	88 (47-100)
8	8	7	88 (47-100)
9	8	5	63 (24-91)
10	8	4	50 (16-84)
11	8	7	88 (47-100)
12	8	5	63 (24-91)
14	8	4	50 (16-84)
17	8	5	63 (24-91)
18	8	3	38 (9-76)
19	8	4	50 (16-84)
20	8	4	50 (16-84)
21	8	5	63 (24-91)
22	8	5	63 (24-91)
Closed Farms			
3	10	6	60 (26-88)
6	10	8	80 (44-97)
13	10	6	60 (26-88)
15	10	5	50 (19-81)
16	10	3	30 (7-65)
Total Open Farm	144	94	65.3 (57-73)
Total Closed Farm	50	28	56.0 (41-70)
Overall Total	194	122	62.9 (56-70)

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Table 10: Proportion of *Salmonella* isolates from various samples in the farms

Farm	Total isolated sample				Total positive (%)			
	Feces	Floor swab	Water	Total	Feces	Floor swab	Water	Overall
Open Farms								
1	10	8	3	21	8 (80)	8 (100)	1 (33)	17 (81)
2	10	7	3	20	7 (70)	6 (86)	1 (33)	14 (70)
4	10	7	3	20	7 (70)	7 (100)	1 (33)	15 (75)
5	10	7	3	20	7 (70)	7 (100)	2 (67)	16 (80)
7	8	7	3	18	7 (88)	7 (100)	1 (33)	15 (83.3)
8	8	7	3	18	7 (88)	7 (100)	1 (33)	15 (83.3)
9	8	7	3	18	5 (63)	6 (86)	1 (33)	12 (66.7)
10	8	7	3	18	4 (50)	7 (100)	1 (33)	12 (66.7)
11	8	7	3	18	7 (88)	7 (100)	2 (67)	16 (88.9)
12	8	7	3	18	5 (88)	7 (100)	1 (33)	13 (72.2)
14	8	7	3	18	4 (50)	7 (100)	0 (0)	10 (55.6)
17	8	7	3	18	5 (63)	7 (100)	1 (33)	13 (72.2)
18	8	7	3	18	3 (38)	5 (71)	1 (33)	9 (50)
19	8	7	3	18	4 (50)	7 (100)	1 (33)	12 (66.7)
20	8	7	3	18	4 (50)	7 (100)	1 (33)	12 (66.7)
21	8	7	3	18	5 (63)	5 (71)	1 (33)	11 (61.1)
22	8	7	3	18	5 (63)	7 (100)	3 (100)	15 (83.3)
Closed Farms								
3	10	7	3	20	6 (60)	6 (86)	3 (100)	15 (75)
6	10	7	3	20	8 (70)	7 (100)	1 (33)	16 (80)
13	10	7	3	20	6 (60)	7 (100)	1 (33)	14 (70)
15	10	7	3	20	5 (50)	7 (100)	1 (33)	13 (65)
16	10	7	3	20	3 (30)	6 (86)	1 (33)	11 (55)
Total Open Farm	144	120	51	315	94 (65.3)	113 (94.2)	20 (39.2)	227 (69.0)
Total Closed Farm	50	35	15	100	28 (56.0)	34 (97.1)	7 (46.7)	69 (72.1)
Overall Total	194	155	66	415	122 (62.9)	147 (94.8)	27 (40.9)	296 (71.3)

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Table 11: Type of water samples and percentage of *Salmonella* positive

Type of water samples	Total samples	No positive (%)
Drinking water	22	3 (13.6)
Cleaning water	22	3 (13.6)
Waste water	22	21 (95.5)

4.1.2. Results of *Salmonella* Serotyping

Table 12 shows the most frequently found serogroups of *Salmonella*. A total of 295 isolates was tested. The serogroup with the highest proportion was *Salmonella* group C (47.1%), followed by group B (32.5%), group E (14.6%), group D (2.0%) and group F-67 (3.7%). The serogroups found in both open and closed farms were basically the same.

Faecal samples and floor swab samples was found to be contaminated with *Salmonella* group C in the highest frequency (54.5% and 43.5%), but in water samples *Salmonella* group B was the most frequently found (37.0%) (Table 13).

From Table 14, there was one 1 farm contaminated with *Salmonella* serogroup C only, 19 farms contaminated with *Salmonella* serogroup B and C, 8 farms contaminated with *Salmonella* serogroup B, C and E and 2 farms contaminated with *Salmonella* serogroup D.

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Table 12: Distribution of *Salmonella* serogroups in the farms

Farm	Number of samples in each group					
	B	**C**	**D**	**E**	**F-67**	**Total**
Open farms						
1	2	5	-	10	-	17
2	3	10	-	1	-	14
4	3	3	-	-	9	15
5	3	7	4	2	-	16
7	2	13	-	-	-	15
8	2	13	-	-	-	15
9	5	2	-	4	1	12
10	7	5	-	-	-	12
11	9	7	-	-	-	16
12	5	8	-	-	-	13
14	5	1	-	4	-	10
17	9	4	-	-	-	13
18	6	2	-	-	-	8
19	4	8	-	-	-	12
20	2	7	-	3	-	12
21	5	6	-	-	-	11
22	-	6	-	9	-	15
Closed farms						
3	-	5	2	7	1	15
6	-	16	-	-	-	16
13	8	6	-	-	-	14
15	9	2	-	2	-	13
16	7	3	-	1	-	11
Total Open Farm (%)	72 (31.9)	107 (47.3)	4 (1.8)	33 (14.6)	10 (4.4)	226
Total Closed Farm (%)	24 (34.8)	32 (46.4)	2 (2.9)	10 (14.5)	1 (1.4)	69
Overall Total (%)	96 (32.5)	139 (47.1)	6 (2.0)	43 (14.6)	11 (3.7)	295

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Table 13: Distribution of *Salmonella* serogroups in each type of samples

Serogrouping	Number and % positive		
	Faecal sample	Water sample	Floor swab sample
B	34 (28.1)	10 (37.0)	52 (35.4)
C	66 (54.5)	9 (33.3)	64 (43.5)
D	3 (2.5)	1 (3.7)	2 (1.4)
E	14 (11.6)	5 (18.5)	24 (16.3)
F-67	4 (3.3)	2 (7.4)	5 (3.4)
Total	121	27	147

Table 14: General distribution of *Salmonella* serogroup

Salmonella Serogroup	Frequency (farm)	Percentage
C	1	4.54
C, E	1	4.54
C, D, E, F-67	1	4.54
B, C	10	45.54
B, C, F-67	1	4.54
B, C, E	6	27.27
B, C, E, F-67	1	4.54
B, C, D, E	1	4.54
Total	22	1.00

Table 15 shows *Salmonella* serotypes isolated from each type of samples. Of the total 295 isolated samples, 19 serotypes were isolated. Of overall samples, *S.* Rissen was the most frequently serotype isolated (45.4% of all isolates), followed by *S.* Typhimuruim (18.3%), *S.* Stanley (11.5%), *S.* Weltevreden (4.1%), *S.* Krefeld (3.1%) and *S.* Anatum (2.0%).

From faecal isolation, 10 serotypes were isolated in the open farm and 5 serotypes were isolated in the closed farms. The most frequently serotypes found were was *S.* Rissen (53.7%), followed by *S.* Stanley (15.7%) and Typhimuruim (9.9%).

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From floor swab isolation, 13 serotypes were isolated in the open farm and 8 serotypes were isolated in closed farms. The most frequently serotypes found were *S.* Rissen (41.5%), followed by *S.* Typhimuruim (23.8%) and *S.* Stanley (8.8%).

The serotypes often found contaminated in water sample were *S.* Rissen and *S.* Typhimuruim; which were found 29.6% for each serotypes.

Table 15: *Salmonella* serotypes of isolates in each type of samples and compare between open farms and closed farms

Salmonella		**Number of isolates in each type of samples**								
Sero-group	Serotypes	Faeces		Floor swab		Type of water			Total	
		Open Farm	Closed Farm	Open Farm	Closed Farm	Drinking Water	Cleaning Water	Waste Water	Total Number	%
B	Typhimurium	8	4	26	8	-	1	7	54	18.3
	Stanley	16	3	10	4	-	-	1	34	11.5
	Agona	2	-	1	-	-	-	1	4	1.4
	Hato	-	-	-	1	-	-	-	1	0.3
	Derby	-	-	1	-	-	-	-	1	0.3
C	Rissen	49	16	47	14	-	-	8	134	45.4
	Afula	-	-	2	1	-	-	1	4	1.4
D	Panama	1	2	2	-	-	-	-	5	1.7
	Israel	-	-	-	-	1	-	-	1	0.3
E	Weltevreden	3	-	7	-	1	1	-	12	4.1
	Krefeld	4	-	5	-	-	-	-	9	3.1
	Anatum	1	3	2	-	-	-	-	6	2.0
	Regent	2	-	1	1	-	-	1	5	1.7
	O3,15:f,g,r:	-	-	3	-	-	-	-	3	1.0
	O3,10:e,h:	-	-	-	3	-	-	-	3	1.0
	Alfort	-	-	-	1	-	1	-	2	0.7
	Langensalza	-	-	1	-	-	-	-	1	0.3
	Rideau	1	-	-	-	-	-	-	1	0.3
	O3,15:f,g:	-	-	-	-	-	-	1	1	0.3
Others		6	-	5	1	1	-	1	13	4.7
Total		93	28	113	34	3	3	21	295	100.0

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4.2. Results of *Salmonella* Antibody Testing from Serum Samples

A total of 428 serum samples from 22 farms was analyzed using the SALMOTYPE® Pig LPS ELISA (Labor Diagnostik Leipzig, Germany) (cut-off value of OD%>40). The results in Table 16 show the distribution of sero-prevalence of *Salmonella*, ranging from 25-95% with an average of 64.4% (95% CI: 60%-69%). Specifically, in the open farms, the sero-prevalence ranged from 30% to 95% with an average of 67.6% (95% CI: 62%-73%), while in the closed farms, the sero-prevalence ranged from 25% to 70% with an average of 54.0% (95%CI: 44%-64%) were obtained. These results were significantly ($p=0.0168$) different.

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Table 16: Results of *Salmonella* antibody testing from serum samples in each farm, using ELISA test witha cut-off value at 40 OD%

Farm	Serum Sample	No Positive	% Proportion (95% CI)
Open Farms			
1	8	4	50 (16-84)
2	20	9	45 (23-68)
4	20	9	45 (23-68)
5	20	18	90 (68-99)
7	20	17	85 (62-97)
8	20	18	90 (68-99)
9	20	11	55 (32-77)
10	20	19	95 (75-100)
11	20	13	65 (41-85)
12	20	6	30 (12-54)
14	20	18	90 (68-99)
17	20	11	55 (32-77)
18	19	8	42 (20-67)
19	20	11	55 (32-77)
20	20	18	90 (68-99)
21	20	16	80 (56-94)
22	20	15	75 (51-91)
Closed Farms			
3	20	13	65 (41-85)
6	20	11	55 (32-77)
13	20	11	55 (32-77)
15	20	5	25 (9-49)
16	20	14	70 (46-88)
Total Open Farm	327	221	67.6 (62-73)
Total Closed Farm	100	54	54.0 (44-64)
Overall Total	427	275	64.4 (60-69)

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4.3. Correlation between a number of *Salmonella* Isolation and ELISA Results

Blood serum and faecal samples were taken from 189 pigs. Table 17 shows the relationship between antibody detection in the serum and *Salmonella* presence throughout the faeces. The antibody detection method used was ELISA with a cut-off value of 40 OD%. 60.8% (115/189) of pigs were ELISA positive and 62.4 (118/189) were isolation positive. 74 pigs were found *Salmonella* positive in both faeces and serum. 30 pigs were negative in both. 44 pigs were found *Salmonella* positive in the faeces but not in the serum. 41 pigs were found negative in faeces but positive in serum. The total number of pigs with the same result (both tests were positive or negative) was 104 pigs. From this result, the correlation between the two methods of examination was found to be very low (kappa = 0.0492, OR=1.23, p=0.5399).

Table 17: Correlation of *Salmonella* isolation results and serological results obtained from ELISA (cut-off value at 40 OD%)

Test		ELISA		Total
		Positive	Negative	
Faecal Isolation	Positive	74	44	118
	Negative	41	30	71
Total		115	74	189

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4.4. Farm Management Characteristics and *Salmonella* Isolation

All the farms included in the survey had similar management because of the regulations given by the particular slaughterhouse the animals were shipped to. The most obvious differences among farms were the type of farm (closed/open house farm), DLD (Department of Livestock Development) certification, the source of water used in farms, waste management, herd size, loss rate and the drinking containing probiotics (EM; Effective Microorganisms).

Results from the questionnaires: the percentage of loss (mortality and culling) in the 22 farms ranged from 1.7% to 14.4% (mean = 4.25%, median = 3.45%). The standard loss rate set by the company was 3%, only 7 farms (31.8%) had a loss < 3%. The number of pigs per pen ranged from 20 to 32 pigs per pen (with mean, median and modes = 25 pigs per pen).

4.4.1. Results from Univariate Analysis

Table 18.1 shows the relationship between the particular management characteristics and the percentage of positive *Salmonella* faecal samples (univariate analysis, Chi-square tests). Among the factors, the type of waste management was the only significant characteristic associated with *Salmonella* isolation: pigs raised in farms with a slurry waste management system had higher *Salmonella* infection than pigs raised in farms with a biogas waste management system (69.2% and 52.7%, OR=2.01, p=0.023).

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Table 18.1: Relationship between farm management characteristics and *Salmonella* detection in faecal samples (univariate analysis)

Factor	Status	No. of samples	% Positive	OR (95% CI)	p-value
Herd Size	< 400 401 - 800 >800	42 132 20	73.8 58.3 70.0	2.01 * (0.93, 4.35)	0.1531
DLD certified	Certified Applying Non-certified	72 68 54	56.9 67.6 64.8	1.58 * (0.79, 3.15)	0.3994
Housing system	Open house Closed house	144 50	65.3 56.0	1.48 (0.77, 2.84)	0.3079 **
Water Source	Tab water Underground water Surface water	10 96 88	60.0 64.6 61.4	1.22 * (0.32, 4.61)	0.8862
Probiotic (EM)	Used Not Used	126 68	64.3 60.3	1.19 (0.65, 2.18)	0.6411 **
Lime Ash	Not used Used	82 112	64.6 61.6	1.14 (0.63, 2.06)	0.7637 **
Waste management	Slurry Biogas	120 74	69.2 52.7	2.01 (1.11, 3.66)	0.0228 **

Remark * Highest OR obtained from 2*2 table of the factors
** p-value from Fisher's Exact

4.4.2. Results from Multivariable Analysis

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Table 18.2 shows the relationship between particular management characteristics and positive results of *Salmonella* in faecal samples (multivariable risk factors analysis, SAS statistic program). All relevant factors (Table 17.2) were included in this calculation. Without the interaction of other farm characteristics, the significant characteristic associated with *Salmonella* isolation was the housing system: the open house system had a significantly higher *Salmonella* isolation than the closed house system (OR=1.59, p=0.0496). Herd size was also a significant characteristic associated with *Salmonella* isolation: a smaller herd size (< 800 pigs/herd) tended to

have lower *Salmonella* isolation than the larger herd size (> 800 pigs/herd) (OR=0.18, p≤0.0002). The lower number of pigs per pen was also significantly associated with lower *Salmonella* infection (OR=0.91, p<0.0001).

Table 18.2: Relationship between all farms management characteristics and *Salmonella* detection in faecal samples (multivariable analysis)

Factor	Status	OR	p-value
Herd size	< 400	0.21	0.0002
	401 - 800	0.18	< 0.0001
	>800	1.00	.
DLD certified	Certified	0.68	0.0412
	Applying	1.72	0.033
	Non-certified	1.00	.
Housing system	Open house	1.59	0.0496
	Closed house	1.00	.
Water source	Tab water	0.95	0.9524
	Underground water	1.76	0.014
	Surface water	1.00	.
Probiotic (EM)	Not Used	0.56	< 0.0001
	Used	1.00	.
Lime ash	Not used	1.03	0.9314
	Used	1.00	.
Waste management	Slurry	1.50	0.1168
	Biogas	1.00	.
No. of pigs/pen	20 to 32 pigs per pen	0.91	< 0.0001
% loss	1.7% to 14.4%	0.98	0.7063

Pigs not fed probiotics (EM) appeared to have a significantly lower risk of harboring *Salmonella* than pigs fed probiotics (EM) (OR=0.56, p<0.0001). There was a higher *Salmonella* isolation rate in farms using underground water than farms using surface water (OR=1.76, p=0.014). Farms certified by DLD had significantly lower *Salmonella* isolation than farms non-certified by DLD (OR=0.68, p=0.0412) while farms in the process of applying DLD certification appeared to have a significantly higher risk of getting *Salmonella* than non-certified farms (OR=1.72, p=0.033). Waste management systems, using lime ash and the percentage of losses had no association with *Salmonella* isolation.

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4.5. Farm Management Characteristics and *Salmonella* Antibody Testing

4.5.1. Results from Univariate Analysis

Table 19.1 shows the relationship between particular management characteristics and the percentage of positive *Salmonella* antibody detection in serum samples (univariate analysis of risk factors, Chi-square tests). Among those characteristics herd size, housing system (open/closed farms), water source, probiotic (EM) feed and waste management affected the sero-prevalence of *Salmonella*.

A herd size with more than 800 pigs per herd had a lower positive percentage of sero-prevalence (60.0%), herds lower than 400 pigs per herd had the highest sero-prevalence (78.4%). This difference was significant (OR=2.42, p=0.0087). Farms with the open house system had a higher sero-positive percentage (67.6%) than farms with the closed house system (54.0%); this was significantly different (OR=1.78, p=0.0168).

Farms that used underground water had a higher sero-positive percentage (74.0%) than farms using tab water (65.0%) or farms using surface water (54.3%). This was also significantly different (OR=2.40, p=0.0002).

Farms that did not feed pigs with probiotics (EM) had higher *Salmonella* sero-positive percentages compared to farms that fed probiotics (EM) (77.5% and 56.6%). These results were significantly different (OR=2.65, p=0.00001).

Pigs raised in farms with a slurry waste management system had higher *Salmonella* infection than pigs raised in farms with a biogas waste management system (67.9% and 58.5%, OR=1.50, p=0.0597).

The *Salmonella* sero-positve percentage in farms certified by DLD was not different from that of non-certified farms and farms that were in the process of

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applying (62.8%, 68.1% and 61.1% respectively, OR=1.36, p=0.4414). Using lime ash in the cleaning and disinfection steps before receiving the new pigs was not different in *Salmonella* sero-prevalence from those farms not using lime ash (64.5% and 64.3%, OR=1.01, p=1.0000).

Table 19.1: Relationship between farm management characteristics and *Salmonella* detection from serum samples using ELISA test with a cut-off value at 40 OD% (univariate analysis)

Factor	Status	No sample	% positive	OR (95% CI)	p-value
Herd Size	< 400 401 - 800 >800	88 299 40	78.4 60.9 60.0	2.42 * (1.08, 5.45)	0.0087
DLD certified	Certified Applying Non-certified	159 160 108	62.9 68.1 61.1	1.36 * (0.82, 2.27)	0.4414
Housing system	Open house Closed house	327 100	67.6 54.0	1.78 (1.13, 2.80)	0.0168 **
Water Source	Tab water Underground water Surface water	20 208 199	65.0 74.0 54.3	2.40 * (1.58, 3.65)	0.0002
Probiotic (EM)	Not Used Used	160 267	77.5 56.6	2.65 (1.70, 4.12)	0.00001
Lime Ash	Not used Used	168 259	64.3 64.5	1.01 (0.67, 1.51)	1.0000 **
Waste management	Slurry Biogas	268 159	67.9 58.5	1.50 (1.00, 2.26)	0.0597 **

Remark * Highest OR obtained from 2*2 table of the factors

** p-value from Fisher's Exact

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4.5.2. Results from Multivariable Risk Factor Analysis

Table 19.2 shows the relationship between particular management characteristics and positive results of *Salmonella* antibody detection in serum samples (multivariable risk factors analysis, SAS statistic program). All relevant factors (Table 18.2) were included in the calculation.

Without the interaction of other farm characteristics, the characteristics significantly associated with *Salmonella* isolation were the housing system in which the open house had a significant higher *Salmonella* isolation than closed housing system (OR=2.84, p=0.0496). The lower number of pigs per pen was also associated with higher *Salmonella* isolation (OR=1.16, p<0.0121).

DLD certified farms had significantly higher results of *Salmonella* infection than non-certified farms (OR=2.76, p=0.0525). Herd size of 400- 800 pigs/farms had lower *Salmonella* infection than farms which more than 800 pigs/farm (OR=0.25, p=0.0252).

Farms not using probiotic (EM) tended to have higher *Salmonella* infection than farms using probiotic (EM) (OR=2.49, p=0.0605).

Waste management, using lime ash, water source and percentage of loss had no association with *Salmonella* antibody detection result.

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Table 19.2: Relationship between all farms management characteristics and S*almonella* detection from serum samples using ELISA test with a cut-off value at 40 OD% (multivariable analysis)

Factor	Status	OR	p-value
Herd size	< 400 401 - 800 >800	0.55 0.25 1.00	0.4733 0.0252 .
DLD certified	Certified Applying Non-certified	2.76 2.15 1.00	0.0525 0.1848 .
Housing system	Open house Closed house	2.84 1.00	0.0475 .
Water source	Tab water Underground water Surface water	1.27 2.23 1.00	0.8351 0.1044 .
Probiotic (EM)	Not Used Used	2.49 1.00	0.0605 .
Lime ash	Not used Used	1.33 1.00	0.4035 .
Waste management	Slurry Biogas	0.55 1.00	0.2148 .
No. of pigs/pen	20 to 32 pigs per pen	1.16	0.0121
% loss	1.7% to 14.4%	0.93	0.4539

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5. DISCUSSION AND CONCLUSIONS

5.1. Discussion

The study units were pig herds of contract pig farms of an integrated pork production company in the region of Chiang Mai province, Thailand. All herds of farms did exclusively receive piglets (3 weeks of age for 'closed' farms; 12 weeks of age for 'open' farms) from the same company breeding farm and pigs were fattened on the farms up to slaughter age and -weight (4.5 months; 90-100 kg). The study design and its time schedule chosen could be carried out without any difficulty. Farms and subsequently the slaughterhouse did supply well any information needed. This reflects the company's approach of a transparent food safety policy for all their production lines. Major pathogenic viruses and bacteria are evaluated in this policy. *Salmonella* are addressed in the list of agents of consideration, but are only tested for in the poultry production line, not in the pork line. The company's policy is also principally supported and regulated by the Ministry of Agriculture and Cooperatives and Ministry of Health of Thailand. The results from this study are expected to provide useful information for further improvements for the company's policy in regards to their pork production.

5.1.1. Materials and Methods

In order to isolate and identify *Salmonella*, Davies *et al.* (2000) recommended pre-enrichment for materials such as foods and environmental samples, because materials are likely to only contain low numbers of *Salmonella* that may have been stressed or injured by factors such as temperature, osmotic shock, or by freezing and thawing. The choice of the most suitable pre-enrichment is debated, although buffered peptone water generally is recommended for routine use, as it maintains a stable pH environment (Axelsson and Sorin, 1997).

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In contrast to investigations of *Salmonella* in foods and in environmental samples, pre-enrichment for faecal samples may be counterproductive. When faecal samples are small, it is better to put the sample directly to selective enrichment (Davies *et al*., 2000). In case of selective enrichment, since no single medium can claim to manage all food matrices and *Salmonella* serotypes equally well, it is often advisable to use two media in parallel.

In this study, tetrathionate broth and Rappaport-Vassiliadis medium were used as selective broth media as recommended by ISO 6579. For subsequent solid selective enrichment, BPLS and XLT4 agar were used. The distinguishing feature of XLT4 is its high degree toward inhibition of other competing bacteria. This allows a significant increase in the recovery of salmonellae, while essentially eliminating false-positive suspected colonies.

The amount of each faecal sample was 25 g which was sufficient for investigation according to ISO 6579 and also agrees with recommendations of Davies *et al*. (2000), who found that *Salmonella* detection increases with sample weight, ranging from rectal swab (estimated 0.5 g) to 25 g faeces.

5.1.2. Results of Isolations

Results of investigations of faecal samples results provide an estimate of herd-level prevalence of current *Salmonella* infection in pre-slaughter pigs. All herds in this investigation were infected with *Salmonella*, the faecal sample prevalence of *Salmonella* between herds ranged from 30% to 88%, with an average of 62.9%.

This result is similar to investigations of Patchanee *et al.* (2002). The authors did determine an average herd-level prevalence of 69.5% for slaughter pigs from investigations of mesenteric lymph nodes of pigs slaughtered at the slaughterhouses in Chiang Mai. Patchanee *et al.* (2002) did attribute this high prevalence though particularly to effects of transport and lairage prior to slaughter. As the study pigs at farm level still had transport and lairage ahead of them, the mean *Salmonella*

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prevalence of 62.9% indicates that pigs throughout farms are already infected to a degree higher than expected so far. This already high farm-level infection rate probably will be further exacerbated by stress factor during transport and lairage and by handling during the slaughter process.

Salmonella isolations from floor swabs and of waste water serve as an indicator of environmental contamination or of the *Salmonella* shedding status of the herds. The contamination levels of both samples, with 94.8% in floor swab samples and 95.5% in waste water samples, were very high and higher than in the faecal samples. High levels of *Salmonella* contamination in environmental samples also were found by Rajic *et al*. (2005) in North Carolina, USA; in their investigation water samples from the draining system were found to be contaminated with *Salmonella* in 31.8%, while faecal samples of pigs were found positive in 14.3%.

In every study farm, water samples were collected. The drinking water and water used for cleaning on the farms came from the same source, but were collected from different locations on the farms. Therefore, when either drinking water or cleaning water was found to be contaminated, this might indicate that each water type independently is contaminated from the environment. In case that both water samples were positive, they probably have been contaminated from the source of water.

5.1.3. Serotypes of Isolates

Of the 22 farms investigated, only one farm was contaminated with a single somatic serogroup (serogroup C), 11 farms with two groups of *Salmonella* (serogroups C and B or C and E) and the remaining 10 farms with at least 3 *Salmonella* serogroups (serogroups B, C, D, E and F-67). The proportions of each serogroup of pigs at farm level compared to those of Patchanee *et al.* (2002) of pigs at slaughter are summarized in the table below.

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Serogroup	Percentage	
	This study	Patchanee *et al.* (2002)
B	32.5	28.5
C	47.1	32.1
D	2.0	9.4
E	14.6	32.1
Others	3.7	0.52

The most frequent serotype determined in this study of pigs for slaughter was *S*. Rissen (45.4% of all isolates) followed by *S*. Typhimurium (18.3%), *S*. Stanley (11.5%), *S*. Weltevreden (4.1%), *S*. Krefeld (3.1%) and *S*. Anatum (2.0%). For comparison, the first 5 of the 10 most frequent *Salmonella* serotypes from human cases were *S*. Weltevreden (12.5%), *S*. Enteritidis (11.4%), *S*. Anatum (7.4%), *S*. Derby (6.6%) and *S*. 1,4,5,12:I:ssp.1 (6.4%) (Bangtrakulnonth *et al.,* 2004). *S*. Rissen and *S*. Typhimurium ranked 7th and 6th in this investigation.

S. Rissen during the last years is increasingly isolated in Thailand (1.6% in 1993 to 8.2% in 2002) in foodborne gastrointestinal infections in humans and 4.7% in 1993 to 14.7% in 2002 in 'other' food products (Bangtrakulnonth *et al.,* 2004). The reservoir of *S*. Rissen has not been identified yet, but the agent so far was frequently found in water and food products (Bangtrakulnonth *et al.,* 2004). The results from this study indicate that pre-slaughter pigs and the environment in pig fattening farms are an important reservoir for *S*. Rissen.

S. Typhimurium is a virulent serotype, and the most frequently serotype found in pigs in many countries such as Denmark, Japan, the United States and Canada (Sorensen *et al*., 2004, Asai *et al*., 2002b, Davies *et al*., 1997, Funk *et al*., 2005, Rajic *et al*., 2005). From the study of Bangtrakulnonth *et al.* (2004) it is suggested, that the importance of *S*. Typhimurium in Thailand in human food borne gastrointestinal infections has not increased, accounting for 5 to 6% of cases. Animals can be a reservoir but no specific respective animal source has been found for Thailand (Bangtrakulnonth *et al*., 2004). The results of this study underline that *S*. Typhimurium exists in pig farms and in farms' environment and pigs subsequently

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could be an important reservoir for respective *Salmonella* contamination of the pork chain.

S. Stanley has been frequently reported in seafood and other food products in Thailand. Ducks were so far the only important reservoir for this serotype according to Bangtrakulnonth *et al.* (2004). However, Bangtrakulnonth *et al.* (2004)'s study did not include pig farms. The present study shows that, pre-slaughter pigs are an important source of *S*. Stanley contamination in the pork chain.

According to the study of Bangtrakulnonth *et al.* (2004), *S*. Weltevreden is the most frequently isolated serotype in human foodborne gastrointestinal infections in Thailand, mainly originating from frozen seafood, human cases, water and from other non-specified food products. In this study, *S*. Weltevreden was found mostly in the environmental samples. *S*. Weltevreden was also isolated from pig faeces, but in lower numbers than in environmental samples. *S*. Enteritidis is reported to be frequently isolated from frozen chicken and is found at a high frequency in human cases (Bangtrakulnonth *et al.*, 2004). In this study, no *S*. Enteritidis was isolated from faeces of pre-slaughter pigs or from their environments at farms.

The remaining serotypes determined in this study were *S.* Panama (1.7%), *S.* Regent (1.7%), *S.* Agona (1.4%), *S.* Afula (1.4%), *S.* O3,15:f,g,r (1.0%), *S.* O3,10:e,h: (1:0%), *S.* Alfort (0.7%), *S.* Hato (0.3%), *S.* Derby (0.3%), *S.* Israel (0.3%), *S.* Langensalza (0.3%), *S.* Rideau (0.3%), *S.* O3, 15:f,g: (0.3%), and further serotypes (4.7%). Of these, *S.* Panama, *S.* Agona and *S.* Derby are also contained in the report of Bangtrakulnonth *et al.* (2004), while the rest of serotypes are not reported.

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5.1.4. Results of Serological Tests

In this study, the Danish Mix-ELISA (SALMOTYPE® Pig LPS ELISA, Labor Diagnostik Leipzig, Germany) was used to estimate the sero-prevalence of *Samonella* in slaughter pigs. Positive serological response is interpreted as indicating a *Salmonella* systemic infection of pigs. From this study, average pig sero-prevalence

of *Salmonella* (64.4%) was similar to the *Salmonella* prevalence in faeces (62.9%). Patchanee *et al.* (2002) in their investigation, using the same test, did obtain a comparably high sero-prevalence of 59.5%. Results are based on the prescribed cut-off value at 40 OD%. The Danish Mix ELISA was developed to help assess the *Salmonella* situation for European countries; at a cut-off value of 40 OD% the test's specificity is particularly emphasized in order to derive at valid sero-negative results. A lower OD%-cut-off value would increase the sensitivity and decrease the specificity of the test. As the result of this study is that the majority of pigs tested were *Salmonella* sero-positive, no benefits are seen of changing this recommended cut-off value of 40 OD% in either direction, decreasing or increasing it, for Thailand. In Denmark, the OD value of the Danish Mix ELISA was meanwhile reduced from 40 OD% to 20 OD% (Nielsen *et al.,* 2001), in order to increase the sensitivity of the test to even better identify the low number of positive herds at the low nationwide herd-level prevalence of 0.7%.

5.1.5. Correlation between Isolation and Serological Tests Results

A total of 189 pigs were examined both blood serum and faecal samples. *Salmonella* prevalences from investigations of faecal samples and of serum in total were not different (62.4% and 60.8%, respectively). However, 45% (85/189) of pigs were found positive only in one but negative in the other test. This result explains the low correlation (kappa=0.0492, p=0.05399) between results of faecal isolation and serological testing. Such result was also found by Davies *et al.* (2003) who also established a poor correlation between bacteriological and serological test results. Other investigations, in contrast (Lo Fo Wong *et al.,* 2003, Sorensen *et al*., 2004, Rajic *et al*., 2005, and Funk *et al.,* 2005) established a moderate to strong correlation between *Salmonella* culture-positive and sero-positive results at herd level. Lo Fo Wong *et al*. (2003) found, that the correlation coefficient between bacteriological and serological results were 62% and 58% at cut-off values of >10 and > 40 OD% of the serological test, respectively. Sorensen *et al.* (2004) found the odds for being culture positive for *Salmonella* to increase 1.3- to 1.5-fold with each increase of 10% in herd

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serology. Funk *et al*. (2005) reported correlations between faecal culture and the Danish Mix-ELISA of 0.40, 0.36, 0.43 and 0.43 ($p<0.0001$) for OD% cut-offs $\geq$ 10, 20, 30 and 40, respectively. Funk *et al*. (2005) also concluded to recommend a higher OD% cut-off if more approximate estimations of the faecal prevalence are desired. It has to be kept in mind, that both test systems not necessarily principally measure the same substrate. Reducing both test systems to their major substrates, cultures of faeces at the minimum indicate that animals carry agents in the intestines, while detection of antibodies points to more systemic carriers of the organism.

The serotypes of *Salmonella* present in herds also are of influence on antibody detection levels. van Winsen *et al*. (2001) found that the antibodies against *S*. Typhimurium and *S*. Brandenburg were well detectable while antibodies against *S*. Goldcoast and *S*. Panama were poorly detected or not at all; this finding is similar to the results of Stege *et al.* (2000), who found, that sero-positivity tended to be related to the presence of *S*. Typhimurium. Funk *et al.* (2005) contradict, in their investigation the association between the predominant serotypes (*S*. Typhimurium) isolated from pigs and sero-prevalence was low. In this study *S*. Typhimurium was detected at low level (9.9%) in faecal samples, however, the corresponding serological test result from the same group of pigs was high (60.8%). Thus, sero-positivity in this study was not related to the presence of *S*. Typhimurium.

Lo Fo Wong *et al*. (2003) offer an explanation why results from bacteriological and serological tests cannot be compared easily, and why the correlation of results of both test systems not only depends on the underlying *Salmonella* prevalence, but also on the sampling method (e.g. sample -size, -volume, -frequency and –location) as well as on the test characteristics of both tests, i.e. their sensitivities and specificities. All factors considered, it is well possible that although the *Salmonella* prevalences of both results are not different, the correlation between both tests can be very low. Further on, differences of LPS antigen composition used in different *Salmonella*-ELISA-systems may result in results deviating from those of the Danish Mix-ELISA, which is based on the predominant 'European' serogroups B, C1 and D1 (van der Wolf *et al*., 1999).

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Nevertheless, for screening purposes, serological testing provides an indication of exposure to *Salmonella*, which forms the basis for more targeted sampling and for interventions and logistic slaughter procedures. Serological screening is useful for identifying whether herds or groups are possibly infected with certain serotypes. It follows that serological testing is of no use to judge the *Salmonella* status of *individual* animals. In these cases, culturing faecal samples for *Salmonella* is a useful tool to determine not only the extent but also the kind of current infections in a pig herd (van Winsen *et al*., 2001, Lo Fo Wong *et al*., 2003, Funk *et al*., 2005).

5.1.6. Farm Management Characteristics and the Prevalence of *Salmonella*

According to questionnaires, all farms studied (i) received the piglets from the same breeding farm, (ii) only used a single house for the fattening pigs (iii) applied all-in/all-out practices (iv) used commercial pellet feed (v) had solid concrete floors with a small water pond in each pen and (vi) used trough-feeding systems. A few farms used both mechanical and trough feeding system within the same pen.

According to Davies *et al.* (1997), the prevalence of *Salmonella* is likely to be lower in pigs raised on slotted floors compared to all other floor types, and highest in pigs raised on dirt lots. van der Wolf *et al*. (1999) found that herds which used trough-feeding systems had a 4 times higher risk of *Salmonella* infection than herds not using this feeding system. Beloeil *et al.* (2004) reported that pigs fed dry feed had higher *Salmonella* isolation rates than pigs fed wet feed. This study did not investigate *Salmonella* contamination levels of floor types, feed or feeding type. However, all study farms used solid concrete floor, pellet feed and the trough feeding system, all being elements which from the above cited studies are associated with high *Salmonella* infection.

The all-in/all-out system principle of farm management might not prevent introduction of an infection into a herd, but rather assists to prevent cross-contamination between batches and allows cleaning and disinfection between batches

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(Lo Fo Wong *et al*., 2004). Davies *et al*. (1997) also conclude that in regards to *Salmonella* infection, modern methods of raising pigs in multiple-site production systems, using all-in/all-out management of finishing pigs, appear to have no benefit in reducing the prevalence of *Salmonella* compared to the conventional farrow-to-finish system.

The effects of management characteristics for aspects of (i) herd size, (ii) DLD certification, (iii) housing system, (iv) water source, (v) feeding of probiotics, (vi) use of lime ash at a step of cleaning and disinfection, (vii) waste management system, (viii) number of pigs per pen and (ix) percentage of losses were analyzed for *Salmonella* prevalence both by univariate (Chi-square test) and by multivariate analysis of logistic regression test. Multivariable analysis permits to estimate the real impact of a particular factor without interaction from other factors.

Herd size: Based on the *Salmonella* results of faecal isolation and from serological testing, pigs raised in farms with smaller herd sizes (<800 pigs/herd) appeared to have a significantly lower chance of *Salmonella* infection ($p<0.05$) than larger farms. Mousing *et al.* (1997) and Carstensen and Christensen (1998) also report that herd size is positively associated with the sero-prevalence of *Salmonella*; increased herd size imposes an increased risk of *Salmonella* infection. The opposite conclusion was drawn from van der Wolf *et al*. (2001), in their study small to moderate sized herds (<800 finishers) had a higher risk of *Salmonella* infection compared to large herds. However, results for the effect of herd sizes do not have to be seen in isolation. Other factors, acting at the herd level, might contribute, such as types of wet feed/dry feed, slurry/manure management, cleaning/disinfection procedures, and pig density in the geographical area around farms (Christensen and Rudemo, 1998).

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Housing system: Pigs raised in a closed house had a significantly lower risk of *Salmonella* infection compared to pigs raised in open farms ($p<0.05$). The closed farms in this study were farms equipped with the ‘Evaporative Cooling System’ (EVAP), a ventilation system that controls the temperature inside the pig house. Closed house systems though cannot prevent infections from outside. According to

Stege *et al*. (2000) and van der Wolf *et al.* (2001), the housing system or housing type might has no impact on large herd sizes, because larger operations generally also have the resources to implement effective biosecurity measures, use health declaration and employ good manufacturing practice schemes.

The DLD (Department of Livestock Development, Ministry of Agriculture and Cooperatives, Thailand) certification: No difference in *Salmonella* prevalence was established for pigs raised in farms certified by DLD and non-DLD-certified farms or farms being in the process of applying for DLD certification. The major difference of the study farms though was not DLD certification; all farms, certified or not, rather used similar basic management and also were under the control of one specific slaughterhouse to which pigs exclusively were supplied.

Water source: There were three types of water sources, (i) tab water, (ii) underground water and (iii) surface water from ponds or wells. No water treatment existed in farms using tab water or underground water. Farms using surface water did employ a program treating water with Chlorine two times per month. Regarding *Salmonella* infection rates at herd level, based on isolation results, farms using underground water had a higher *Salmonella* infection risk compared to farms using surface water (p=0.014).

Use of probiotics (Effective Microorganisms (EM)): Feeding probiotics is another intervention strategy to reduce food-borne pathogens in food animals (Callaway *et al.*, 2003). The probiotic used in this study on some farms was EM, first used in Japan and Denmark (Pinto, 2005), and widely used in raising animals in Europe and more than 100 countries (Harnes-Parton, 2005). EM is composed of three general groups of organisms, being lactic acid bacteria, yeasts and phototrophic bacteria (Pinto, 2005). Contrary to expectations, farms feeding EM in this study were associated with higher *Salmonella* isolations than farms not feeding EM (p<0.0001). However, serum titers of pigs given probiotics were lower than of pigs not fed probiotic; this difference was not significant (p=0.060).

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Lime ash: All farms employed similar cleaning and disinfections procedures. Disinfectants used were identical and all provided by one particular company, except for lime ash. The use of lime ash did not benefit farms regarding their *Salmonella* infections.

Waste management system: Farms using slurry waste management and biogas waste management or not were not different in their *Salmonella* prevalences. *Salmonella* were found in a very high proportion; 95.5%, in waste water samples (water from drainage systems) and in 94.8% in floor swab samples. As *Salmonella* can survive for 47 days in manure storage or even years in suitable organic material (Schneider *et al*., 2003) they are a constant source of re-infection in farms, either by vectors, humans or by oral exposure to faecal materials. Husbandry technology like waste water management may help keep infection within limits, but may not decisively help reduce infection levels.

Number of pigs per pen: The number of pigs per pen ranged from 20 to 32 (mean, median and modes = 25 pigs per pen). Based on isolation results of individual pigs' faeces, a smaller number of pigs/pen was associated with a significant lower risk of *Salmonella* infection ($p<0.0001$). In contrast, a smaller number of pigs/pen was associated with a higher number of serological positive animals ($p=0.0121$). It may be possible that these obviously disagreeing results may be impacted by overall total herd size or other unknown factors associated with the distribution of *Salmonella* in herds or in pens. For example, number of pens in the house, the draining system within the pens, spreading of manure and the contact of pig between pens (Lo Fo Wong *et al*., 2004). Berends *et al*. (1996) concluded that in case of a pen is infected, the current probability of transmission to other pens (pen transmission) would be about 90%.

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Percentage of losses: The percentages of losses include mortality losses and culling losses. Losses ranged from 1.7% to 14.4% (mean = 4.25%). The standard loss rate set by the company was 3%; only 7 farms (31.8%) did reach this target of <3%. Losses though most likely were not due to *Salmonella*, the percentages of losses in

this study were not associated with prevalences of *Salmonella,* regardless whether determined by culture or by serology.

Finally, all study farms and herds were managed by one company and delivered slaughter pigs to one particular slaughterhouse. Results of this investigation for *Salmonella* can not be generalized for pigs raised by other companies or even by backyard farms in the Chiang Mai region or even all of Thailand. It is nevertheless not unreasonable to assume that *Salmonella* prevalences in pigs in other farms, having no or lower-standard provisions for pig fattening, may be even higher than the already high prevalences in the 'top-selection' of farms used in this study. It is understood that levels of *Salmonella* infection on farms might change over time and a single sampling may not be sufficient to depict the *Salmonella* status of a herd or a farm entirely (Rajic *et al*., 2005). A longitudinal sampling scheme would be useful to evaluate the dynamics of *Salmonella* infections on farms as well as the impacts of on-farm interventions against *Salmonella* (Funk *et al*., 2005).

5.2. Conclusion

All farms investigated were infected with *Salmonella enterica*. *Salmonella* serogroups C and B were the major serogroups isolated. 19 serotypes in total were isolated with this study. The most frequent serotype isolated was *S*. Rissen, which was present in every farm investigated. Other serotypes found in high frequencies were *S*. Typhimurium, *S*. Stanley, *S.* Weltevreden, *S.* Krefeld and *S.* Anatum.

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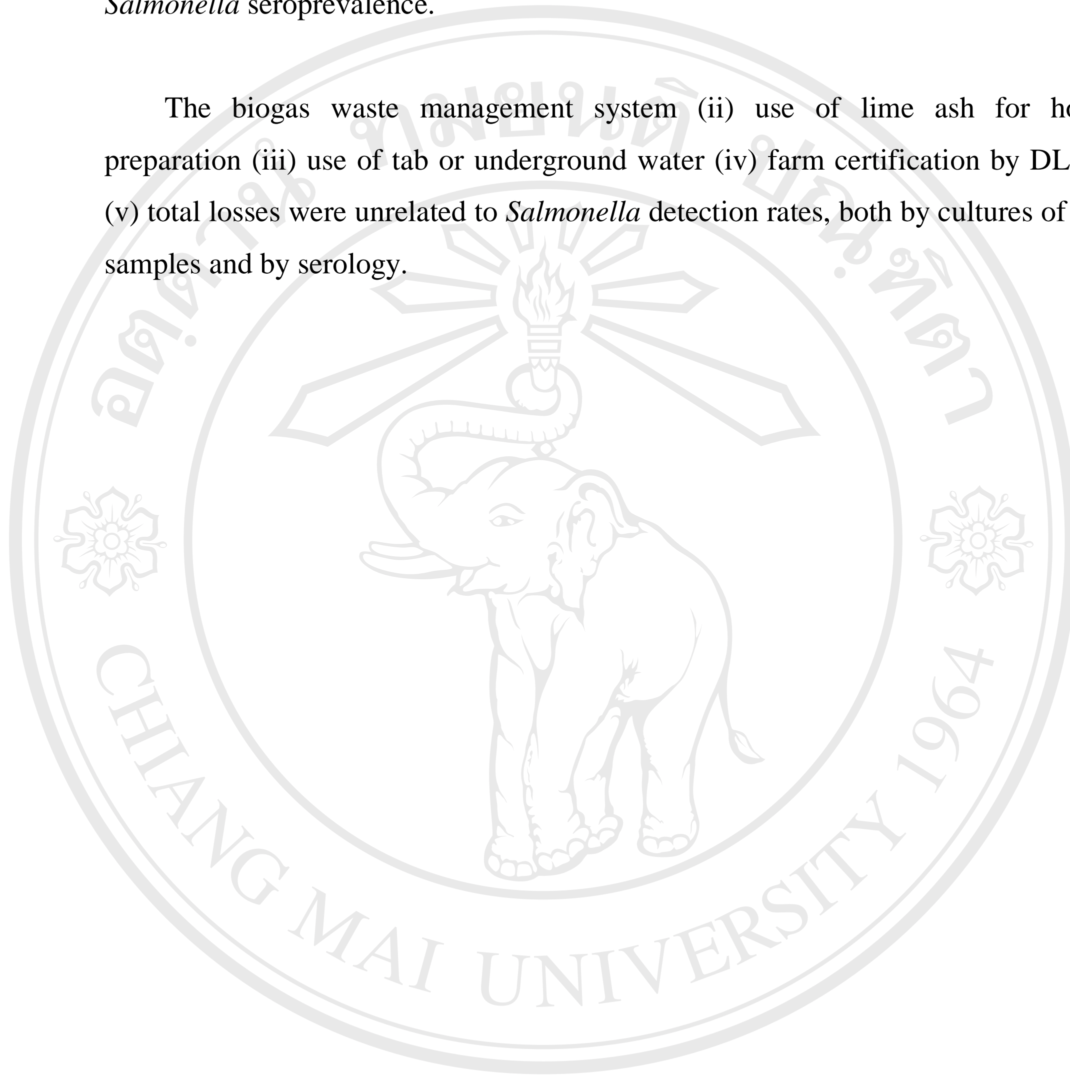
Correlation between investigation results of faecal isolation and of serolgy was poor, although prevalences of both test systems were equally high. Farm management characteristics, such as (i) herd size (<800 pigs per herd) and (ii) a closed house system were significantly associated with lower *Salmonella* infection. Feeding of EM probiotics rather did increase *Salmonella* faecal isolation rates but resulted in a higher level of antibodies. Also, keeping of a higher number of pigs per pen was associated

with high *Salmonella* isolation rates but appears to be associates with lower *Salmonella* seroprevalence.

The biogas waste management system (ii) use of lime ash for housing preparation (iii) use of tab or underground water (iv) farm certification by DLD and (v) total losses were unrelated to *Salmonella* detection rates, both by cultures of faecal samples and by serology.



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APPENDIX

Appendix A: Questionnaires and Check List

Fattening Farm Information

Date of investigation……………………..Investigator………………………………

Farmer…………………………………Address………………………………..

---

1. Animal

1.1 Breed	2X	3X	
1.2 Number/farm	<400	401-800 pigs	>800 pigs

2. DLD certification certified applying non-certified

3. Health problem

no problem

respiratory disease

enteric disease

other……………………………………………

4. Feed and feeding system

4.1 Type of feed	pellet	powder
4.2 Type of feeding system	trough	mechanical
	automatic	
4.3 Frequency of feeding/day	1 time	2 times
	$\geq$ 3 times	

4.4 Sanitation of feeding system

……………………………………………………………………

……………………………………………………………………

4.5 Feed storage

……………………………………………………………………

……………………………………………………………………

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4.6 Antibiotic in feed

………………………………………………………………………
………………………………………………………………………

5. Drinking water

5.1 Source of water tap water underground water
surface water

5.2 Testing of water quality (e.g. hardness, bacterial count)
Yes ……………………………………...
No

5.3 Treatment of water
Yes ……………………………………...
No

5.4 Type of watering system nipple through

6. Water for cleaning

6.1 Source of water tap water underground water
surface water

6.2 Treatment of water
Yes/with……………………………………...
No treatment

7. Housing

7.1 System of farm all in/all out continuous

7.2 Type of housing Open house Closed house (Evap)

7.3 Type of floor solid floor slatted floor both

7.4 Number of pen in house ……………………………………………….

7.5 Number of pig/pen …………………………………………………….

7.6 Pen size…………………………………………………………………

8. Medication

8.1 vaccination FMD SF Mycroplasma
AD Others.........................

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8.2 Therapeutic antibiotic (dose and duration)

1. ……………………………………………………………………

2. ……………………………………………………………………

3. ……………………………………………………………………

4. ……………………………………………………………………

8.3 Vitamin and Minerals

Yes/with………………………………………...

No

8.4 Probiotic usage

Yes/with………………………………………...

No

8.5 Deworm program

Yes/with………………………………………...

No

8.6 Isolation of sick animal

Yes/with………………………………………...

No

9. Hygiene and sanitation

9.1 Sanitation of pen and housing

……………………………………………………………………

……………………………………………………………………

9.2 Personal hygiene

……………………………………………………………………

……………………………………………………………………

9.3 Cleaning and disinfection procedure

……………………………………………………………………

……………………………………………………………………

9.4 Frequency of cleaning

……………………………………………………………………

……………………………………………………………………

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9.5 Type of disinfectant

…………………………………………………………………….

…………………………………………………………………….

9.6 Pest control procedure

…………………………………………………………………….

…………………………………………………………………….

10. Production parameter

10.1 Age at start (wk) …………………………………………………………

10.2 Age at the end (wk) ……………………………………………………….

10.3 Live weight at start (kg) ……………………………………….……….

10.4 Live weight at the end (kg) ……………………………………...….

10.5 Total feed used (kg) ………………………………………………...….

10.6 Average daily gain (ADG) …………………………………………….

10.7 Feed conversion rate…………………………………………….….…

10.8 Percentage of loss……………………………………………..………

11. Waste management

Biogas………………………………………………

Slurry………………………………………………

Nothing……………………………………………

12. Veterinary services

…………………………………………………………………….

…………………………………………………………………….

Number of visiting per month………………………………….

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Appendix B: Equipment, Materials, Media and Reagents

1. Lab Equipment and Materials

- Sterile 500, 1000 and 2000 ml Erlenmeyer flasks, sterile 250 and 500 ml beakers, and containers of capacity to accommodate samples
- Balance with a 2000 g-weights capacity and a sensitivity of 0.1 g
- Incubator, 37 and 42 C
- Laboratory refrigerator, - 20 C and -1 to 4+ C
- Water bath
- Sterile spoons for transferring faecal samples and media
- Sterile culture dishes, 15*100 mm, glass or plastic
- Sterile pipettes
- Inoculating needle and inoculating loop (10 micrometer)
- Culture tubes, 16*150 and 20*150 m
- Test or culture tube racks
- Vortex mixer
- Stomacher machine.
- Sterile scissors, scalpel, and forceps
- Bunsen burner
- Stomacher bags and plastic bags
- Appendop
- Autoclave

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2. Equipment and Material for Sample Collection

- Sterile cotton sock swabs
- Disposable hand gloves
- Stomacher bags and plastic bags
- Buffered peptone water (BPW)
- Sterile 1000 ml. Duran bottle
- Marker pens

- Alcohol, cotton, lighter
- Normal saline
- Disposal gloves, boots and lab coat
- Ice box with ice
- Snare

3. Media, Reagents and Chemicals

- Buffered Peptone Water (BPW)
- Nutrient agar (NA)
- Brilliant-green Phenol-red Lactose Sucrose Agar (BPLS)
- Xylose Lysine Tergitol 4 agar (XLT4)
- Muller Kaufmann Tetrathionate broth (MKTT)
- Rppaport-Vassiliadis broth (RV)
- Triple Sugar Iron Agar (TSI)
- Urea Agar
- Motility Indole Lysine Decarboxylation (MIL)
- Voges-roskauer Reaction (VPR)
- *Salmonella* polyvalent somatic (O) antiserum A- E
- *Salmonella* polyvalent somatic (O) antiserum F- 67
- *Salmonella* somatic (O) antiserum- *Salmonella* group B (O4, O5 , O27)
- *Salmonella* somatic (O) antiserum- *Salmonella* group C (O7, O8)
- *Salmonella* somatic (O) antiserum- *Salmonella* group D (O9, Vi)
- *Salmonella* somatic (O) antiserum - *Salmonella* group E (O3, O19)
- Anti- *Salmonella* flagella (H) e.g. e, f, g, h, i, k, l, m, p, q, r, s, t, u, v, w, x, z_4, z_{23}, z_6, z_{29}, z_{32}, 1, 2, 5, 6, 7

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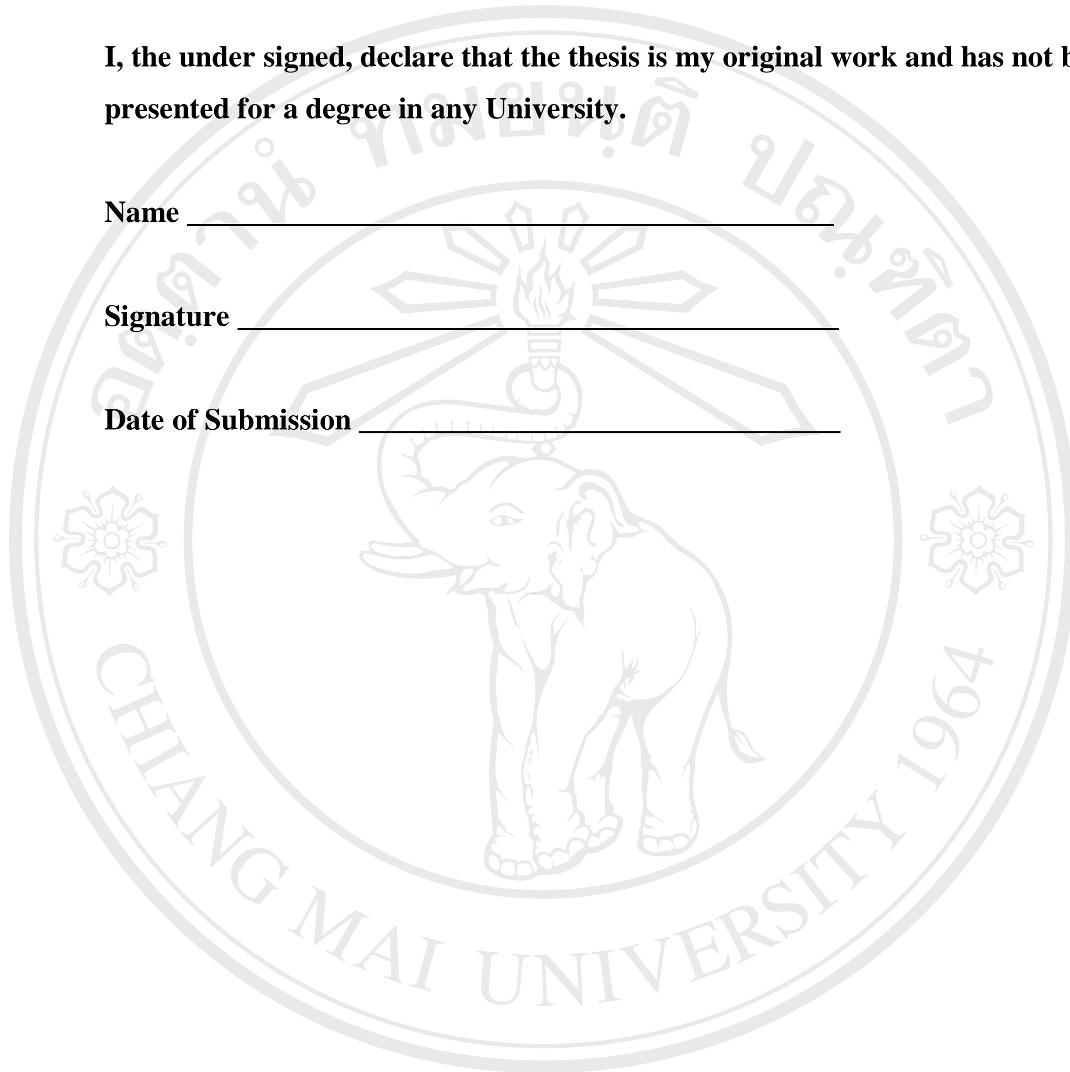
DECLARATION



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

Name ______________________________________

Signature __________________________________

Date of Submission ___________________________

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