MICROBIOLOGICAL QUALITY OF PIG CARCASS AT DORN DU SLAUGHTERHOUSE IN VIENTIANE MUNICIPALITY, LAO PDR

PHOUTH INTHAVONG

MASTER OF SCIENCE
IN VETERINARY PUBLIC HEALTH

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ABSTRACT

The purpose of this study was to determine and evaluate the microbiological quality of pig carcasses and to assess the hygienic status of "Dorn Du" slaughterhouse in Vientiane Capital of Lao People Democratic Republic (Lao PDR). Furthermore, associations between some potential risk factors for microbiological contamination were determined. Potential risk factors were gathered using a questionnaire survey at farm level and at the slaughterhouse level. The design of the study was a cross-sectional survey.

Between November 2004 and April 2005, 62 pig carcasses were randomly selected at the "Dorn Du" slaughterhouse. Two-pooled swab samples (Swab1 and Swab 2) and 25 g tissues of mesenteric lymph node from each carcass were collected. Swab samples were taken from 4 sites (from back, jowl (or cheek), hind limb medial (ham), and belly. Swab1 was taken immediately after dehairing and Swab2 was taken after splitting and washing the carcasses. The swab samples were enumerated for aerobic and *Enterobacteriaceae* bacteria. The lymph nodes were cultured for *Salmonella* only.

Swab1 had a mean aerobic bacterial count of 4.70 $\log_{10}$cfu/cm^2 and a range of 4.4 to 4.9 $\log_{10}$cfu/cm^2, whereas Swab2 had a mean aerobic bacterial count of 4.85 $\log_{10}$cfu/cm^2 and a range of 4.5 to 5.31 $\log_{10}$cfu/cm^2. These two means were significantly (p=0.0001) different. The means of *Enterobacteriaceae* counts were 2.81

log10cfu/cm^2 with a range of 2.1 to 3.3 log10cfu/cm^2 for Swab1, and 2.98 $\log_{10}$cfu/cm^2 with a range of 2.3 to 3.1 $\log_{10}$cfu/cm^2 for Swab2. These two means were also significantly (p=0.0001) different.

The proportion of *Salmonella* isolated from Swab1 was 46.8% and from Swab2 66.1%, and mesenteric lymph nodes 53.2%. Eight different *Salmonella* serotypes were identified. The most frequent (29,1 %) serotype was *Salmonella* Rissen, followed by *S.* Anatum (26.2%), *S.* Derby (19.4%), and *S.* Elisabethville (8,7%). The other serotypes identified were *S.* Amsterdam (7,8%), *S.* Typhimurium (3,9%), *S.* Agona (2,9%), and *S.* Enteritidis (1,9 %).

From the results of this study, it can be concluded that the pig carcasses were contaminated with high levels of aerobic bacteria and *Enterobacteriaceae*, as well as contaminated with pathogenic bacteria like *Salmonella.* Based on the findings of this study, improvements of hygienic practices in the slaughterhouse are recommended.

ชื่อเรื่องวิทยานิพนธ์	คุณภาพทางจุลชีววิทยาของซากสุกรที่โรงฆ่าสัตว์ดอนดู่ในเทศบาลนครเวียงจันทร์ ประเทศสาธารณรัฐประชาธิปไตยประชาชนลาว
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บทคัดย่อ

การศึกษานี้มีจุดประสงค์ที่จะตรวจและประเมินคุณภาพทางจุลชีววิทยาของซากสุกรและประเมินภาวะทางสุขาภิบาลของโรงฆ่าสัตว์ “ดอนดู่” ในกรุงเวียงจันทร์ สาธารณรัฐประชาธิปไตยประชาชนลาว นอกจากนี้ยังมุ่งหาความสัมพันธ์ระหว่างปัจจัยเสี่ยงกับการปนเปื้อนของจุลชีพ ปัจจัยที่อาจเป็นปัจจัยเสี่ยงจะรวบรวมโดยการสำรวจด้วยแบบสอบถามที่ระดับฟาร์มและที่โรงฆ่าสัตว์ การศึกษานี้ออกแบบเป็นการสำรวจแบบตัดขวาง ระหว่างเดือนพฤศจิกายน 2547 ถึงเดือนเมษายน 2548 ซากสุกร 62 ตัวอย่างถูกสุ่มเลือกที่โรงฆ่าสัตว์ “ดอนดู่” จะเก็บตัวอย่างพูลด์สวอป 2 ตัวอย่าง (สวอป 1 และสวอป 2) และเนื้อเยื่อปุ่มน้ำเหลือง 25 กรัม จากแต่ละซาก สวอปจะป้ายจาก 4 แห่ง (จากหลัง คางหรือแก้ม ขาหลังด้านในหรือแฮมและท้อง) สวอป 1 จะเก็บทันทีหลังจากการขูดขน และสวอป 2 หลังจากการผ่าซีกซากและล้างซาก ตัวอย่างสวอปจะทำการตรวจนับจำนวนแอโรบิคแบคทีเรีย และเอนเทอโรแบคทีเรียซีอี ชิ้นปุ่มน้ำเหลืองจะถูกนำไปเพาะหาเชื้อซัลโมเนลล่าเท่านั้น

สวอป 1 พบค่าเฉลี่ยจำนวนนับแบคทีเรียเท่ากับ 4.70 ล็อกสิบโคโลนีฟอร์มมิ่งยูนิตต่อตารางเซนติเมตร (log10cfu/cm2) ขณะที่สวอป 2 มีค่าเฉลี่ยแอโรบิคแบคทีเรียลเคานท์เท่ากับ 4.85 log10cfu/cm2 และอยู่ในช่วง 4.5 – 5.3 log10cfu/cm2 ค่าเฉลี่ยทั้งสองนี้แตกต่างกันอย่างมีนัยยะสำคัญทางสถิติ ค่าเฉลี่ยของเอนเทอร์โรแบคทีเรียซีอี่เคานท์เป็น 2.81 log10cfu/cm2 อยู่ในช่วง 2.1 ถึง 3.3 log10cfu/cm2 สำหรับสวอป 1 และ 2.98 log10cfu/cm2 ในช่วง 2.3 ถึง 3.1 log10cfu/cm2 สำหรับสวอป 2 ค่าเฉลี่ยทั้งสองแตกต่างกันอย่างมีนัยยะสำคัญทางสถิติเช่นกัน (p=0.0001)

สัดส่วนของซัลโมเนลลาแยกได้จากสวอป 1 เท่ากับ 46.8% และสวอป 2 เท่ากับ 66.1% และจากปุ่มน้ำเหลืองมีเซนเทอริคเท่ากับ 53.2% ซึ่งแยกซีโรไทป์ของซัลโมเนลลาได้ 8 ชนิด เรียงลำดับจากมากไปน้อยคือ Salmonella Rissen (29.1%), S. Anatum (26.2%), S. Derby (18.4%), S. Elisabethville (8.7%), S. Amsterdam (7.8%), S. Typhimurium (4.9%), S. Agona (2.9%), and S. Enteritidis (1.9 %)

จากการศึกษาสามารถสรุปได้ว่า ซากสุกรมีการปนเปื้อนในระดับสูงของแอโรบิคแบคทีเรียและเอนเทอโรแบคทีเรียซีอี่ และปนเปื้อนเชื้อก่อโรค เช่น ซัลโมเนลลา อาศัยผลการศึกษานี้การปรับปรุงสุขาภิบาลของโรงฆ่าเป็นสิ่งที่พึงกระทำ

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ABBREVIATION AND SYMBOLS

APC	Aerobic Plate Count
EBC	*Enteribacteriaceae* count
°C	Celsius centigrade
CDC	Center of Disease Control
CFU	Colony forming unit
cm	centimeter
cm^2	square centimeter
et al.	et alii
DLF	Department of Livestock and Fisheries
FSIS	Food Safety and Inspection Service
g	gram
GMP	Good manufacturing practice
h	hour
HACCP	Hazard Analysis and Critical Control Point
ISO	International Organization for Standardization
Lao PDR	Lao People's Democratic Republic
log	logarithm
max.	maximum
ml	millilitre
MLN	mesenteric lymph node
NAHC	National Animal Health Center
neg.	Negative
pos.	Positive
S.	*Salmonella*
TVCs	Total Viable Counts
spp.	Species
WHO	World Health Organization

1. INTRODUCTION AND OBJECTIVES

Meat is an important element in most people's diets and its safety depends upon the application of effective control measures at all stages of the production chain, literally from 'farm to fork'. In order to assure meat quality and safety, there has to be co-operation from all parties involved in the food production chain. These are farmers, feed manufacturers, livestock market operators, livestock haulers, abattoir operators and those working in food processing plants. Regulatory authorities that conduct meat inspection and those who work in food-borne disease surveillance and disease control play an important role in the national surveillance system.

There are three categories of food safety hazards: chemical, physical and microbiological hazards. The last one causes the highest incidence of food-borne illness (WHO, 2005). Microbiological contamination can originate from living animals or plants or from cross-contamination at pre-harvest and post-harvest levels. Many microorganisms are ubiquitous in nature; consequently pathogenic microorganisms can enter the food chain in many stages. Therefore, the entire supply chain has to be involved in controlling microbiological risks (Berends *et al.*, 1998). Microorganisms thrive best in high protein, non-acid environments such as meat, which makes meat a serious risk for food-borne illness. In general, the bacteria *Salmonella* and *Campylobacter* cause most food-borne illness (CDC, 2003).

Good hygienic practices (GMP) can prevent and control zoonoses and food-borne diseases as well as pollution of the environment. Food hygiene also contributes significantly to the improvement of food quality as well as to reduction in food losses, the elimination of adulteration and fraud; the prevention of dumping contaminated or substandard food. Proper hygiene practices promote development of the food industry and improvement of food making systems.

Sanitation and cleaning are an integral part of slaughtering and the handling of meat and should already be taken into consideration at the planning and construction stage of slaughter facilities. Well-planned, well-executed controlled cleaning and

sanitation programs for rooms, machines and equipment are important elements in achieving hygienic standard. Cleaning and sanitation alone, however, will not assure a hygienic standard in production. Processing hygiene as well as personal hygiene is also important factors.

Many human enteric diseases are associated with the consumption of food of animal origin, caused by organisms present in the guts of healthy animals (WHO, 2005). These organisms are not detected by routine meat inspection. Organisms, initially present in low numbers, proliferate when the food product is incorrectly handled during processing, distribution or preparation. Prevention of food-borne illness therefore depends on control measures at all points in the food chain, from live animals through processing to consumption. Emphasizing control only at the kitchen level will therefore never succeed. This also reflects the major role of veterinary food hygienists in protecting consumer safety.

Regulatory authorities have often been forced to apply the classical rules of food inspection, because there is insufficient information to support changes to a more science-based program. To be more reliable, regulatory authorities have an inevitable task in designing and operating modern food inspection programs, which are well-defined and proven to be scientifically based.

The classical zoonoses, such as tuberculosis, were eradicated through efficient inspection of slaughter animals and meat inspection. As earlier mentioned, at and after slaughter *Salmonella, Campylobacter,* enterohaemorrhagic *Escherichia coli* and *Yersinia* can be released from clinically healthy animals, thus contaminating carcasses and meat. *Salmonellae, E. coli* and *Listeria* can also survive in the abattoir environment (Rostagno *et al.*, 2003; Borch *et al.*,1996).

In order to overcome the problems of food-borne diseases, the production line must be kept in good hygienic fashion. Sampling for bacteriological analysis to detect the contamination rate in different stages of the food chain is the best way to monitor and to keep the hygienic status of slaughterhouses and food processing plants. Also

visual inspection of the hygienic condition of live animals, the surface of working materials, workers' clothes, meat inspectors and meat inspection tools, as well as consideration of additional aspects that can bring cross contamination are needed.

In HACCP systems, the indicator organisms that suggest quality in process control, validation and verification are the total aerobic count, coliforms, *Escherichia*, *Aeromonas* and *Listeria*; the latter two are more pathogenic and hygienic indicators. The total aerobic count is an indicator for the general microbiological condition of the product and equipment (Gill, 2000). *Enterobacteriaceae* counts are accepted as an indicator for fecal contamination (McEvoy *et al.*, 2004; Nel *et al.*, 2004; Zweifel *et al.*, 2005; Byrne *et al.*, 2005).

In Lao PDR, The Department of Livestock and Fisheries (DLF) is responsible for animal heath and production including safety aspects of animal products for human consumption. DLF has developed a national plan for the improvement of the quality of products of animal origin to meet international standards. Local veterinary authorities supervise the slaughterhouses and slaughter facilities. Permanent staffs work under a qualified manager. The meat inspector is independent of the manager who is responsible to the local or governmental veterinary or livestock authorities.

Until now there has been, however, no report on hygienic studies of slaughterhouses in Lao PDR. The DLF as the organization responsible for the quality and safety of animal products needs science-based knowledge in order to come up with proper solutions. The findings of this study will serve as initial data for The Department of Livestock and Fisheries and for carrying out further research and development.

The objective of the study

The purpose of this study was to determine the microbiological contamination of pig carcasses in order to evaluate the microbiological quality of pig carcasses and assess the hygienic status of the selected slaughterhouse. Isolation of most important

microorganisms will indicate the microbiological quality and safety of such products. For this purpose, according to the working document “The development of a risk based on a meat inspection system, EN SANCO/4403/2000”, it is recommended that the routine analysis should be based on Total Viable Count and *Enterobacteriaceae.* In this study the indicator bacteria would be Total Viable Count (Aerobic Plate Counts), *Enterobacteriaceae* counts, and more specifically the *Salmonella* isolation and identification of the samples. The specific objectives of this study are:

- To determine and compare the contamination level of Total Bacteria Counts and *Enterobacteriaceae* counts in pig carcasses between pre- and post-evisceration;
- To estimate the prevalence of *Salmonella* isolated from pig carcasses and lymph nodes, and to assess the association between the prevalence and groups of pigs with regard to herd size, transport time, source of water and source of pigs, together with information on farm management practices.
- To discuss the critical steps in the slaughtering procedure in order to formulate necessary actions for improvement.

2. LITERATURE REVIEW

2.1 Microorganisms in meat

2.1.1 Meat spoilage and pathogenic microorganisms

Many factors affect the storage life of fresh meat and the keeping quality of meat and poultry products. It can be predicted by monitoring for spoilage microorganisms (Gill and Bryant, 1992). Also temperature plays a vital role in meat spoilage (Narashimha Rao *et al*., 1998) and is considered most important. Based on temperature requirements, microorganisms are classified as psychotrophs, mesophiles and thermophiles (Table 2.1).

Table 2.1: Cardinal temperatures for microorganisms (Narashimha Rao, 1998)

Group	Temperature (ºC)		
	Minimum	Optimum	Maximum
Thermophiles	40 – 45	55 – 65	60 – 90
Mesophiles	5 – 10	30 – 45	35 – 47
Psychrotrophs	-5 – +5	25 – 30	30 – 45
Psychrophiles	-5 – +5	12 – 15	15 – 20

Meat is recognized as a source of several bacterial pathogens that cause food poisoning in humans although the source of infection is not determined in the majority of outbreaks of food-borne infectious disease investigated (Hinton, 2000). There are several reasons for this, an important one being that the food responsible for the problem has usually been consumed completely, or has been disposed of before microbiological investigations are instituted.

There are three important factors determining the microbiological quality of the meat sold by the butchers: the condition of the animal slaughter, the spread of

contamination during slaughter and processing, and the temperature, time and other conditions of storage and distribution (Nortje *et al.*, 1990).

Currently the most important pathogens associated with raw meat are *Campylobacter* spp., *Clostridium perfringens*, and pathogenic serotypes of *Escherichia coli,* for example *E. coli* O157:H7, *Salmonella* and certain serotypes of *Yersinia enterocolitica*. *Listeria monocytogenes* is also a common contaminant of meat. The most important microorganisms associated with the meat of different animals are shown in Table 2.2 (Borch *et al.*, 1996; Qiongzhen *et al.*, 2004; Nel *et al.*, 2004). Many of these bacteria are confined to the intestinal tract of the animal, while others occur, for example, in the nasopharynx or on the skin. All of them may contaminate carcasses during dressing and further handling (Yashoda *et al*., 2000).

Usually, the organisms are capable of prolonged survival on meat surfaces, although *C. jejuni* is sensitive to drying. With the exception of spores of clostridia and aerobic bacilli, food-borne pathogenic bacteria are heat sensitive and should be killed by proper cooking, especially when present as surface contaminants.

In the process of pig slaughter a wide range of potential pathogens, such as *Salmonella* (Currier *et al.*, 1986; Borch *et al.*, 1996; Berends *et al.*, 1996), and *Listeria monocytogenes* (Reij and Aantrekker, 2004; Borch *et al*., 1996; Nel, *et al*., 2004) can contaminate the surface of carcasses. There are many opportunities for carcass contamination to occur during slaughter. The main emphasis of control is applied at the end of evisceration in the form of washing. Nevertheless, the initial scalding and singeing steps that are performed to de-hair carcasses have also been demonstrated to remove a substantial proportion of the carcass surface microflora (Borch *et al.*, 1996; Warriner, *et al.*, 2002); and can be considered to act as barriers to minimize the transfer of pathogens through the line. However, for more effective control of pathogen spread there is a need to develop a hazard analysis critical control point scheme within the pig slaughter process (Gill and John, 1997; Goodfellow, 1995).

Many reports have been published that highlight the potential for carcass contamination during dehairing and evisceration operations (Gill and Bryant 1993; Pearce *et al.*, 2004; Swanenburg *et al.*, 2001a). Such studies have been based on enumerating total aerobic and indicator organism counts from samples recovered from carcasses (Nel *et al.*, 2004; Palumbo *et al.*, 1999). However, although such methods permit the gross changes in carcass microflora to be determined, this does not provide sufficient data to elucidate the origins of pathogens. In addition, as pathogens typically occur in low numbers, contamination of carcasses is not necessarily reflected by an increase in bacterial counts.

Table 2.2: Pathogens of primary concern in raw meat and poultry (Pearson and Dutson, (1995).

Meat	Pathogen
Poultry:	*Salmonella*
	Campylobacter jejuni
	L. monocytogenes
	C. perfringens
	C. botulinum
Pork:	*Salmonella*
	Yersinia enterocololitica
	Campylobacter jejuni
	L. monocytogenes
	C. perfringens
	C. botulinum Trichenella spiralis
	Toxoplasma gondii
Beef:	*Salmonella*
	Escherichia coli O157:H7
	L. monocytogenes
	C. perfringens
	C. botulinum

2.1.2 *Enterobacteriaceae*

Members of the genera belonging to the *Enterobacteriaceae* family have been placed among the most pathogenic and most often encountered organisms in clinical and food microbiology (Quinn *et al.*, 1998; Miliotis and Bier, 2003). These gram-negative straight rods are usually associated with intestinal infections, but can be found in almost all natural habitats. They are the causative agents of such diseases as dysentery, typhoid, and food poisoning.

All members of this family are oxidase negative, glucose fermenters and nitrate reducers. In most cases, the pathogenicity of a particular enteric bacterium can be determined by its ability to metabolize lactose. Non-utilizers are usually pathogenic while the lactose utilizers are not (Holt *et al*., 2000).

They are distributed worldwide, they found in soil, water, fruits, vegetables, grains, flowering plants and trees, and animals from worms and insects to humans (Holt *et al*., 2000). There is substantial heterogeneity in the ecology, host range, and pathogenic potential to humans and animals, insects, and plants. A number of species cause diarrheic diseases including typhoid fever and bacillary dysentery. Many species not normally associated with diarrheic diseases are often referred to as opportunistic pathogens (Holt *et al*., 2000). Most of these, as well as the species causing diarrheic disease, can cause a variety of extra-intestinal infections including meningitis, bacteremia in the urinary and respiratory tracts, as well as wound infection.

Enterobacteriaceae are often used as hygiene indicators of foods of animal origin (Anon., 2001; Crowley *et al.*, 2005; Warriner *et al.*, 2002; Nel *et al.*, 2004; Zweifel *et al.*, 2005). Their presence on processed food may give a better indication than coliforms of inadequate treatment or post-process contamination from the environment, and may help to indicate the extent of fecal contamination (Anon., 2001). However, the greatest application of *Enterobacteriaceae* and other indicator

organisms is the assessment of the overall quality of a food and the hygiene conditions present during the food processing.

Various sampling methods have been utilized to determine the number of bacteria on the surface of food processing equipment and red meat animal carcasses (Palumbo *et al.*, 1999). The principal sampling methods are swabbing and excision; in addition rinse techniques, contact (Rodac) plates and different tape methods have been used (Pearce, *et al.*, 2005). Each has its advantages and disadvantages. Because they are easier to use, require the least amount of specialized material and provide data, which are generally more reproducible, swabbing and excision have found the widest acceptance and use.

A possible procedure for objectively assessing the hygienic performance of the carcass dressing process with respect to both safety and storage stability has been proposed. The procedure involves the collection of swab samples from randomly selected sites on randomly selected carcasses at appropriate points at the end of a process (Gill *et al.*, 1996).

At the abattoir, *Enterobacteriaceae* and pseudomonads were the biggest contributors to psychotrophic count, at the wholesalers' the *Enterobateriaceae* and micrococci counts, and at the retailers' the micrococci and pseudomonads respectively (Nortje *et al.*, 1990). This indicates the *Enterobateriaceae* might be common psychotrophs in the meat production chain, originating from the abattoir and from the environments.

The presence of *Enterobacteriaceae* in meat or meat products indicates possible fecal contamination (Pearce, *et al.*, 2005). The steps in pig slaughter that lead to an increased *Enterobacteriaceae* count are dehairing, polishing, and evisceration. Scalding and singeing are steps, which result in considerable decrease in the numbers of microorganisms on carcass surfaces. After singeing, the surface is probably almost free of *Enterobacteriaceae,* and evisceration leads to the recontamination of carcasses

with *Enterobacteriaceae* (Morgan *et al*., 1987 and Berends *et al*., 1996), since after singeing there are no steps that lead to a decrease in numbers of bacteria.

Aerobic and *Enterobacteriaceae* counts are used as indicator organisms in meat and food products. A high APC on carcasses usually indicates the degree of care taken during slaughter and unsuitable time or temperature conditions during the production and storage of the meat. It can also indicate heavy post-slaughter and post-processing contamination. The counts of *Enterobacteriaceae* and *E. coli* have been used as an indicator of direct contamination of carcasses with fecal material. The detection of such microorganisms on carcasses could also indicate indirect contamination from the intestinal tract during slaughter (McEvoy *et al.*, 2004; Nel *et al.*, 2004; Zweifel *et al.*, 2005; Byrne *et al.*, 2005). In the European Union (Anon. 2001) it is recommended that the routine analysis should be based on Total Viable Count and *Enterobacteriaceae* (Table 2.3)

Table 2.3: Daily log mean value for bacterial performance criteria for cattle, sheep, goats, horses and pigs according to the Commission Decision 2001/471/EU.

	Acceptable Range (m)		Marginal Range (>m but ≤ M	Unacceptable Range (M)
	cattle/sheep/ goats/horses	pigs	cattle/pigs/sheep/ goats/horses	cattle/pigs/sheep/ goats/horses
Total viable counts (TVC)	< 3.5 log	< 4.0 log	3.5 log (pig: 4,0 log) - 5.0 log	>5.0 log
Enterobacteriaceae	< 1.5 log	< 2.0 log	1.5 log (pig: 2,0 log) – 2.5 log (pig:3.0 log)	> 2.5 log (pig: > 3.0 log)

2.1.3 *Salmonella* spp.

Taxonomy

The genus *Salmonella*, family *Enterobacteriaceae,* is comprised of anaerobic, facultative anaerobic, catalase-positive gram-negative rod-shaped bacteria and contains two species: *Salmonella enterica* and *Salmonella bongori*, based on the phenotypic criteria (D' Aoust *et al.*, 2001). As shown in Table 2.4, the species *S. enterica* is divided into six subspecies: subspecies *enterica* (I), subspecies *salamae* (II), subspecies *arizonae* (IIIa), subspecies *diarizonae* (IIIb), subspecies *houtenae* (IV), and subspecies *indica* (VI). The actual number of serovars in all *Salmonella* species and subspecies is 2501 (Popoff, 2004). Most isolates of *Salmonella* from warm-blooded animals belong to the subspecies *enterica* (I). The other subspecies are found in cold-blooded animals and in the environment.

The nomenclature for the genus *Salmonella* has evolved from the initial one serotype-one species concept proposed by Kaufmann (D' Aoust *et al.*, 2001). Serotype identification, delivered from agglutination reactions with specific antisera, is based upon the organism's component of somatic (O antigen), capsular, and flagella (H antigen) antigens. The **O antigens** are the lipopolysaccharides (LPS) of the outer membrane, similar to the O antigens of other *Enterobacteriaceae*. The **H antigens** are the proteins that make up the peritrichous flagella of the bacteria; they can be expressed in one of two forms (termed phases) (D' Aoust *et al.*, 2001).

Phase1 H antigen is specific and associated with the immunological identity of that serovar. However, *Salmonella* strains can alter flagella antigens to phase2 (containing a different antigenic subunit protein), which is shared by many serovars. Certain *Salmonella* express a surface-bound polysccharide capsular antigen, which typically blankets the O antigen and blocks O-agglutination; however, the capsular can be selectively removed by heat treatment prior to O-agglutination assay. The virulence (Vi) capsular antigen occurs in *Salmonella* serovars Typhi, Paratyphi C and Dublin (D' Aoust *et al*, 2001) upon primary isolation.

Table 2.4: *Salmonella* species, subspecies, numbers of serotypes in each subspecies, and their usual habitats (Popoff *et al.*, 2004).

Salmonella species and subspecies	No. of serotypes	Usual habitat Within subspecies
S. enterica subsp. *enterica* (I)	1,478	Warm-blooded animals
S. enterica subsp. *salamae* (II)	498	Cold-blooded animals and the environment
S. enterica subsp. *arizonae* (IIIa)	94	Cold-blooded animals and the environment
S. enterica subsp. *diarizonae* (IIIb	327	Cold-blooded animals and the environment
S. enterica subsp. *houtenae* (IV)	71	Cold-blooded animals and the environment
S. enterica subsp. *indica* (VI)	12	Cold-blooded animals and the environment
S. bongori (V)	21	Cold-blooded animals And the environment
Total	2,501	

Serotype names designated by antigenic formulae include the following: (i) subspecies designation (subspecies I through VI), (ii) O (somatic) antigens followed by a colon, (iii) H (flagellar) antigens (phase 1) followed by a colon, and (iv) H antigens (phase 2, if present) (for example, *Salmonella* serotype IV 45:g,z51:2. For formulae of serotypes in S. bongori, V is still used for uniformity (for example, S. V 61:z35:2).

The name usually refers to the geographic location where the serotype was first isolated. For named serotypes, to emphasize that they are not separate species, the serotype name is not italicized and the first letter is capitalized (Table 2.5). At the first citation of a serotype the genus name is given followed by the word “serotype” or

the abbreviation “ser.” and then the serotype name (for example, *Salmonella* serotype or ser. Typhimurium). Subsequently, the name may be written with the genus followed directly by the serotype name (for example, *Salmonella* Typhimurium or *S.* Typhimurium (Popoff *et al.*, 2000; Popoff and Le Minor, 1997). Both versions of the serotype name are listed as key words in manuscripts to facilitate the search and retrieval of information on *Salmonella* serotypes from electronic databases.

Table 2.5: *Salmonella* nomenclature in use in literatures.

Taxonomia position	Nomenclature
Genus (italics)	*Salmonella*
Species (italics)	• *enterica*, which includes subspecies I, II, IIIIa, IIIb, IV and V • *bongori* (formerly subspecies V)
Serotype (capitalized, not italicized)	• The first time a serotype is mentioned in the text; the name should be preceded by the word “*serotype*” or “ser.” • Serotypes are named in subspecies I and designed by antigenic formulae in subspecies II to IV, and VI and S. bongori • Member of subspecies II, IV and VI and *S. bongori* retain their names if named before 1966

Table 2.6: Examples of antigenic structure formulae for some common Salmonellae, modified from Krieg and Holt (1984).

Serovars	Somatic	Antigens		Combination
	(O) Antigens	Phase 1	Phase 2	
	Group 02 (A)			
S. Paratyphi *A*	1,2,12	A	[1,5]	1,2,12:a:1,5
S. Nitra	2,12	g,m	-	2,12:g,m:-
	Group 04 (B)			
S. Kisangani	1,4,[5],12	A	1,2	1,4,5,12:a:1,2
S. Canada	4,12	B	1,6	4,6:b:1,6
S. Derby	1,4,12	f,g	[1,2]	1,4,12:f,g:1,2
S. Agona	1,4,[5],12	f,g,s	-	1,4,5,12:g,f,s:-
	Group 06,7 (C_1)			
S. Paratyphi C	6,7[Vi]	C	1,5	6,7:c:1,5
S. III arizonae	6,7	-	1,6	6,7:-:1,6
	Group 09,12(D_1)			
S. Endai	1,9,12	A	1,5	1,9,12:a:1,5
S. Typhi	9,12[Vi]	D	-	9,12,Vi:d:-
S. Enteritidis	1,9,12	g.m	[1,7]	1,9,12:g,m:1,7
	Group 03,10 (E_1)			
S. Aminatu	3,10	A	1,2	3,10:a:1,2
S. Amsterdam	3,10	g,m,s	-	3,10:g,m,s:-
	Group O67			
S. Crossness	67	R	1,2	67:r: 12

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

Salmonellosis in pigs

Salmonellosis is an important cause of human gastroenteritis in western countries (Danilo *et al*., 2000). Pork contaminated with *Salmonella* is recognized as one of the causes of human salmonellosis. Pigs are an important reservoir of *Salmonella* for humans. Infection of man follows either through direct contact or more frequently concludes from pork and pork products (Feddorka-Cray *et al.*, 2000). Pigs can become infected with *Salmonella* at the breeding and/or fattening farm (van der Wolf *et al.*, 1999 and 2001). However, from the moment the pigs leave the farm, there are also many opportunities to become infected or contaminated with *Salmonella* during transport, lairage or slaughter (Warriner *et al.*, 2002; Botteldoorn *et al.*, 2003; Søren *et al.*, 2003). This implies that control measures taken on the farm in order to decrease *Salmonella* prevalence should be combined with measures to prevent pigs and pork from contamination after the pigs have left the farm.

Infected pigs remain healthy carriers in most of the cases and as a consequence are of great importance to public health. *Salmonella* infections in swine are of concern for two reasons. The first is the clinical disease in pigs (salmonellosis), and the second is that pigs are susceptible to infection with a broad range of *Salmonella* serotypes constituting a potential source of human exposures and illness (Schwartz, 1998).

Recent investigations have shown that *Salmonella* could be isolated from 23% of finishing pig herds in the southern part of the Netherlands (Van der Wolf *et al.*, 1999), and from 26% of rectal samples of slaughtered pigs (Swanenburg *et al.*, 2001a,b). In Europe and the USA, the predominant not species-adapted *Salmonella* serovars found in pigs are *S.* Typhimurium and *S.* Derby. In Germany, the most common serovar following *S.* Typhimurium in 1961–1965 was *S.* Dublin and in 1998 it was *S*. Agona, while in Denmark it was *S.* Infantis (Feddorka-Cray *et al.*, 2000). In England in 1997, of the 338 *Salmonella* incidents reported in pigs, 62% were caused by *S.* Typhimurium, and 12% by *S.* Derby. In Denmark, 6.2% of fecal samples were found positive, usually with one phage type predominating from each farm source

(Baggsen *et al.*, 1996). From the isolated species-adapted *Salmonella* serovars *S.* Choleraesuis was the most common strain recovered.

Salmonella in pigs at slaughter

Pigs can become infected with *Salmonella* during transport and lairage due to stress, mingling with salmonellae excreting pigs, and contact with a *Salmonella*-contaminated environment, if the truck/lairage was not cleaned and disinfected well (Berends *et al.*, 1996; Morgan *et al.*, 1987; Isaacson *et al.*, 1999; Swanenburg *et. al*., 2001a). It has been shown that the proportion of pigs in a herd that excrete *Salmonella* increased after transport (Isaacson. *et al.*, 1999). Fedorka-Cray *et al.* (1995) showed that *Salmonella* could be isolated from mesenterial lymph nodes and caecal and rectal contents already 3 hours after infection, which makes it possible for pigs to pick up *Salmonella* during transport or in lairage, and start excreting before they are slaughtered. In this way, they can infect other pigs, as well as the environment of the truck and lairage. Carcasses can become contaminated with *Salmonella* during the slaughter procedure by contaminated slaughter equipment (Oosterom *et al.*, 1985; Gill and Bryant, 1993; Sammarco *et al.*, 1997). A study by Käsbohrer *et al*. (2000) from seven abattoirs located in different states of Germany reported that *Salmonella* were isolated from 3.7% of the fecal samples, 3.3% of the lymph nodes and 4.7% of the surface swabs.

Salmonella prevalence in slaughter pigs has been investigated in many parts of the world. *Salmonella* could be isolated from portal lymph nodes, mesenterial lymph nodes or rectal contents. Swanenburg *et al.*, (2001a,b) reported that *Salmonella* could be isolated from either rectal contents (26.5%), tonsils 19.6%, and 9.3% in mesenterial lymph nodes, livers and tongues of slaughtered pigs. Oosterom *et al.* (1985) found *Salmonella* in the intestinal tract of 21% of slaughtered pigs and on 13% of carcasses after evisceration.

Results from other countries showed prevalences of *Salmonella* in samples of slaughtered pigs that sometimes differed from each other. Finlay *et al.* (1986) isolated

Salmonella from 2% of muscle samples and 3.7% of fecal samples of slaughtered pigs in Canada. Currier *et al.* (1986) isolated *Salmonella* from 13.5% of fecal samples in the USA, whereas Letellier *et al.* (1999) isolated *Salmonella* from 5.2% of fecal samples in Canada. Morse and Hird (1984), Lammerding *et al.* (1988), Keteran *et al.* (1982) and Lazaro *et al.* (1997) isolated *Salmonella* from 4.3% USA, 14.2% Canada, 31.3% USA and 40% (Brazil) of mesenteric lymph nodes, respectively. Lazaro *et al.* (1997) isolated *Salmonella* from 77.5% of tonsils of slaughtered pigs. Unfortunately, these results are hard to compare, because *Salmonella* isolation procedures and kinds of samples collected differed among these different studies.

The number of *Salmonella* organisms on the surfaces of carcasses of pigs may be reduced as a result of careful slaughter procedures, such as scalding individually, careful removal of intestines (Oosterom and Notermans, 1983; Berends *et al.*, 1997), a plastic bag over the rectum (Nesbakken *et al.*, 1994; Sørensen *et al.*, 1999), and a decontamination step after slaughter (Snijders et al, 1985; Snijders, 1988). Separate slaughter of pigs free from a certain pathogen, to avoid introduction of certain bacterial zoonoses into the slaughter-line and to avoid cross-contamination between herds during slaughter (Swanenburg *et al.*, 2001a,b). Maffia *et al.* (1989) and Sammarco *et al.* (1997) both investigated the slaughterhouse environment and concluded that *Salmonella* can be present on floors and working tables. Oosterom and Notermans (1983) showed that fewer pigs were contaminated with *Salmonella* after slaughter in The Netherlands if they were singed individually and the guts were removed carefully.

2.2 Possible sources of microorganisms in meat

Muscles of healthy animals are free from microorganisms because of defensive mechanisms associated with skin and mucous membranes, hair and cilia, gastric juice, the intestine and urine. Inflammatory processes and humoral antibodies play a role (Narasimha Rao *et al*. 1998). All these defence mechanisms present barriers to the entry of microorganisms into the muscles of live animals. Microorganisms inevitably gain access to meat at slaughter when the defences break down, and also during processing. So, minimization of microbial contamination is essential in meat handling systems in order to retard meat spoilage as well as to prevent health hazards that may arise from meat consumption. Therefore there is a need to know how microorganisms enter meat and to determine critical control points of contamination.

Microorganisms contaminating meat are derived from the environment (soil and water), gastrointestinal contents, hide, skin, or feathers of animals, processing equipment and personnel. A survey performed by the WHO (1995) in Europe indicated that 25% of the food-borne outbreaks could be traced back to recontamination. The most important factors contributing to the presence of pathogens in processing food were insufficient hygiene (1.6%), cross-contamination (3.6%), processing and storage in inadequate rooms (4.25%), contaminated equipment (5.7%), and contamination by personnel (9.2%).

Sources of microbial contamination in fresh meat have been documented (Gill and Lander, 2004; Lo Fo Wong *et al.*, 2002; Nel *et al.*, 2004; Gill *et al.*, 1998; Mossel *et al.*, 1998). Hides and skin, hooves, fleece and hair of live animals, gut microflora, the sticking-knife, scalding tank, equipment, instruments and tools (overhead rail, gambrels, stainless steel platforms, s-hooks, trays, tables, knives, axes, saw blades), chopping blocks (wooden), floor, walls, air, water, cloths, hands and boots have been identified as sources of microbial contamination of carcasses and meat cuts (Warriner *et al.*, 2002; Botteldoorn *et al.*, 2003). Bacteria can contaminate meat during the following operations: sticking, skinning, scalding, de-hairing, evisceration, and splitting and quartering.

During post-mortem meat inspection, palpation and incision of lymph nodes, infected tissues or tissues with abnormalities can give rise to cross contamination. Incision should be avoided where possible, and palpation of organs should be as minimal as possible (Borch *et al.*, 1996). Pathogenic bacteria that will subsequently be transferred to the carcass are likely on contaminated knives, cutters and other tools/equipments used.

3. MATERIAL AND METHODS

3.1. Study design

This study was a combined cross-sectional study design and laboratory work with an overall aim of determining the microbiological quality of pig carcasses at a slaughterhouse. Associations with some risk factors for contamination were determined using a questionnaire survey on farm management practices (Annex B) and sanitary control measures in the slaughterhouse (Annex C).

3.2. Location of study

Samples were taken from the “Dorn Du” pig slaughterhouse, microbiological work was carried out in the Bacteriology Laboratory of the National Animal Health Center (NAHC), Department of Livestock and Fisheries (DLF) in Vientiane, Lao PDR and in the Faculty of Veterinary Medicine Chiang Mai University, Thailand.

The “Dorn Du” slaughterhouse is the biggest slaughterhouse in Lao PDR at the moment. It was established in 1979. The slaughterhouse comprises two slaughter lines - one for cattle and another one for pigs. Both of them are located in the same building. The slaughtering process is carried out mostly at night. About 80-100 cattle and buffaloes and 60-80 pigs are slaughtered per day.

Slaughtering of pigs in particular includes stunning, bleeding, scalding and de-hairing, evisceration and splitting. Inspection is performed both ante-mortem on live animals and post-mortem on carcasses and visceral organs. Without chilling, the carcasses are distributed to the market immediately after slaughtering procedures have been completed.

3.3. Study population

Target animals were all pigs slaughtered at the “Dorn Du” slaughterhouse. The particular pigs were from different farms located in the Vientiane Municipal Region and other provinces. Transportation of the animals from farms to slaughterhouse took about 1-5 hrs.

3.4. Sampling strategy

3.4.1. Sample size estimation

The sample size was calculated using the computer program “Winepiscope 2.0” by the formula given as below:

$$n = \frac{1.96^2\, p(1-p)}{d^2}$$

Because the actual *Salmonella* prevalence in Laos was unknown, for sample calculation the prevalence (p) of *Salmonella* was estimated p=50%, the accepted absolute error or precision being 5% (d=0.05) and level of confidence at 95%(t=1.96), therefore:

$$n = \frac{1.96^2 * 0.5(1-0.5)}{(0.05)^2} = 385$$

In principle, the sample size should be 385 pigs, but in this slaughterhouse only 60 -80 (average 70) pigs are slaughtered per day, and according to this, the sample size is calculated by the formula:

$$n_{adj.} = \frac{N*n}{N+n} = \frac{385*70}{385+70} = 60$$

Where: n_{adj} = adjusted sample size.

In this study 62 carcasses were sampled.

3.4.2. Type of samples and laboratory analysis

From each pig three samples were collected and microbiological analysis as shown in Table 3.1 was performed.

Table 3.1: Type of sample and laboratory analysis

Type of sample	Laboratory analysis
Carcass swab 1*	Aerobic plate count, *Enterobacteriaceae* count and *Salmonella* isolation
Mesenteric lymph nodes	*Salmonella* isolation
Carcass swab 2**	Aerobic plate count, *Enterobacteriaceae* count and *Salmonella* isolation

* Collected after de-hairing of the pig

** Collected at the end of the slaughtering process.

3.4.3. Sampling method

The random sampling method was performed. Pig carcasses were randomly selected during the slaughtering process. Because of the laboratory capacity to analyze only a limited number of samples per day, the number of samples per day was not more than 20 samples. According to this, up to seven carcasses per sampling day were sampled.

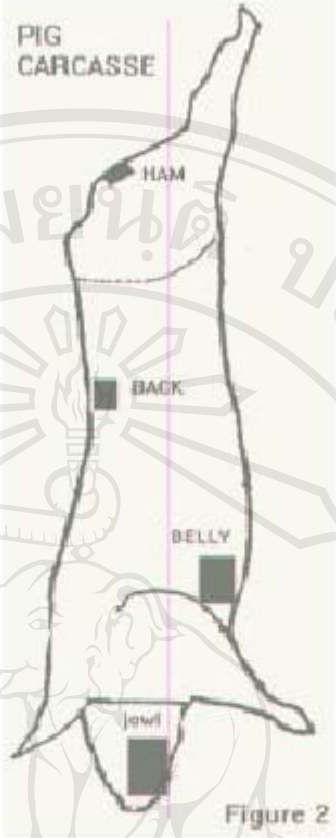

Figure 3.1: Location for swab sampling
Source: Commission decision 2001/471/EC.

3.4.4. Samplecollection

Swab samples

The cotton wool swabs were used, as described by Van den Elzen and Snijders (1993), Palumbo *et al.* (1999) and Byrne *et al.* (2005), and which were validated for *Salmonella* isolation by Swanenburg (2000). Swabs were moistened with buffered peptone water (BPW) and rubbed initially vertically, then horizontally, then diagonally across the entire surface delineated by a sterile template. Each carcass was swabbed 4 sites (from the back, jowl (or cheek), hind limb medial (ham), and belly (Figure 3.1) with one swab per sampling site. The sampling area for swabs was 10 cm by10 cm, which covered 100 $cm^{2;}$ total area swabbed was 400 cm^2 per carcass.

Mesenteric lymph nodes

Mesenteric lymph nodes were collected immediately after evisceration of the pig in a separate room beside the slaughter line by excision using sterile scissors and forceps. About 25 g of mesenteric lymph node were cut out and kept in a plastic bag.

The samples were stored in a cool box and transported to the laboratory, where after the microbiological procedures were performed the same day.

3.5. Microbiological analysis

3.5.1. Preparation of first macerate

Swab samples were homogenized in plastic bags (Stomacher bag) for at least two minutes in 100 ml of buffered peptone water at about 250 cycles of a peristaltic Stomacher. Thereafter serials of ten-fold dilution for Aerobic plate counts and *Enterobacteriaceae* counts were prepared.

Mesenteric lymph nodes were put into boiling water for 3 seconds to eliminate superficial contamination (Swanenburg *et al.* (2001), and thereafter-cut into small pieces with sterile materials. Thereafter, 25g were transferred into a stomacher bag, and 225 ml of BPW were added and homogenized at about 250 cycles per minute for two minutes.

3.5.2. Aerobic plate count

In general the aerobic plate count is designed to detect an estimate of the total number of aerobic organisms in a particular sample. There are two methods available for Aerobic plate counts, namely: Surface inoculation (Spread Plate) and Pour-plate procedures (David *et al.*, 1995).

In this study the pour-plate procedure was performed. A series of dilutions (10^{-2}, 10^{-3} and 10^{-4}) of the sample homogenate was mixed with an agar medium and incubated at 35 ºC for 24-48 hrs. Calculation of APC was done as follows:

$$N = \frac{\Sigma C}{[(1 * n_1) + (0.1 * n_2)] * (d)}$$

Where N = Number of colonies per ml or g of product

ΣC = Sum of all colonies on all plates counted

n_1 = Number of plates in first dilution counted

n_2 = Number of plates in second dilution counted

d = Dilution from which the first counts were obtained

3.5.3. Enumeration of Enterobacteriaceae.

Enumeration of *Enterobacteriaceae* followed the guidelines given in the standard operating Procedure "Enumeration of *Enterobacteriaceae* by the colony count technique" issued by the Health Protection Agency UK (2003). The method involves inoculation and confirmation.

Inoculation and incubation

Transfer 1 ml of each decimal dilution to a sterile Petri dish. Pour about 15 ml of molten violet red bile glucose agar (VRBGA), tempered in a 45°C water bath, into each Petri dish. The time elapsing between the end of the preparation of the initial suspension and the time when the medium is poured shall not exceed 15 minutes. Carefully mix the inoculums with the medium and allow the mixture to solidify. Invert the prepared dishes and place in an incubator at 37°C for 24 hours.

Counting of colonies

Colonies of *Enterobacteriaceae* produce purple red colonies with a diameter of 0.5 mm or greater and sometimes surrounded by a red zone of precipitated bile. Count

and record the characteristic *Enterobacteriaceae* colonies on each plate containing not more than 150 colonies. Above this number it is likely that colonies will have an atypical appearance. The number of *Enterobacteriaceae* was calculated the same as the calculation of the Aerobic plate count.

Confirmatory tests

Subculture five suspect *Enterobacteriaceae* colonies onto a segment of a nutrient agar (NA) plate and incubate at 37^{o}C for 24 +2 hours. Use the growth obtained for biochemical confirmation.

Oxidase test

Prepare a fresh solution of the reagent for each time of use. Immerse a swab in oxidase reagent and touch lightly to the surface of the colony to be tested. The immediate appearance of a dark purple colour at the point of contact denotes a positive reaction but no colour change or a purplish colour which develops later are both negative reactions.

Fermentation test

Prior to use, steam or boil the glucose agar for 10 minutes and allow to set.

Perform a fermentation test on oxidase negative subcultures by a deep stab inoculation of tubes of glucose agar and place in an incubator at 37^{o}C for 24 +2 hours. *Enterobacteriaceae* produce a yellow colour throughout the medium.

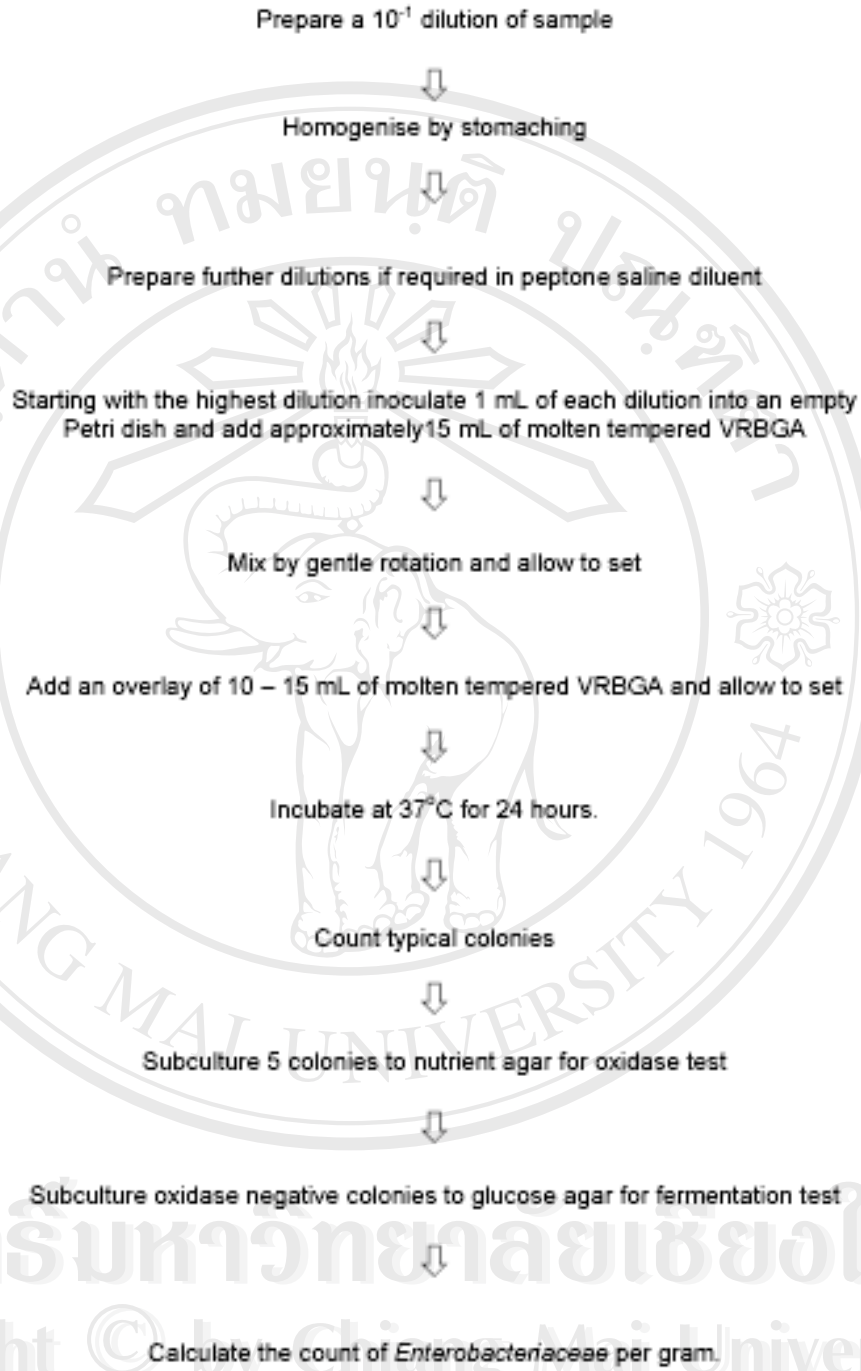

Figure 3.2: Flowchart of the enumeration of *Enterobacteriaceae* by the colony counts technique

3.5.4. *Salmonella* isolation and identification

The conventional method for *Salmonella* isolation and identification followed guidelines given from the Institute of Meat Hygiene, Freie Universitat Berlin (July, 2004) and ISO 6579-2002. The procedure consists of the following steps.

Non-Selective Enrichment (Pre-enrichment).

The test samples were initially inoculated into a non-inhibitory liquid medium to favor the repair and growth of stressed or sub-lethally injured salmonellae. The required volume of analytical unit was dispersed into nonselective enrichment broth (BPW). Incubate the pre-enrichment mixture at 35±0.5^{o}C for 18-24 hrs.

Selective Enrichment:

Replicate portions of each pre-enrichment culture are inoculated into Muller-Kauffmann tetrationate broth and Rappaport-Vassilidiadis (RV) to favor the proliferation of salmonellae through a selective repression or inhibition of the growth of competing microorganisms, according to ISO 6579: 2002.

Selective plating:

A loopful from each selective enriched culture was streaked onto XLD and BPLS agar plates. The plates were incubated at 37^{o}C for 24 hrs. Five suspected colonies having the typical appearance of *Salmonella* were transferred to nutrient agar plates and incubated at 37^{o}C for a further 18-24 hrs. (Manufacture of all media used in this study: Merck, Darmstadt, Germany.

Serological Identification:

The serotyping consisted of the determination of the O (somatic), H (flagella with biphasic strain) and Vi (capsular if necessary) antigens according to the Kauffmann-White Scheme. Polyvalent and/or somatic grouping antisera were initially used for preliminary identification of isolates as members of the genus *Salmonella*. Secondly, the isolates were screened with Enteroclon Anti-*Salmonella* polispecific A-E and Enteroclon Anti-*Salmonella* polyspecific F-67, and thirdly, determination of the

O group by means of group-specific Enterocolns was performed. These antisera were produced by the *SIFIN* Company, Berlin, Germany.

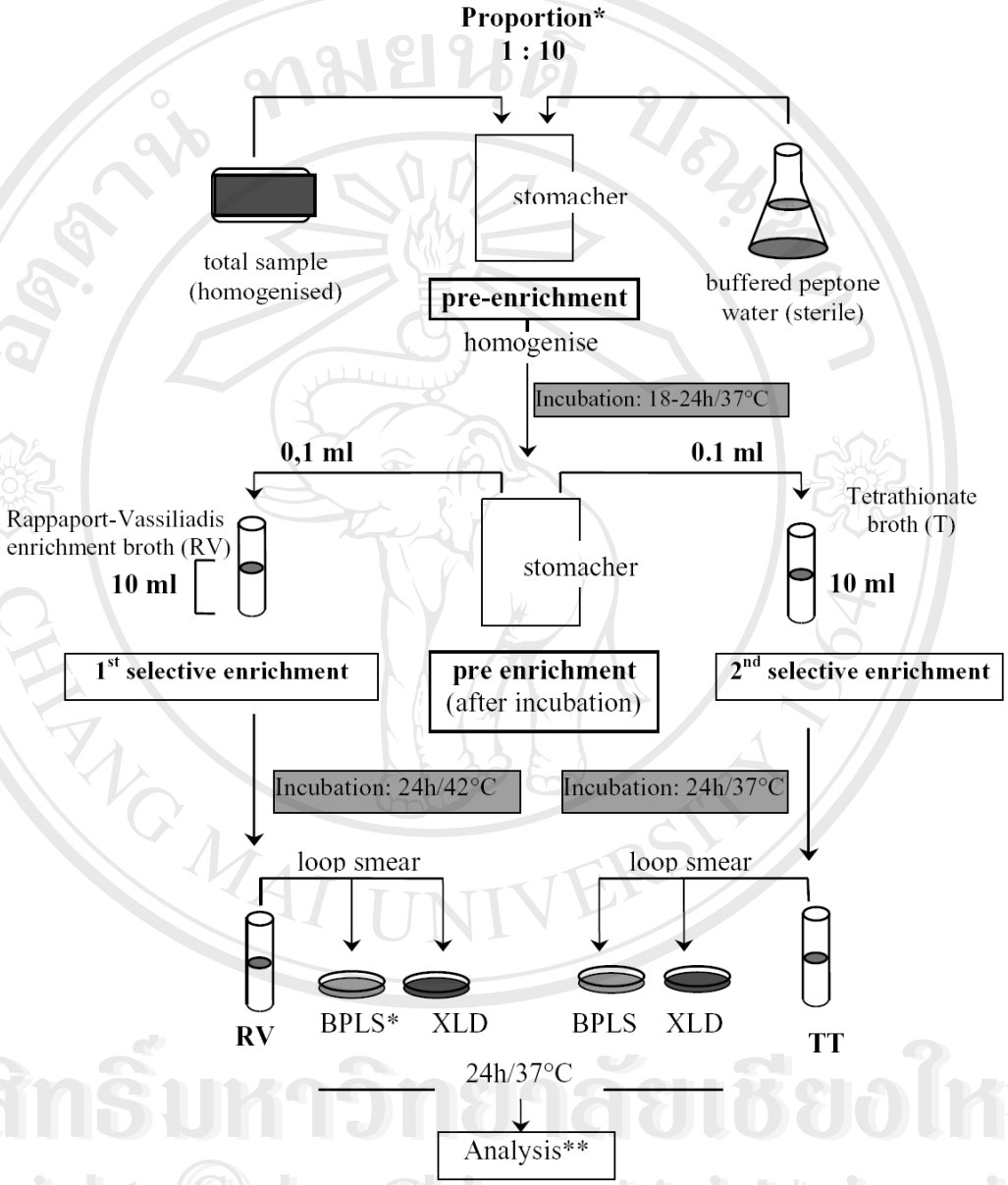

Figure 3.3: *Salmonella* Isolation and Identification Flow Chart

* Swab sample 400 cm^2 in 100 ml of BPW; Mesenteric Lymph Nodes 25g with 225 ml BPW

** In case of finding suspicious colonies on the selective media, a biochemical and serological confirmation must follow.

- for biochemical confirmation 5 cfu, testing for pure culture on standard media, followed by biochemical testing.
- for serological confirmation 5 colonies are used for agglutination test with polyvalent serum I/II and III

3.6. Data management and analysis

The data were managed in Microsoft Excel and NCSS-PASS (Dawson). Analysis and calculation of sample-specific prevalence of S*almonella* and their differences were performed using the computer program EpiCalc version 2000. Testing for differences between two means was performed to compare the difference between the Aerobic Plate counts and the *Enterobacteriacea*e Counts of the swab samples collected from carcasses before and after evisceration of the pigs. Multivariate (Binary Logistic Regression) testing was performed for *Salmonella* prevalence among samples, and univariate analyses of the potential risk factors and occurrence of *Salmonella* in mesenteric lymph nodes were performed by using Statistics program MINITAB-13.

4. RESULTS

4.1. Aerobic plate counts (APCs)

The aerobic plate counts were obtained by culturing pooled samples taken by sponge swabs from the back, jowl, ham and belly of the 62 pig carcasses. Histogram of the Log_{10}cfu of APCs per cm^2 are presented in Figure 4.1. Generally, the $Log_{10}cfu/cm^2$ of APCs ranged from 4.4 to 4.9 with a mean of 4.70 $Log_{10}cfu/cm^2$ in Swab1. In Swab2, these ranged from 4.5 to 5.3 with a mean of 4.85 $Log_{10}cfu/cm^2$. The $Log_{10}cfu/cm^2$ in Swab2 had the largest variability and many outliers. Overall, the difference between the $Log_{10}cfu/cm^2$ of two swabs was significant (p=0.0001).

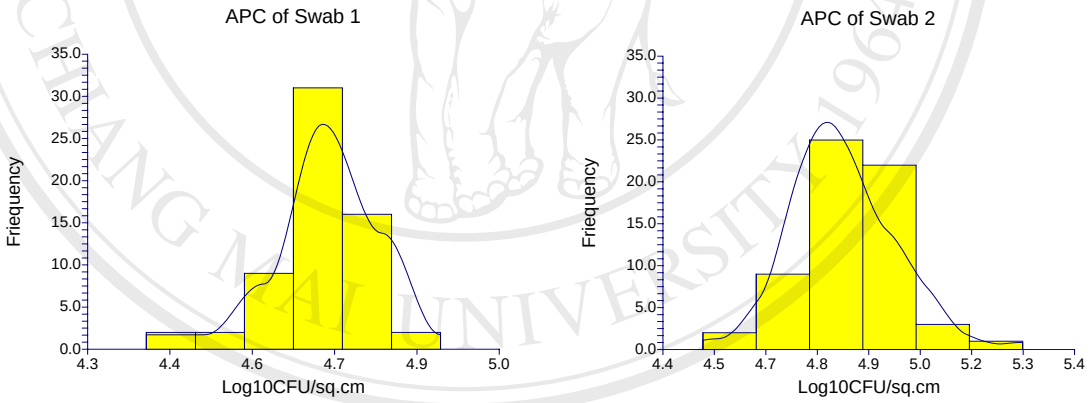

Figure 4.1: Histograms of $log_{10}cfu/cm^2$ of aerobic plate counts of pig carcasses in the Dorn Du slaughterhouse, 2004 – 2005.

4.2. *Enterobacteriaceae* counts (EBCs)

The *Enterobacteriaceae* counts (EBCs) were obtained by culturing pooled samples taken by sponge swabs from the back, jowl, ham and belly areas of 62 pig carcasses Figure 4.2 shows Box-and-Whisker plots of the Log_{10}cfu of *Enterobacteriaceae* counts per cm^2 ($Log_{10}cfu/cm^2$). In Swab1, the Log_{10}cfu of EBCs ranged from 2.3 (an outlier) to 3.1 with a mean of 2.81 and a median of 2.8, whereas, Swab2 had values of Log_{10}cfu of EBCs that ranged from 2.1 (an outlier) to 3.3 with a mean of 2.98 and a median of 3.0. Visually, many values in Swab2 were negatively skewed. Overall, the $Log_{10}cfu/cm^2$ of EBCs were significantly (p=0.0001) different between Swba1 and Swab2.

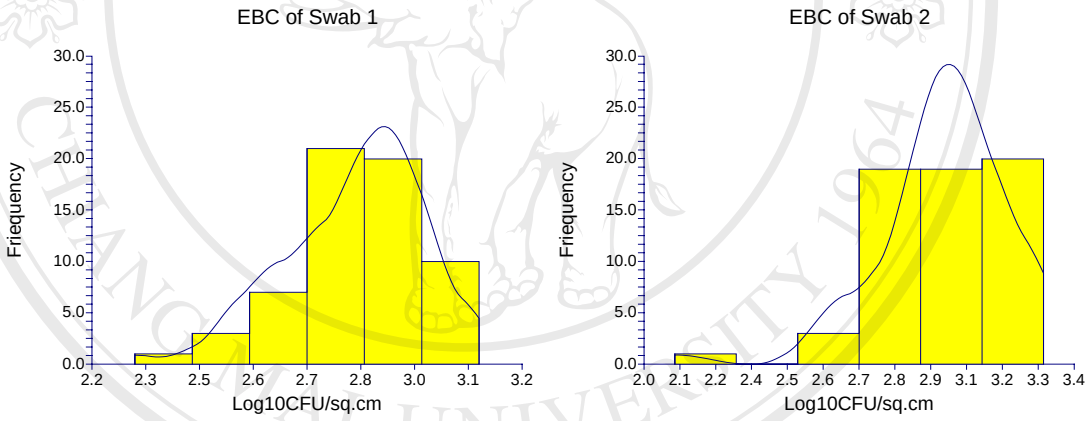

Figure 4.2: Histograms of log10cfu/cm^2 of *Enterbacteriaceae* counts of pig carcasses in the Dorn Du slaughterhouse, 2004 – 2005;

4.3. *Salmonella* detection and Identification

4.3.1. *Salmonella* isolation

A total number of 186 samples were collected from 62 pig carcasses over the study period and examined for *Salmonella*. Three samples were collected from each randomly selected carcass (Swab1, Swab2 and mesenteric lymph node (MLN)). Table 4.1 shows the prevalence of *Salmonella* obtained from each type of sample. In all of the samples, 55.4% (95% CI: 47.9-62.6) were *Salmonella* positive. S*almonella* was found more frequently (66.1%, 95% CI: 52.9 - 77.4) in Swab2, followed by 53.2% (95% CI: 40.2 - 65.8) in MLN and lowest in 46.8% (95% CI: 34.2 - 59.8) in Swab1. No significant (P=0.088) differences were observed among these prevalences.

Table 4.1: Prevalence of *Salmonella* in Swab1 and Swab2, and Mesenteric lymph nodes of pig carcasses at "Dorn DU" Slaughterhouse.

Sample type	n	Positive	Prevalence (%)	95% CI
Swab1	62	29	46.8	34.2 - 59.8
Swab2	62	41	66.1	52.9 - 77.4
MLN	62	33	53.2	40.2 - 65.8
Overall	186	103	55.4	47.93 - 62.6

n = number of sample
CI =Confidence interval
MLN = mesenteric lymph node

4.3.2. Pre-harvest *Salmonella*

Table 4.2 shows the results of the univariate analyses of the potential risk factors and occurrence of *Salmonella* in mesenteric lymph nodes. There was a significant association (OR=2.15, 95% CI: 0.76-6.07, P= 0.19) between the presence of *Salmonella* in mesenteric lymph nodes and transportation time: more than 4 hours of transportation had higher prevalence those less than 4 hours. But, herd size greater than 500, water sources and sources of piglets had odds ratios of less than one. Herd size greater than 500 had a higher sample prevalence compared to small sized herds. Thus, it was 2.9 (1/OR) times of having *Salmonella* isolated from mesenteric lymph nodes. Similarly, "other water sources" and sources of piglets outside the farms were 3.3 and 4.5 times, respectively; of having *Salmonella* isolated from mesenteric lymph nodes.

Table 4.2: Summary results of univariate analysis on association between *Salmonella* isolation from Mesenteric lymph nodes and various potential risk factors

Risk factor	Sample	Positive sample	% Positive	OR (95% CI)	$\chi 2$ corrected (P-value)
Herd Size					
<500	32	13	41	0.34	3.24
>500	30	20	67	(0.11-1.09)	0.0072
Transportation time					
>4hrs	38	23	61	2.15	1.41
<4hrs	24	10	42	(0.67-6.95)	0.2346
Water Source					
Tab water	38	16	42	0.30	3.79
Others*	24	17	71	(0.09-1.01)	0.05154
Sources of piglets					
Within farm	38	15	39	0.22	6.10
Outside farm	24	18	47	(0.06-0.76)	0.0135

n = number of samples (MLN) with *Salmonella* positive

OR= Odds Ratio

p = p-value

*Underground and surface water

The summary results of the multiple logistic regression analysis of the potential risk factors that were significantly associated with *Salmonella* isolations from mesenteric lymph nodes in the univariate analysis are shown in Table 4.3. The multiple logistic regression model obtained was:

Logit (p/1-p) = -0.4938 + 0.3698 (Herd size) + 1.717 (Source of piglets) – 0.487 (Source of water):

Log-likelihood = -38.789, df 3, p = 0.044;

Goodness of-fit-Tests

Method	Chi-square	DF	p-value
Pearson	2.202	2	0.333
Deviance	2.888	2	0236
Hosmer-Lemeshow	0.947	2	0.623

The model fitted the data (p = 0.333).

The source of piglets was strongly associated despite the fact that its OR = 5.57 was not statistically significant (p = 0.160). The rest of the risk factors gave low ORs.

Table 4.3: Summary results of multiple logistic regression analysis for the associations between *Salmonella* isolation from Mesenteric lymph nodes and various potential risk factors

Risk factor	Sample	Positive sample	% Positive	OR (95% CI)	P-value
Herd Size					
<500	32	13	41	1.45	
>500	30	20	67	(0.38-5.56)	0.590
Water Source					
Tab water	38	16	42	0.61	
Others*	24	17	71	(0.06-6.58)	0.687
Sources of piglets				5.57	
Within farm	38	15	39	(0.51-	
Outside farm	24	18	47	61.04)	0.160

Table 4.4: Proportions of *Salmonella* isolation from Swabs1, Swabs2 and mesenteric lymph nodes from pork carcasses in the Dorn Du slaughterhouse

n	Swab1	M L N	Swab2	Proportion (%)
6	+	+	+	9,7
6	+	+	-	9,7
13	-	+	+	21,0
11	+	-	+	17,7
6	+	-	-	9,7
8	-	+	-	12,9
11	-	-	+	17,7
1	-	-	-	1,6
62				100.0

+ = *Salmonella* positive; - = *Salmonella* negative

MLN = Mesenteric lymph node

n= Number of pigs with salmonella positive result.

Table 4.4 shows the distribution of samples positive for *Salmonella.* Of all different samples collected from the 62 pork carcasses, 9.7% were positive for *Salmonella*. Swab1 and MLN were both positive in 9.7% of the carcasses while MLN and Swab2 were positive in 21.0% of the carcasses. Swab1 and Swab2, as the indicator of carcass contamination with *Salmonella* and slaughterhouse hygiene, were isolated in 17.7% of all carcasses.

4.3.3. Serotyping

Out of 103 isolates, 26.2% belonged to Somatic group B, 29.1% to group C, 42.7% to group E and 1.9% to group D (Table 4.5). Totally eight different serotypes were obtained (Table 4.6). The most frequent (29,1 %) serotype was *Salmonella* Rissen, followed by *S.* Anatum (27.2%), *S.* Derby (19.4%), and *S.* Elisabethville (7.8%). The other serotypes identified were *S.* Amsterdam (7.8%), *S.* Typhimurium (3.9%), *S.* Agona (2.9%), and *S.* Enteritidis (1.9 %). These serotypes were found in all types of samples, except the last one, (*S.* Enteritidis), which was isolated from Swab2 only.

Table 4.5: *Salmonella* Somatic (O) group in carcass Swab1 and Swab2 and mesenteric lymph nodes

Sample	Somatic (O) group				Total
	B	C	D	E	
Swab1	8* (27.6)	11 (37.9)	-	10 (34.5)	29 (100.0)
MLN	11 (33.3)	10 (30.3)	-	12 (36.4)	33 (100.0)
Swab2	8 (19.5)	9 (22.0)	2 (4.9)	22 (53.7)	41 (100.0)
Overall	27 (26.2)	30 (29.1)	2 (1.9)	44 (42.7)	103 (100.0)

( ) = Percentage;

* = number of samples with positive *Salmonella* result

MLN = Mesenteric lymph node

Table 4.6: *Salmonella* serotypes in carcass Swab1 and Swab2 and Mesenteric lymph nodes

Salmonella serovar	Number of strains	%
S. Rissen	30	29.1
S. Anatum	28	27.2
S. Derby	20	19.4
S. Elisabethville	8	7.8
S. Amsterdam	8	7.8
S. Typhomurium	4	3.9
S. Agona	3	2.9
S. Enteritidis	2	1.9
Total	103	100.0

5. DISCUSSION AND CONCLUSION

5.1. Discussion

This study was conducted to determine the microbiological contamination in pig carcasses in order to evaluate the microbiological quality of pig carcasses and the hygienic status of this slaughterhouse, which is an important aspect of public health in Vientiane Capital, Lao PDR. For that purpose, 62 pigs were randomly selected during the sampling days (10). In each carcass, two swabs and mesenteric lymph nodes were sampled for bacteriological analysis. All swabs were analyzed for Total plate counts, *Enterobacteriaceae* counts and *Salmonella*. The lymph node samples were tested for *Salmonella* only.

From the questionnaire and visual observation in the slaughterhouse, the slaughter procedures in particular, pigs were stunned mechanically by hitting on the head, and then bled. After bleeding, scalding was carried out in a hot (temperature 62-68ºC) water tank. Following scalding, dehairing was done using machine. Sometimes the machine couldn't remove all hair, so additional manual deharing is performed. The scalding water was changed on daily basis. This was done prior to commencing slaughter. The de-haired pigs were washed with tap water and eviscerated. During bleeding and evisceration pigs were suspended on a rail. They were then dropped on the floor for splitting, after which they were hung on the rail again, washed and transferred to next room for dripping. Lastly the carcasses were distributed to the market at the same day.

Aerobic plate counts and *Enterobacteriaceae* counts on carcasses swabs

Although the flesh of healthy slaughtered animals can be expected to be sterile, it is difficult to avoid contamination of carcasses and meat delivered from carcasses during slaughter procedures. Slaughter techniques determine the extent of carcass contamination. In this study, the microbiological status of carcasses was assessed in

order to compare the bacterial counts in terms of APC and EBC at stage of post-de-hairing and post-evisceration along the slaughter line.

Aerobic plate counts and *Enterobacteriaceae* counts are often used as hygiene indicators of foods of animal origin (Anon., 2001; Berends *et al.*, 1997; Crowley *et al.*, 2005; Warriner *et al.*, 2002; Nel *et al.*, 2004; Zweifel *et al.*, 2005). Aerobic plate counts are widely used to determine the general of microbial contamination, while *Enterobacteriaceae* counts are indicative for possible fecal contamination. World Health Organization (WHO) and FSIS considered fecal materials as the main source of pathogens such as *Escherichia coli* (*E. coli*) O157:H7, *Salmonella* or *Campylobacter spp*. (Anon., 1990).

In this study, the means of overall aerobic plate counts ranged from 4.4 $\log_{10}$cfu/cm^2 to 5.3 $\log_{10}$cfu/cm^2. These findings are very close to those by Pearce *et al.* (2004). Pearce *et al.* obtained Aerobic mesophlic counts of 4.46 $\log_{10}$cfucm^2 (belly) and 4.75 $\log_{10}$cfu/cm^2 (neck) at dehairing. Nevertheless, the mean of aerobic plate counts in Swab2 (at the end of the process) of 4.85 $\log_{10}$cfu/cm^2, was much different form that by Pearce *et al.* (2004) of 3.65 $\log_{10}$cfu/cm^2 (belly) and 3.53 $\log_{10}$cfu/cm^2 (neck). The decrease is probably due to the singeing, because according to several studies (Gill and Bryant 1993; Warriner *et al*., 2002; Rivas *et al*., 2000) in this step decreases of microbial load on the surface of carcass usually take place. Similar results were reported in Zweifel's (2005) study in five Swiss abattoirs, where the GMP measures were applied and TVCs were low (2.2 to 3.7 $\log_{10}$cfu cm^2). However, the mean of 4.85 $\log_{10}$cfu/cm^2 observed in this study is fairly above the acceptable level of 4.0 $\log_{10}$cfu/cm^2 recommended by EU Commission Decision 2001/471/EC. The overall numbers of APCs counts were significantly ($p = 0.0001$) different between Swab1 and Swab2.

Similar results were obtained from *Enterobacteriaceae* counts. All samples had a number of EBCs above the acceptable value of 2.0 $\log_{10}$cfu (according to the EU Commission Decision 2001/471/EC); they ranged from 2.3 to 3.1 and 2.1 to 3.3 logcfu/cm^2 for Swab1 and Swab2, respectively. 95% of Swab1 and 69% of Swab2

had number of EBCs in the marginal range (2.0 - 3.0 log_{10}cfu/cm^2). Accordingly, 31% of Swab2 had EBCs results above the maximum (unacceptable) value of 3.0 log_{10}cfu/cm^2. Means of log_{10}cfu from Swab1 and Swab2 were 4.70 and 4.85log_{10}cfu/cm^2, respectively. The overall numbers of EBCs counts were significantly ($p = 0.0001$) different between Swab1 and Swab2. Increase in the number of EBCs was probably caused by contamination during subsequent operations, for example evisceration, washing and splitting (after evisceration carcasses were laid down on the floor for splitting).

Contaminations would certainly occurred during all steps in the slaughter line. This was because the carcasses were in many times exposed to unclean surfaces and equipment. Moreover carcasses were put on the floor for splitting. All these could lead to contaminations of carcasses with a variety of biological (e.g. microorganisms), chemical (e.g. cleaning and disinfection substances) and physical hazards.

Salmonella isolation

Sources of S*almonella* in pork carcasses and products have been investigated over the years in many developed countries. For example, the SALINPORK Project (Danilo *et al.*, 2000) explored various epidemiological and economic aspects of *Salmonella* in pork. In general, these included pre-harvest and harvest epidemiology of *Salmonella* and economic assessment of possible control scenarios along the pork production chain in specific countries in the European Union. Danilo *et al* pointed out that the epidemiology of *Salmonella* in pork at the slaughterhouse level is basically due to direct or indirect fecal contamination of live pigs or carcasses. Thus, in live pigs, presence of *Salmonella* could stem from the farm-level and cross-contamination during transportation. But in the slaughterhouse, carcasses may be cross-contaminated from *Salmonella* positive pigs slaughtered earlier on the same day, from contaminated slaughter equipment and/or human carriers. In this study, swabs (Swab1 and Swab2) and lymph nodes were used to isolate *Salmonella*. Swab1 was taken following de-hairing whereas Swab2 was taken at the dripping stage after the carcass was washed.

The lymph node samples were taken during the evisceration step. These samples are commonly used in studies of this type, e.g. in the study of Danilo *et al*. (2000).

The occurrence of *Salmonella* in Swab2 was higher (66.1%) than in Swab1 (46.8%). This increase in carcass surface contaminations during the slaughter process, indicated by this finding, is well documented (Gill and John, 1997). This has been attributed to the contamination of carcasses by bacteria in the gastrointestinal tract, mouth and tonsils during the course of slaughtering (Gill and John, 1998). *Salmonella* can finally also come from the slaughterhouse environment as well as from humans if the hygienic standards are extremely low (Warriner *et al.*, 2002; Botteldoorn *et al.*, 2003). Therefore, the high proportion of positive Swab2 samples compared to that obtained in Swab1 samples is probably due to an increased contamination of carcasses along the slaughter line. It is worthy noting that Swab1 samples were taken after de-haring of the carcasses in order to monitor the contaminations at this step and to compare with microbiological findings in the subsequent Swab2 samples. The rotating flails that are used to remove hairs may squeeze feces from the anus, potentially contaminating the equipment with fecal microorganisms, including *Salmonella*, and hence contaminate the carcasses (Borch *et al.*, 1996). Thus, the presence of *Salmonella* in Swab1 samples strongly suggest carcass contamination during de-hairing and/or during earlier stages (Berends *et al.*, 1997).

The next possible contamination is in the dressing of the carcasses. In particular two steps have been identified as critical control points: the evisceration process, including bung dropping, and the removal of the pluck-set. The carcass splitting process is not normally considered to be an important source of carcass contamination (Berends et al., 1997; Gill and John 1997). In this study, Swab2 was taken at the post splitting stage after washing the carcass. This was done to monitor the presence of *Salmonella* in final pork carcasses or products. The percentage of *Salmonella* isolation was 66.1%, which was higher then the percentage obtained from Swab1.

The finding of *Salmonella* in fecal and lymph node samples is considered as an estimate of pre-harvest prevalence of *Salmonella* at the farm-level and shedding due to various stress factors like transportation (Pachanee *et al*., 2002). In this study, the proportion of positive samples of mesenteric lymph nodes was 53.2%. The finding of *Salmonella* in these samples indicated a "long time infection" of the slaughter pigs that could have occurred at the farm-levels (Pachanee *et al*., 2002). This hypothesis was supported by the positive and significant associations between high numbers of positive lymph-node samples and various potential risk factors examined in this investigation, which were herd size, transportation time, source of water and sources of piglets. The multiple logistic regression analysis showed that the sources of piglets was strongly associated with presence of Salmonella in lymph nodes despite the fact that its OR = 5.57 was not statistically significant (p = 0.160). The rest of the risk factors gave low ORs. However, results of the univariate analysis showed that transportation time of more than 4 hours was biologically associated (OR = 2.15) with *Salmonella* in lymph nodes. Berends et al. (1996) studied the effect of transportation time on the prevalence of *Salmonella* in pork. Within 2-6 h of transport and lairage, the number of animals excreting *Salmonella* was 1-2.4 times higher.

Herd size greater than 500 had higher sample prevalence than small sized herds. Examination of the reciprocal of the OR of these herd sizes showed that they were 2.9 times of having *Salmonella* isolated from mesenteric lymph nodes. Similarly, "water sources" and sources of piglets outside the farms were 3.3 and 4.5 times, respectively; of having *Salmonella* isolated from mesenteric lymph nodes.

***Salmonella* serotype distribution**

From 103 isolates, 27 isolates (26.2%) belonged to Somatic group B; 30 (29.1%) to group C; 44 (42.7%) to group E and 2 isolates (1.9%) belonged to group D (Table 4.4). Out of them, eight serotypes were identified (Table 4.5). The most frequent serotype was *Salmonella* Rissen (29,1 %), followed by *S.* Anatum (26.2%), *S.* Derby (19.4%), and *S.* Elizabethville (8,7%). The other serotypes identified were *S.*

Amsterdam, *S.* Typhimurium, *S.* Agona, and *S.* Enteritidis (7,8%, 3,9%, 2,9% and 1,9 %, respectively).

In Laos, *Salmonella* would commonly be found in all types of meat and cooking materials (Nakamura *et al.*, 2004). But to date, there is no available information of *Salmonella* serotype in Laos. Nevertheless, these values indicate similar finding patterns in the neighbouring Thailand. In this country, the most common serovars from all sources (human, pig, poultry) were *S.* Weltevreden, *S.* Enteritidis, *S.* Anatum, *S.* Derby, *S.* Typhimurium, *S.* Rissen, *S.* Stanley, *S.* Panama, *S.* Agona, *S.* Paratyphi B var Java (Aroon, 2004). In Vietnam, Tran *et al*. (2004) in a study in the Mekong Delta established that the most predominant *Salmonell* serotypes were *S.* Javiana, *S.* Derby, and *S.* Weltevreden. *S.* Javiana and *S.* Weltevreden in pigs, chickens, and ducks. In the Netherlands, Duijkeren *et al.* (2001) reported that the most prevalent serotypes in pigs were Typhimurium (44%), Enteritidis (24%) in humans, serovars Typhimurium (69%), Panama (5%) and London (4%). In the USA, Gebreyes *et al.* (2004) found out that *Salmonella* Derby was the predominant serovar in fecal samples whereas in slaughter pork samples, *Salmonella* Typhimurium var. Copenhagen was predominant (49%) followed by *Salmonella* Derby.

5.2. Conclusions

This study provides baseline data on the microbiological status of pig carcasses at the Dorn Du slaughterhouse in Vientiane Capital Lao PDR. The results indicate that microbiological contamination of pork carcasses during the slaughter processing is high. The carcasses were contaminated with aerobic and *Enterobacteriaceae* bacteria, as well as with *Salmonella*. Moreover, *Salmonella* Enteritidis, which is considered world wide as risk for human health, was among the *Salmonella* isolates. The microbiological contamination could be due to various aspects that include environmental slaughterhouse conditions, pigs and farm-level managerial factors.

From the questionnaire survey and visual observation of sanitary control measures in the slaughterhouse, it was found out that there were no GMP and HACCP programs in place. There were no facilities for microbiological testing. The control of as well as reducing the microbial contamination, especially *Salmonella*, in pork at the slaughterhouses, require identification of sources and processes of cross-contamination. The findings would then be used to modify slaughter procedures and improve hygienic standards in the whole of the slaughterhouse. Therefore, the introduction of a diagnostic laboratory at the Dorn Du slaughterhouse in Vientiane Capital Lao PDR is strongly recommended.

The strict maintenance of good practices of slaughter hygiene in meat production is considered of central importance for the prevention of microbial carcass contamination in the interest of ensuring both health protection and meat quality. To enable risks involved to be estimated and appropriate measures to be taken, analysis of the slaughtering process has to be complemented by collection of abattoir-specific microbiological monitoring data in accordance with hazard analysis critical control point (HACCP) principles. This work is a first step toward such a system.

APPENDIX

Appendix A: EQUIPMENT, MATERIALS, MEDIA AND REAGENTS

1. Lab Equipment and materials

Usual laboratory equipment and in addition:

1) Sufficient work area, level table with ample surface in room that is clean, well lighted and well ventilated, and reasonably free of dust and drafts
2) Storage space, free of dust and insects and adequate for protection of equipment and supplies
3) Petri dishes, glass or plastic (at least 15 x 90 mm)
4) Pipettes with pipette aids or pipettors, 1, 5, 10, and 25 ml graduated in 0.1 ml units
5) Dilution bottles, 6 oz (160 ml), borosilicate-resistant glass, with rubber stoppers or plastic screw caps
6) Pipette and petri dish containers, adequate for protection
7) Water bath, for tempering agar, thermostatically controlled to 45 ± 1°C
8) Incubator, 35 –37 °C and 40-42 °C
9) Colony counter, dark-field, Quebec, or equivalent, with suitable light source and grid plate
10) Refrigerator, to cool and maintain samples at 0-5°C
11) Thermometers (mercury) appropriate range and/or electronic
12) Top pan scale capable of weighing to 0.1g
13) Stomacher machine
14) Vortex mixer

2. Equipment and material for sample collection

1) Scissors, forceps, Stomacher (sterile) bags
2) Sterile cotton wool swabs
3) Buffered peptone water (BPW)
4) Marker pens.
5) Alcohol, cotton, lighter
6) Gloves, boots and lab coat
7) Ice box with ice

3. Media, reagents and chemicals

- Peptone saline diluent (Maximum recovery diluent)
- Violet red bile glucose agar (VRBG)
- Glucose agar
- Nutrient agar (NA)
- BPLS Agar (Brilliant-green Phenol-red Lactose Sucrose Agar)
- XLD (Xylose Lysine Deoxycholate) Agar
- Buffered Peptone Water (BPW)
- Muller Kaufmann Tetrathionate broth (MKTT).
- Rappaport -Vassiliadis broth (RVS)
- Triple Sugar Iron Agar (TSI)
- Lysine Iron Agar (LIA)
- Urea Agar (Christensen's)
- Polyvalent and single grouping somatic (O) and flagellar (H)., and virulent (Vi) antisera
- Physiological Salin (85% NaCl)
- Oxidase reagente

Appendix B: QUESTIONNAIRE FOR COLLECTING DATA ON FARM MANAGEMENT PRACTICES

Date _____/_____/_____

Interviewee name: _____ title_____

Interviewer name: _____ title_____

1. Name of the farm_____
2. Name of owner_____
3. Location_____
4. Date of establishment_____/_____/_____
5. How many pigs are there in the farm? _____, Piglets in different age groups: 1) _____ 2) _____ 3) _____
6. What is the Source(s) of piglets
7. 1) Breeding in the farm? Yes_____ No_____

   2) Purchasing from outside? Yes_____ No_____

   3) If yes, where? _____
8. Do the pigs have individual numbers? Yes_____ No_____
9. Feed and feeding system
   - Is the feed being mixed in the farm? Yes_____ No_____
   - Purchased from outside? Yes_____ No_____
   - Manual feeding_____ automatic feeding _____
   - Drinking: - Tap water Yes_____ No_____
     - Underground water Yes_____ No_____
10. Vaccination program

    1) _____

    2) _____

    3) _____
11. Main health problem (disease)

    1) respiratory_____ 2) Gastro-intestinal_____

    3) others_____
12. Is the treatment effective? Yes_____N_____
13. What drugs are used?

1) _____
2) _____
3) _____

14. Condition of the holding:
    1) Roofing: galvanized iron _____ tile_____; other_____
    2) Floor: Concreted_____ un-concreted _____
       clean_____ dirty_____
    3) Ventilation: Yes_____ No_____
    4) Waste management: good_____ poor_____
    5) Other
       comments_____
15. How many pigs are being sent to the slaughterhouse in one shipment? _____
16. Who is responsible for the transportation
    1) farmer Yes_____ No_____
    2) middle man Yes_____ No_____
    3) slaughterhouse Yes_____ No_____
17. Condition of transport (vehicle): Good_____ poor_____
18. Other comments

_____
_____
_____

Appendix C: QUESTIONNAIRE FOR COLLECTING INFORMATION IN THE SLAUGHTERHOUSE

1. General Information on Slaughterhouse

Registration number	
Registration date	
Name of slaughterhouse	
Address	
Owner's name	
Date of establishment	
Species of animals approved for slaughtering	
Average number of animals slaughtered per day	
Capacity of slaughtering per day	
Number of inspectors - Veterinarians from central government	
- Veterinarians from local government	
- Assistant veterinarians	
Number of employees	
Operation days per year	
Countries importing meat derived from the animals slaughtered in this slaughterhouse	
The others	

2. Questionnaire on the facilities of slaughterhouse

	Checking points	Result of checking		**Comments**
		o	x	
1	Is there a mooring place (the place where pigs are			

	kept before slaughtering), inspection place to check condition of pigs to be slaughtered, slaughtering room, disinfection preparation room (the place to prepare disinfection), waste water disposal facility, waste disposal facility, place for disinfection of vehicles for transports of pigs and the place where employees can take a rest and employees can change clothes (locker room)?			
2	Conditions of drainage at the floor of slaughtering room excellent?			
3	Is there a safety device for lighting equipment at the slaughtering room so that broken pieces of the lighting equipment could not be contaminated in the meat?			
4	- Is there a device to prevent entrance of insects at the windows at the slaughtering room and the meat storage room? - Is there a device to prevent entrance of mice at the drain-outlet of the slaughtering room and the meat storage room?			
5	Is there a system to supply at least 83-centigrade water to disinfect the knives used for slaughtering lines?			
6	Is the water-supply facility equipped with a system to supply the water of which quality is in accordance with related regulations?			
7	– Are toilets located at a certain place so that the toilet would not affect the sanitary conditions of slaughtering room? – - Is there a device to prevent entrance of insects and mice to the toilets?			

8	Is there a system to control the temperature for preservation of meat at the refrigerator and freezer?			

3. Questionnaire on sanitary administration of slaughterhouse

	Checking points	Result of checking		**Comments**
		o	x	
1	- Is there a guideline for sanitary administration of slaughterhouse? - Are regular examinations conducted in accordance with the guideline for sanitary administration of slaughterhouse?			
2	Is the examination record of the sanitary administration of slaughterhouse kept more than 6 months?			
3	Are all internal facilities and equipment at the slaughtering room always cleaned before and after slaughtering?			
4	Are slaughtering workers always dressed in sanitary wear, sanitary cap and sanitary shoes during slaughtering work?			
5	Is slaughtering conducted under the status that pigs are hung?			
6	Do workers often disinfect slaughtering knives and utensils with at least 83-centigrade water to prevent contamination of carcass during slaughtering work?			
7	Do managers of the slaughterhouse implement education programs for workers on the basis of their own sanitary administration guideline?			

8	- Does the slaughterhouse have the HACCP administration applied to the slaughterhouse? - When was this slaughterhouse approved by the HACCP? Date, Month, Year			
9	Are antibiotic residual tests conducted as a form of random sampling tests against the pigs? Are the results of the antibiotic residual tests kept more than 6 months?			
10	Are microbial tests such as the total number of bacteria conducted as a form of random sampling tests against the carcass? Are the results of the antibiotic residual tests kept more than 6 months?			

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DECLARATION

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

Name: Phouth Inthavong

Signature.....

Date of submission.....20th Sept. 2005