CHAPTER I

INTRODUCTION

Salmonelloses are some of the most prevalent bacterial diseases of humans and animals worldwide. All domestic and wild animals are at risk of contracting salmonellosis. The disease spreads easily from animals and animal products to humans. It is caused by *Salmonella* microorganisms (Van der Wolf et al., 1999) and is the major significant food-borne disease for humans (Stohr and Meslin 1996; Anonymous 1998b; Blaha 1998; Van der Wolf et al., 1998; Erdman et al., 2000).

In many countries, explosive human outbreaks of salmonellosis, originating from infection in animals and animal products have been reported in recent years (Wegener and Bager 1997; Wierup 1997). The impacts of such incidences on human health, animal industry and the international trade have created momentum for research on salmonellosis (Baggesen et al., 1996; Van der Wolf et al., 1999; Stebben, 2001). The socio-economics of both the human and animal health effects of this disease are of interest to producers, veterinarians, consumers and public health regulatory authorities (Stohr, 1995; Van der Wolf et al., 1998).

Salmonellae are gram-negative, facultative and ubiquitous anaerobic bacilli (Quinn et al., 1999). Generally, they are categorized into typhoidal and non-typhoidal groups, having more than 2,435 known serotypes (Bopp et al., 2000; Quinn et al., 1999). The typhoidal group contains, for example, *Salmonella* Typhi and *Salmonella* Paratyphi. These cause typhoid and paratyphoid disease in humans, respectively. These infections are generally transmitted from person to person without animal involvement.

The members of the non-typhoidal group are extremely important in regards to food quality and safety in that they easily develop anti-microbial resistance to the commonly used antibiotics (Duijkeren and Houwers, 2000).

For animals, there are many different species of bacteria in the *Salmonella* family. Some only infect a single species of animals, most species of *Salmonella*; however, can infect a wide variety of vertebrates (especially mammals and birds). Thus, they are divided into host-adapted and non host-adapted *Salmonella* (Blaha, 1998; Sirinavin, 1998). The host-adapted *Salmonella* generally are only pathogenic for a single host species. For example, the host-adapted *Salmonella* serotypes of pigs, namely *Salmonella* Cholerasuis and *Salmonella* Typhisuis are highly pathogenic for pigs and rarely cause disease in humans. Therefore, they are of minor importance in terms of food-borne infection for humans (Blaha, 1998; Sirinavin, 1998; Duijkeren and Houwers, 2000).

The non host-adapted serotypes, in contrast, such as *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Agona, *Salmonella* Virchow, and others, are of major importance from the food-hygiene point of view (Blaha, 1998; Duijkeren and Houwers, 2000). Humans as well as pigs are susceptible. However, while infections in humans lead to serious illness, infections of pigs only leads to in apparent or untypical clinical disease. In humans, diarrhea, septicemia and encephalitis in children, old people and in immuno-compromised people, caused by these non host-adapted serotypes, develop while infections in pigs occur without clinical signs.

For the non host-adapted *Salmonella*, pigs primarily serve as latently infected carriers and act as portal of entry points into the pork food chain. Therefore, infections of pigs with the non host-adapted *Salmonella* are not macroscopically recognizable

during routine ante- and postmortem meat inspection. This leads to infected pigs being slaughtered and their infected products enter the food chain. Prevalences of such cases in the past have not frequently been investigated since the critical control points have not been delineated in many countries, especially in developing countries. In recent years, as the volume of the international trade and consumer demands on food quality and safety increased, research on *Salmonella* bacteria at various points in the Farm-to-Table Chain of primary pig production and pork products though has gained increased attention (Wegener and Bager, 1997). Raw pork and insufficiently cooked pork products today are seen major sources of human infections with *Salmonella* bacteria (Stohr, 1995; Stohr and Meslin, 1996; Lo Fo et al., 1997; Sirinavin et al., 2001).

In Thailand, the non host-adapted *Salmonella* are the most important causes of outbrakes of human salmonellosis (Sirinavin et al., 1991; Boonmar et al., 1998; Bangtrakulnonth et al., 1999). Pork has rarely been suspected as cause of such human infections. It is worth nothing that the in apparent infections in pigs mostly have been acknowledged predominantly in light of anti-microbial drug resistance and their indirect negative effects on production and income losses to farmers and not for their food safety aspects. Considering the important role of the pig industry in Thailand and the fact that pork is the predominant meat consumed, urgent needs exist to establish the status of the *Salmonella* bacteria in pigs and pork products, with the objective of subsequently set up food quality standards and export-risk assessment in Thailand.

For this, missing in particular are structured protocols of prevalence investigations and the diagnosibility of *Salmonella* bacteria in farm- and slaughter-pigs as well as in various pork products. Results of such investigation are

preconditions for surveillance and monitoring systems, which will eventually lead to the formulation of suitable and cost-effective *Salmonella* management programs. Therefore, investigation of latent *Salmonella* infections in pre-to-post harvest pigs and in pork products are urgently warranted.

The Significance of the Study

To generate information on the prevalence of so far unrecognized *Salmonella* infections of herds of pigs at slaughter.

LITERATURE REVIEW

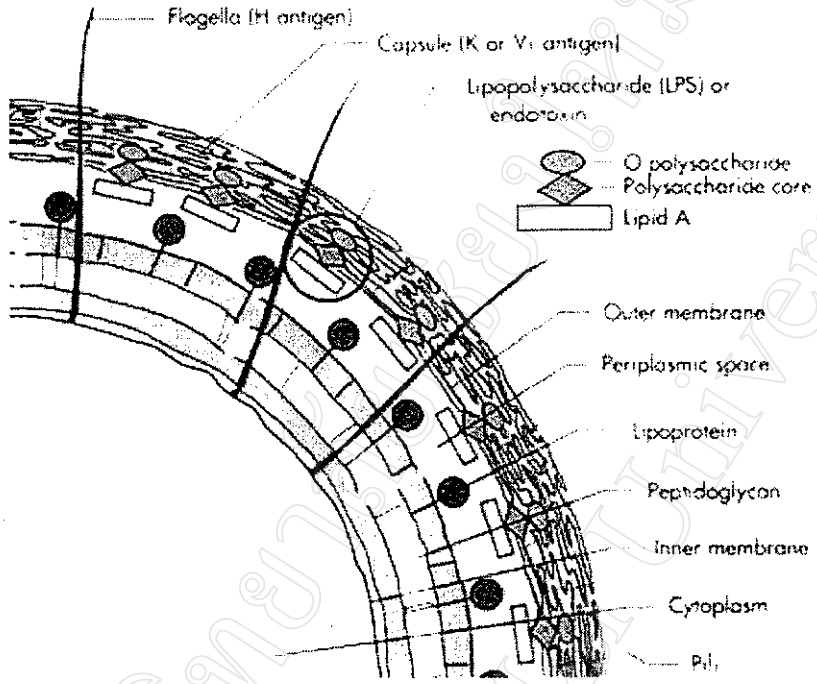
Genus *Salmonella*

The genus *Salmonella* belongs to family Enterobacteriaceae and consists of two species namely, the *Salmonella enterica* and *Salmonella bongeri.* The former is composed of six subspecies {*Salmonella enterica* subsp. enterica [Subspecies I], *Salmonella enterica* subsp. salamae [Subspecies II], *Salmonella enterica* subsp. arizonae [Subspecies IIIa], *Salmonella enterica* subsp. diarizonae [Subspecies IIIb], *Salmonella enterica* subsp. houtenae [Subspecies IV], *Salmonella enterica* subsp. indica [Subspecies VI], and *Salmonella bongeri* (formerly subspecies V)}.

Subspecies I strain are usually isolated from humans and warm-blooded animals. Subspecies II, IIIa, IIIb, IV, and VI strains and *Salmonella bongeri* are usually isolated from cold-blooded animals and the environment (rarely from humans). These species and subspecies can be distinguished on the basis of differential characters listed in table 1and 2.

Morphology and function of the envelope in *Salmonella*

Salmonella are gram-negative rods of size 2-4 μm in lengths and 0.5 μm in width. They are motile except *Salmonella* Gallinarum and *Salmonella* Pullorum (Quinn et al., 1999). The outer envelope consists of three layers: the plasma membrane (cytoplasmic membrane), the peptidoglycan (murein) and the outer membrane (Figure 1). The space between the two membranes is called periplasm.



Source : http://www.bioan.dk/Projekter/salmonella.htm

Figure 2. Membrane Structure of *Genus Salmonella*

Species	***S. enterica***						***S. bongeri***
Subspecies	enterica	salamae	arizonae	diarizonae	houtenae	indica	
Characters							
Dulcitol	+	+	-	-	-	d	+
ONPG(2h)	-	-	+	+	-	d	+
Malonate	-	+	+	+	-	-	-
Gelatinase	-	+	+	+	+	+	-
Sorbitol	+	+	+	+	+	-	+
Culture with KCN	-	-	-	-	+	-	+
L(+)-tartrate[a]	+	-	-	-	-	-	-
Galacturonate	-	+	-	+	+	+	+
γ-glutamyltransferase	+(*)	+	-	+	+	+	+
β-glucuronidase	d	d	-	+	-	d	-
Mucate	+	+	+	-(70%)	-	+	+
Salicine	-	-	-	-	+	-	-
Lactose	-	-	-(75%)	+(75%)	-	d	-
Lysine by phage O_1	+	+	-	+	-	+	d
Usual habitat							

(a) = d-tartrate
(*) = Typhimurium d, Dublin –
\+ = 90% or more positive reactions
\- = 90% or more negative reactions
d = different reactions given by different serovars

Source: WHO (1997) Antigenic formulas of the *Salmonella* Serovars 1997. WHO Collaborating Centre for Reference and Research on *Salmonella* institut pasteur, France

Table 1. Differential characters of *Salmonella* species and subspecies

Test serotype reaction	Nontyphoidal *Salmonella* Subsp. I reaction	*Salmonella* serotype Typhi reaction	*Salmonella* Paratyphi A
TSI	K/Ag	K/Ag	K/Ag
H2S(TSI)	+	+weak	-or+weak
Indole	-	-	-
Citrate(cimmon)	+	-	-
Urea	-	-	-
Lysine decarboxylase	+	+	-
Arginine dihydrolase	+	d	(+)
Motility	+	+	+
Mucate	+	-	-
Malonate	-	-	-
L(+)-Tartrate	+	+	-
Growth in KCN	-	-	-
Glucose	Ag	A	Ag
Lactose	-	-	-
Salicin	-	-	-
Dulcitol	Ag	-	Ag2days
Sorbitol	Ag	A	Ag
ONPG	-	-	-
Galacturonate	-	-	-

Source: Bopp et al. (2000)

Table2. Biochemical tests in differentiating *Salmonella* from other members of the family Enterobacteriaceae and for identifying *Salmonella* serotypes Typhi and Paratyphi A

The plasma membrane of *Salmonella* is composed of phospholipids and proteins. Phospholipids are structurally a symmetric with polar (hydrophillic) and non-polar (hydrophobic) ends and are called amphiphatic. This property of phospholipids enables the bacteria to form the bilayer membranes with transports nutrients. Furthermore, it is the site of oxidative phosphorelation and for the synthesis of phospholipid, peptidoglycan and lipopolysaccharides (LPS).

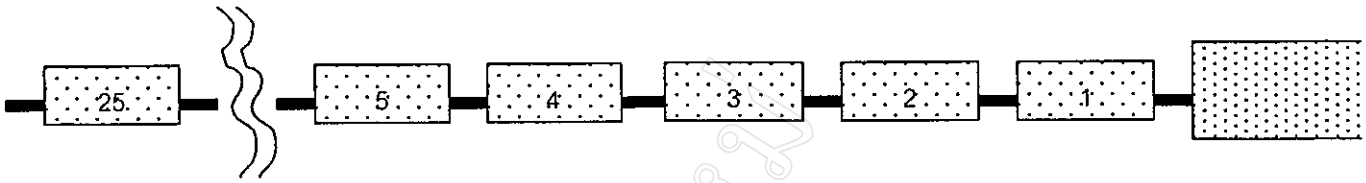
The peptidoglycan is a thin layer in gram-negative bacteria. It is composed of residues of N-acetyl muramic acid and N-acetyl glucosamine, forming long glycan chains that are convalently cross-linked by peptide bridges. The function of peptidoglycan is to stabilize the cell against osmotic lysis.

The outer membrane is a highly complex lipid-bilayer membrane structure, which surrounds the peptidoglycan layer and protect the periplasm from the external environment. It also prevents leakage of the periplasmic proteins away from the immediate environment of the cytoplasmic membrane.

Structurally, the most important components of the outer membrane are the lipolysaccharides (LPSs). Lipopolysaccharides contain both lipid and carbohydrate, and the O-side chain (O antigen) (Figure2).

The lipid A region contain two glucosamine sugar derivertives , each with three fatty acids and phosphate and/or pyrophosphate.

The O-side chain or O antigen is a short polysaccharide chain extending outwards from the core. The O-side chain is used to differentiate the *Salmonella* serotypes. Serotyping is based on the grouping of strains according to the structure of their O antigenic side-chain polysaccharide, coupled with determination of the serological



Source: Rycroft (2000)

Figure 2. Schematic representation of the structure of S-form lipopolysaccharide. Lipid A is joined, via the inner-and outer-core oligosaccharide, to the repeating oligosaccharide that forms the O side-chain. The length of the O side-chain is variable, but between 25 and 40 units is common in *Salmonella.*

specificity of the H (flagella) antigen. The White-Kauffmann scheme is based on this priciple. Besides the O and H antigen, some serotypes of *Salmonella* produce the Vi antigen (capsule). It was termed the Vi antigen because of its association with virulence. It is produced by strains of *Salmonella* Typhi, *Salmonella* Paratyphi, and *Salmonella* Dublin. *Salmonella* carrying the Vi antigen are not agglutinable with anti-O antiserum until the bacteria have been heated at 100 °C for 60 min in order to remove the masking effect of the capsule and to reveal the O-specific antigen (Rycroft, 2000).

Diagnosis of *Salmonella*

Isolation and identification of the bacteria and/or detection of antibodies using serological examination must substantiate accurate diagnosis. The isolation and subsequent identification of *Salmonellae* depend not only on the quality of the sample but also on the culture medium and growth characteristics of the serotype, particularly those adapted to the hosts.

Testing for the detection of *Salmonella* is a key point in surveillance and control programs. A perfect test is characterized by having a very high specificity and sensitivity, easy to perform, rapid and amenable to automation, cheap, and having the possibility of large-scale application (Nielsen and Baggesen, 1997). So far no single test has been able to fulfil all these requirements.

Isolation and Identification (Bacteriological Diagnosis)

The "gold standard" for *Salmonella* diagnosis is bacterial culture (Kapur et al., 1998). Many different culture media and methods have been developed and used for *Salmonella* detection (Table 3). Isolation procedures for *Salmonella* consist

Culture step	Commonly used components
1. Non-selective pre-enrichment	- Buffered Peptone Water(BPW)
2. Selective enrichment	- Rappaport Vasiliadis broth(RV) - Rappaport Vasiliadis Soya broth(RVS) - Modified Semi-solid Rappaport Vasiliadis(MSRV) - Selenite broth - Selenite brilliant green broth - Tetrathionate broth - Tetrathionate brilliant green broth - Hajna Tetrathionate broth
3. Plating agars	- Brilliant green agar(BGA) - Desoxycholate Citrate agar(DCA) - Ranbach agar - Xylose lysine deoxycholate(XLD) - Xylose lysinetergitol(XLT4)
4.Verification	- Biochemistry - Triple Sugar Iron agar - Rapid test systems
5.Identification	- Biochemistry and serotyping

Sorce: Van winsen et al. (1999)

Table 3. Priciples of conventional culture for *Salmonella*.

principally of four distinct phases: 1) non-selective pre-enrichment; 2) selective enrichment; 3) selective isolation and elective growth to produce suspect isolates, and 4) biochemical and serological confirmation (Van winsen et al., 1999; Waltman, 2000).

Pre-enrichment

Early studies showed that direct selective enrichment of samples were often unsuccessful for the detection of *Salmonella* bacteria. In these samples, *Salmonella* could be present but were "sub-lethally damaged". Although these *Salmonella* bacteria still are viable and able to cause disease under the right conditions they are easily killed in the environment of selective-enrichment broth, especially when incubated at high temperature. Non-selective pre-enrichment broth allows these "sub-lethally damaged" cells to resuscitate prior to transfer into the selective enrichment media. For most samples, buffered peptone water (BPW) is the medium of chioce (Nielsen and Baggesen, 1997).

Selective Enrichment

Selective-enrichment media, each with various modifications, are formulated to selectively inhibit other bacteria while allowing *Salmonella* bacteria to multiply to levels that may be detected following plating. The most common selective-enrichment media are generally based on tetrathionate, selenite, and Rappaport-Vassiliadis enrichment media. In Europe, however, selective-motility enrichment Rappaport-Vassiliadis medium is widely used (Waltman, 2000)

Incubation Conditions

Pre-enrichment:

Since the pre-enrichment step increases the time required for the isolation of *Salmonella*, several investigators have attempted to shorten this pre-enrichment time. Most of studies, however, have found that incubating for less 18 hrs reduced sensitivity. Therefore, incubating the pre-enrichment for 18-24 hrs is recommended to allow resuscitation of *Salmonella* bacteria before transfer into selective-enrichment media.

The recommended incubation temperature for pre-enrichment is 35-37 °C. Since the purpose of pre-enrichment is the resuscitation of damaged *Salmonella* bacteria, a higher temperature should be avoided (Waltman, 2000).

Selective-enrichment:

Generally, samples such as internal organs or tissues, are incubated at 35-37°C. A higher temperature is not necessary to suppress contaminants in these samples. However, intestinal and surface samples, which generally have higher levels of competing micro-flora, may be incubated at higher temperatures (40-43 °C), because *Salmonella* bacteria are more tolerant to the high temperature. Moreover, since both tetrathionate and Rappaport-Vasiliadis broths can be stored and refrigerated, these must be allowed to warm up to at least 4-8 hrs to allow the sample to reach the appropriate temperature.

Plating Media

The enrichment process is designed to increase the number of *Salmonella* bacteria in the culture to a level that may be detected on plating medium. Various plating media have been developed for the isolation of these bacteria by using the principles of selectivity and differentiation. Selectivity involves the incorporation of inhibitory substances into the media that selectively inhibit other bacteria. The differential characteristics of the specific plating medium involve the addition of substance(s) that differentiate *Salmonella* colonies from other bacteria. The principal differential biochemical characteristics are the production of acid forms of some sugar, for example lactose, and the production of hydrogen sulfide (H_2S). Nevertheless, it is recommended that at least two plating media be used, with different selective and differential properties.

Differential plating media are incubated at 35-37°C for 20-24 hrs and observed for the presence of the colonial characteristics of *Salmonella*.

Biochemical Identification

Occasionally, other bacterial species will react serologically with the *Salmonella* antiserum, especially somatic sera. Typical biochemical reactions of *Salmonella* and some other commonly bacteria are shown in Table 4 (Waltman, 2000).

Serotyping

Bacteria for serotyping should be taken from a TSI agar or from the nutrient agar. This is, because bacteria from selective media is often unsuitable for serotyping

Bacteria	Biochemical test results										
	TSI	LIA	ONPG	LAC	MAN	SAL	SUC	XYL	LYS	MLN	URE
Citrobacter freundii	A/AG,H2S	K/A	+	+	+	-	-	+	-	-	-
Enterobacter cloacae	A/AG	K/A	+	+	+	+	+	+	-	-	+/-
Escherichia coli	A/AG	K/K	+	+	+	+/-	+/-	+	+	-	-
Klebsella pneumonia	A/AG	K/K	+	+	+	+	+	+	+	+	+
Morganella morganii	K/AG	R/A	-	-	-	-	-	-	-	-	-
Proteus mirabilis	K/AG,H2S	R/A	-	-	-	+/-	+	+	-	-	+
Proteus vulgaris	A/AG,H2S	R/A	-	-	-	+/-	+	+	-	-	+
Providencia rettgeri	K/A	R/A	-	-	+	+/-	+/-	-	-	-	+
Salmonella spp.	K/AG,H2S	K/K	-	-	+	-	-	+	+	-	-
Salmonella arizonae	K/AG,H2S	K/K	+	+	+	-	-	+	+	+	-

+, > 75% positive reaction; +/-, 25-75% positive reaction; -, <25% positive reaction. Tests: TSI, Triple Sugar Iron; LIA, Lysine Iron Agar; ONPG, Ortho-Nitro-Phenyl-Galactopyranoside (beta-galactosidase); LAC, Lactose Fermentation; MAN, Mannitol Fermentation; SAL, Salicin Fermentation; SUC, Sucrose Fermentation; XLY, Xylosw Fermentation; LYS, Lysine Decarboxylase; MLN, Malonate Utilisation; URE, Urease. TSI: A, acid (yellow); K, Alkaline (red); G, gas (bubbles); H2S, Hydrogen Sulphine (black).LIA: A, acid (yellow); K, alkaline (purple); R, Lysine Deaminase (red)
Sorce: Wray (2000)

Table 4. Biochemical reactions of *Salmonella* and other commonly bacteria

(Quinn et al., 1999). Serotyping is based on the O (somatic) and H (flagellar) antigens (Table 5). The slide agglutination test is used for this purpose.

Alternative method:

Bacteriological test procedure, as all other microbiological tests, is time consuming. Results are available only after 3 to 6 days. Therefore, a worldwide search for new tests that save time and costs has been on going. Such tests developed and investigated include the modified Rappaport-Vassiliadis-medium (MSRV) (Goossens et al., 1984), the immuno-magnetic separation (IMS) (Fluit et al., 1993), the DNA-hybridization test (Flowers et al., 1987), the Immunodiffusion Test (Van der Zee and Huis in't Veld, 2000), the Polymerase Chain Reaction (Stone et al., 1994) and the Enzyme Immunoassays (Van der Zee and Huis in't Veld, 2000). The majorities of these tests are still at developmental phases or still are too expensive for the routine diagnosis. In Denmark, a Mix-ELISA (Eia Foss) has been developed which identifies *Salmonella* antibodies in serum and in meat juices of infected pigs. This ELISA provides both qualitative and quantitative results. Advantageously, this ELISA detects herds with a history of *Salmonella* infection (Protz et al., 1997). This is quite important since the sensitivity of fecal examination for *Salmonella* bacteria decreases within a few weeks.

The Denmark Mix-ELISA system is part of investigations in the countries of the European Union (EU) since 1995 to improve the diagnosis *of Salmonella* infections of slaughter pigs. *Salmonella* infection in animals and humans stimulate antibody responses towards a variety of *Salmonella* antigens (Nielsen and Baggesen, 1997). These antibodies can be detected by use of ELISA.

Serotype	Serogroup	Somatic(O) antigens	Flagella(H) antigens	
			Phase 1	Phase 2
S.Paratyphi A	A	1,2,12	a	[1,5]
S.Typhimurium	B	1,4,[5],12	i	1,2
S.Derby	B	1,4,[5],12	f,g	[1,2]
*S*Aagona	B	4,12	f,g,s	-
S.Saint paul	B	1,4,[5],12	e,h	1,2
S.Heidelberg	B	1,4,[5],12	r	1,2
S.Abortusovis	B	4,12	c	1,6
S.Typhisuis	C1	6,7	c	1,5
S.Choleraesuis	C1	6,7	[c]	1,5
S.Montevideo	C1	6,7,14	g, m, [p], s	-
S.Oranienburg	C1	6,7	m, t	-
S.Newport	C2	6,8	e, h	1,2
S.Bovismorbificans	C2	6,8	r	1,5
S.Kentucky	C3	8,20	i	z6
S.Typhi	D1	9,12,[Vi]	d	-
S.Enteridis	D1	1,9,12	g,m	[1,7]
S.Dublin	D1	1,9,12,[Vi]	g,p	-
S.Gallinarum	D1	1,9,12	-	-
S.Pullorum	D1	9,12	-	-
S.Anatum	E1	3,10	e,h	1,6
S.Newington	E2	3,15	e,h	1,6
S.Senftenberg	E4	1,3,19	g,[s],t	-
S.Worthington	G2	1,13,23	z	l,w

Source: Quinn et al. (1999)

Table 5. Antigens of Some *Salmonella* serotypes

The Mix-ELISA serological test potentially offers advantages over bacteriological tests in terms of the improvement of the diagnosis of in apparent *Salmonella* infections of slaughter pigs and also shortens the time before the test results are obtained.

The Denmark Mix-ELISA uses lipopolysaccharide from *Salmonella* Typhimurium (O-antigen 1,4,5, and 12) and *Salmonella* Choleraesuis (O-antigens 6,7) (Burkhart et al., 1997; Nielsen and Baggesen, 1997; Nielsen and Wegener, 1997; Baum, 1998; Baum et al., 1998a). By this antigen combination, the test managed to detect 95% of the *Salmonella* serotypes found in Danish pigs (Nielsen et al., 1996; Nielsen and Wegener, 1997). Results from several other investigations of the Mix-ELISA in the diagnosis of *Salmonella* infections in pigs have shown that this enzyme immunoassay is also suitable to be used in the United States as a herd test (Baum, 1998a; Baum et al., 1998b) and in Australia (Widders et al., 1997).

The screening of breeding and finishing pig herds is done using pig sera and the Mix-ELISA.The screening of slaughter pigs, in contrast, is done by the examination of the "meat-juice" and the Mix-ELISA (Nielsen et al., 1996; Nielsen and Baggesen, 1997; Nielsen and Wegener, 1997; Nielsen et al., 1997; Nielsen et al., 1998). For this, at the slaughterhouse, 10 grams of muscle tissue are taken from the carcass and placed into a container. The container is frozen at –20°C overnight and subsequently allowed to thaw at 4°C for 24 hrs. The resulting "meat juice" is diluted at 1:30 and examined using the Mix-ELISA (Nielsen and Baggesen 1997; Nielsen et al., 1998). In the EU, the purpose of investigations presently carried out is to compare the applicabilty (safety) of the ISO 6579 Bacteriological Standards with this Mix-

ELISA. A diagnosis of *Salmonella* infections in pigs with an improved Mix-ELISA compared to bacteriological tests is expected to offer considerable cost advantages.

Nomenclature for *Salmonella* and distribution of serotypes

In 1884, Gaffky cultured the typhoid bacillus from spleen and mesenteric lymph node samples from a patient who died from typhoid disease. The microorganism now known as *Salmonella* Cholerasuis was first isolated from pigs by Salmon and Smith (1886). They attributed the microorganism to be the cause of swine fever. The initial serodiagnosis of *Salmonella* was performed in 1896, using antiserum from a *Salmonella* patient to agglutinate with the pathogen from the patient.

In 1926, analysis of O and H antigens, initiated by White and extended by Kauffmann in 1941, resulted in the description of a number of serotypes (Grimont et al., 2000; Bangtrakulnonth, 2001).

Between 1997 and 1998, the WHO Collaborating Center for Reference and Research on *Salmonella*, located at the Pasteur Institute in Paris, France, gave the name of serotypes belonging to *Salmonella* Enterica subspecies as the enterica (subspecies I). This name related to the geographical place where the serotype was first isolated.

The serotype name is written in roman (not italicized) letter (for example, *Salmonella* serotype Typhimurium or *Salmonella* Typhimurium). Serotypes belonging to other subspecies are designated by their antigenic formulae following the subspecies name (for example, *Salmonella enterica* subsp. salamae ser. 50: Z: e,n,x or *Salmonella* serotype II 50: z: e,n,x).

The National *Salmonella* Reference Laboratory at the Center for Disease Control (CDC), Atlanta, Georgia, USA, uses this nomenclature with deviations. It

uses the term "Serotype" instead of "Serovar" and encourages its use because it communicates the appropriate taxonomic relationship of the more than 2,400 antigenically distinct members of the two species of the genus *Salmonella*.

Currently, 2,463 *Salmonella* serotypes have been identified (Table 6). Most of these serotypes, including *Salmonella* serotype Typhi, belong to subspecies I (1,435 Serotypes) and are found in O groups A, B, C, C1, C2, D, E1, E2, E3, and E4. Serotypes belonging to subspecies II (485 serotypes), IIIa (94 serotypes), IIIb (321 serotypes), IV (69 serotypes), VI (11 serotypes, and to *Salmonella* Bongeri (20 serotypes) are primarily found in O groups F (O11) through O 67.

Salmonella infections in pigs

Salmonellae have been isolated from all vertebrate hosts with the possible exception of fish in unpolluted waters. Although many of the more than 2,400 *Salmonella* serotype have a broad range and are widely distributed, several serotypes are adapted to specific hosts, such as *Salmonella* Typhi (humans), *Salmonella* Dublin (bovine), and *Salmonella* Cholerasuis (pigs). Epidemiologically, *Salmonella* infections of pigs are of major concern because they cause human mortality and pigs can be infected with a wide range of *Salmonella* serotypes that can be a source of infection to other animals. The epidemiology of *Salmonella* infections in pigs is quite important in that pigs can be infected with a variety of serotypes that do not cause disease in pigs but do represent a source of infection for humans.

Disease associated with host-adapted serotypes such as *Salmonella* Choleraesuis is associated with septicemia and enterocolitis, or leads to bacterimic

localization as pneumonia and hepatitis or occasionally as meningitis, encephalitis, and abortion.

Year	1997	1998
Samonella enterica		
1. Subsp. enterica	1435	1454
2. Subsp. salamae	485	489
3. Subsp. arizonae	94	94
4. Subsp. diarizonae	321	324
5. Subsp. houtenae	69	70
6. Subsp. indica	11	12
Salmonella bongeri	20	20
Total	2435	2463

Source: Updating the nomenclature of *Salmonella* serovar (WHO Collaborating Center for Reference and Research on *Salmonella*, Institute Pasteur, France).

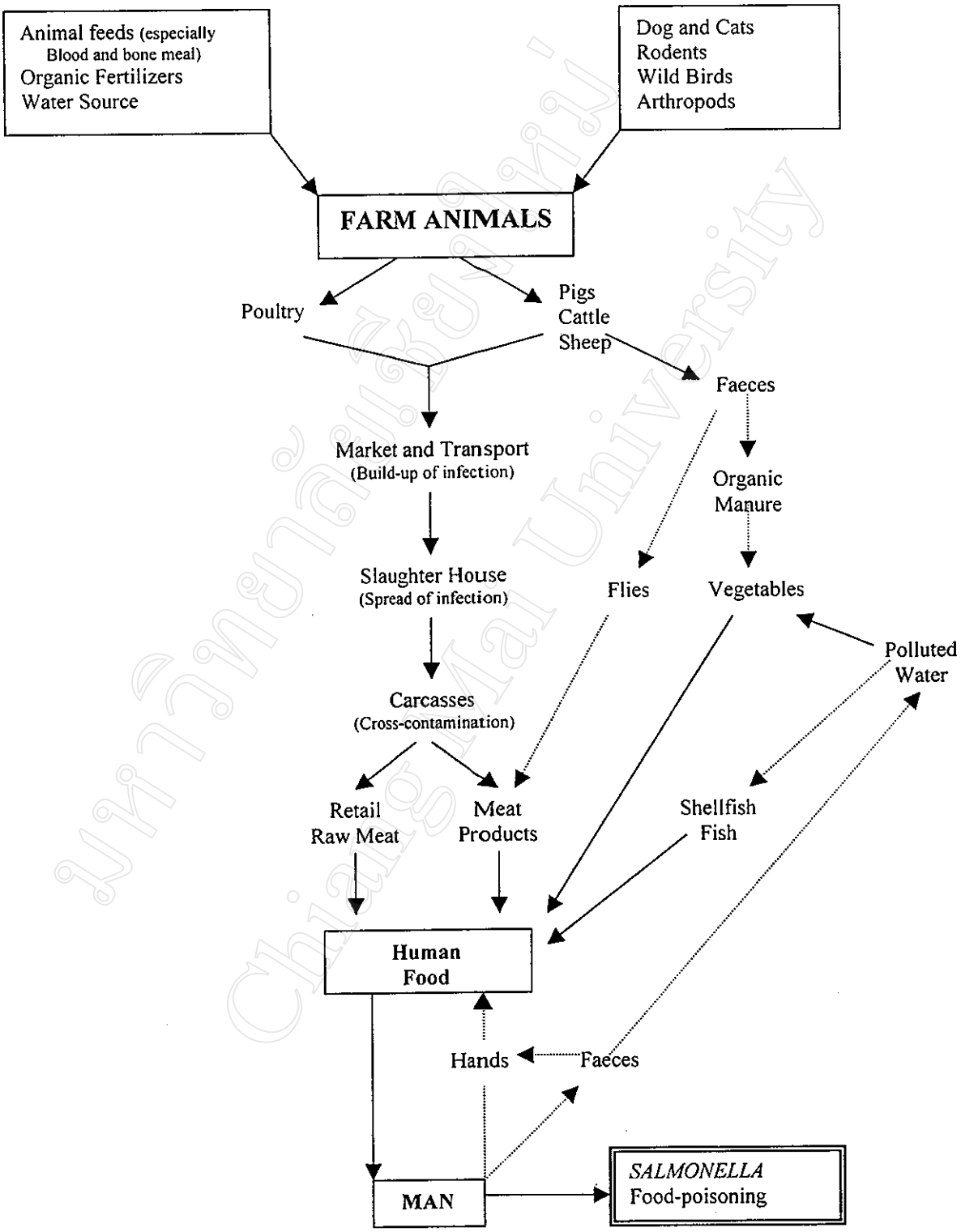
Table 6. Present number of serovars in each species and subspecies

As the majority of *Salmonella* infections in pigs are in apparent, they are characterized by a non-visible clinical course that does not lead to patho-anatomic changes. These in-apparent *Salmonella* infections in pigs are of particular epidemiological importance (Van der Gaag et al. 1999; Fedorka-Cray et al., 2000) infected animals may either periodically shed bacteria or may temporarily shed bacteria when they are under stressful conditions (e.g. transportation, starvation) (Figure 3). Naturally, the reservoir for *Salmonella* is the intestinal tract of warm and cold-blooded animals. *Salmonellae* have evolved to virtually endure various attributes. These attributes are necessary to ensure wide distributions, including abundant reservoir hosts, efficient fecal shedding from carrier animals, persistence within the environment, and the effective use of transmission vectors (feed, fomites, vehicle, etc.).

In-apparent *Salmonella*-infections of pigs

Salmonella infections in pigs, which do not lead to a clinical picture, can be called in apparent or latent infections (Mayr et al., 1993). As these infections take an undetected course, they can pose a most serious food hygiene problem. Several serotypes of *Salmonella* are agents of in apparent infections of pigs, with different serovars being of importance in different parts of the world.

The investigations of organs of slaughter pigs in Germany identified the serotypes *Salmonella* Indiana, *Salmonella* Mbandaka, *Salmonella.* Newington and *Salmonella* Worthington as most predominant. Both et al. (1982) and Pietsch (1981) in his investigations of fecal samples from healthy pig herds for *Salmonella,* in Germany identified the serotypes *Salmonella* Typhimurium *var. copenhagen*, *Salmonella* Dublin, *Salmonella* Derby, *Salmonella* Agona, *Salmonella*



Source: Quinn et al. (1999)

Figure 3. Spread of *Salmonellae* and sources of contamination for human food

Indirect contamination

Salmonellae carried directly

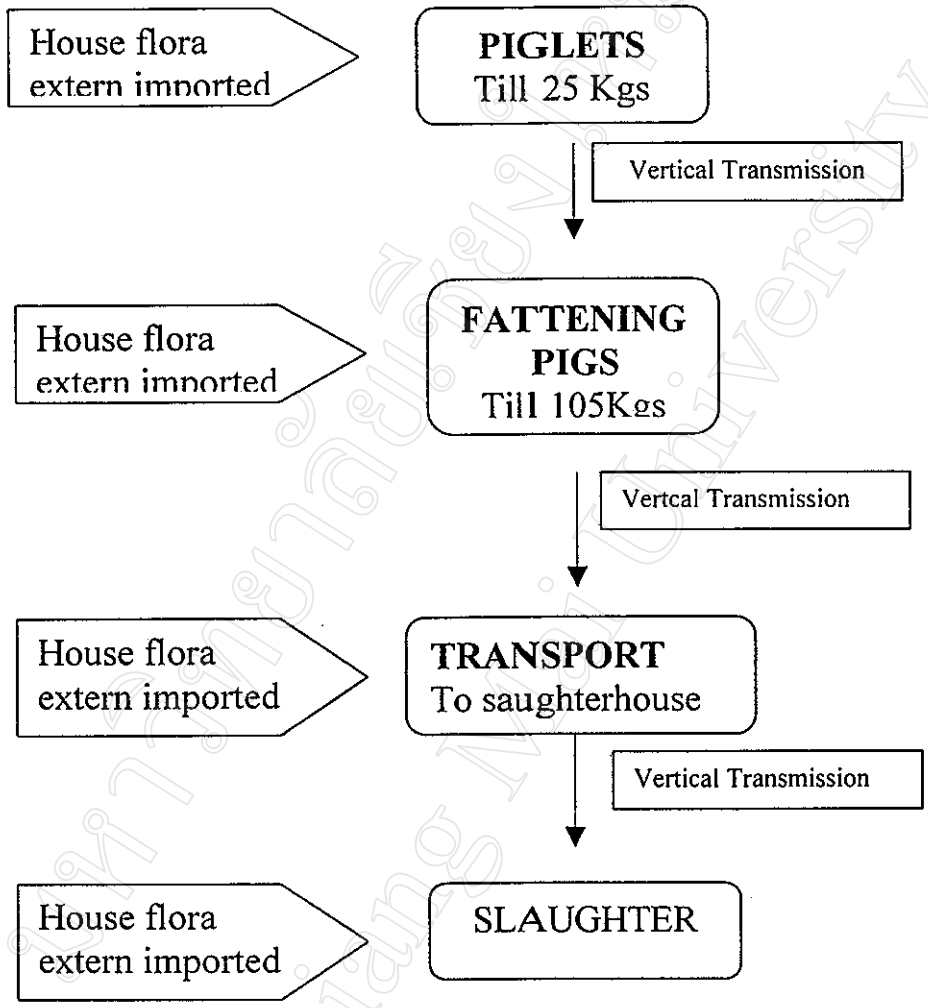


Figure 4. Pork production chain and the *Salmonella* transmission routes

Enteritidis, *Salmonella* Infantis, *Salmonella* Livingston, *Salmonella* Duisburg, *Salmonella* Panama, *Salmonella* Bornum and *Salmonella* Saintpal. Blaha (1993a, 1995), the prevalent *Salmonella* serotypes in slaughter pigs were *Salmonella* Typhimurium *var copenhagen*, *Salmonella* Dublin, *Salmonella* Derby, *Salmonella* Agona, *Salmonella* Enteritidis, *Salmonella* Infantis and *Salmonella* Livingston.

In Great Britain, the serovars *Salmonella* Typhimurium, *Salmonella* Infantis, *Salmonella* Dublin, *Salmonella* Bredeney and *Salmonella* Virchow were identified in slaughter pigs (Gilbert, 1987). The serotypes *Salmonella* Anatum, *Salmonella* Derby and *Salmonella* Infantis from fecal samples of slaughter pigs in Portugal were isolated. Lintermans and Pohl (1983) identified the serotypes *Salmonella* Typhimurium, *Salmonella* Typhimurium *var copenhagen* and *Salmonella* Dublin as most prevalent in in apparent *Salmonella* infections in healthy pig herds in Belgium. The serotypes *Salmonella* Typhimurium, *Salmonella* Anatum, *Salmonella* Bovismorbificans and *Salmonella* Heidelberg in fecal samples from pigs were identified in Italy.

In 1984, human *Salmonella* cases associated with infections of slaughter pigs with *Salmonella* Goldcoast were reported in France. Fecal samples of pigs in Hungary were infected with *Salmonella* Typhimurium, *Salmonella Derby*, *Salmonella* Agona, *Salmonella* Infantis, *Salmonella* London, *Salmonella* Bredenery and *Salmonella* Panama. The importance of infections of pigs with *Salmonella* Typhimurium, *Salmonella* Anatum, *Salmonella* Enteritidis and *Salmonella* Tennessee was describe in Poland. In Tchechoslowakia, Simko (1984) identified *Salmonella* Typhimurium, *Salmonella* Agona, *Salmonella* Enteritidis, *Salmonella* Bareilly, *Salmonella* Arizona, *Salmonella* Anatum, *Salmonella* Cholerasuis *var decatur* and *Salmonella* Heidelberg

from healthy pig herds. In the USA, *Salmonella* Typhimurium, *Salmonella* Agona, *Salmonella* Anatum, *Salmonella* Derby, *Salmonella* Java, *Salmonella Manhatten* and *Salmonella* Thompson were identified from slaughter pigs.

In India, in addition to the serotypes recorded in Europe and the USA prior to their investigation, additionally identified *Salmonella* Newport, *Salmonella* Stanley, *Salmonella* Oranienburg, *Salmonella* Senftenberg and *Salmonella* Weltevreden. Infections with *Salmonella* Havanna in Australia were reported.

In Thailand, the serotypes *Salmonella* Weltevreden, *Salmonella* I.3,9, *Salmonella* Agona, *Salmonella* Typhimurium, *Salmonella* Cerro, *Salmonella* Ohio, *Salmonella* Choleraesuis, *Salmonella* Anatum, *Salmonella* Krefeld, *Salmonella* Derby and *Salmonella* I.1,4,5,12 were identified in slaughter pigs (Bangtrakulnont,1994).

Infections of pigs with these serotypes listed above often remain in apparent since their hosts are not susceptible. Either due to low dosages, the levels of resistance or due to prevailing physiological conditions of animals development of clinical disease is prevented and/or the specific incubation periods are not attained. Thus, *Salmonella* multiply uncontrolled and undetected. In order to minimize the infection levels in such pigs it is necessary to introduce widespread surveillance and monitoring systems in herds. These enable early detection of carriers as well as of corresponding risk factors. Based on these results, suitable management programs can be formulated and implemented.

Salmonella as meat hygiene problem

Undetected infections of pigs with *Salmonella* pre-slaughter lead to primary contamination of carcasses and subsequent post-slaughter contamination of meat and meat products. These latter contaminations of food products are the main sources of human infections with *Salmonella* bacteria (Selbitz et al., 1985).

In Thailand, food-borne diseases, particularly those caused by non-typhoidal *Salmonella* have the highest negative impacts on public health and on farming, especially on pig farming. These diseases have led to rejections of pigs and pork products in the International Trade and have also led to the imposition of trade barriers by importing countries. Results from initial epidemiological studies in Thailand have recorded high prevalences of *Salmonella* sero-types isolated from humans and animals. However, such studies only involved microbiological investigations of slaughtered pigs, which only detect a low proportion of the true infections. Furthermore, only clinically sick animals were typically examined, leaving the in-apparently infected pigs un-investigated. This situation is be supported by work done in Germany in which the microbiological investigations carried out on clinically sick slaughter pigs only identified 0.5% of pigs as *Salmonella* positive, whereas the true infection rates of pigs delivered to the slaughter houses ranged between 0.4% and 76.3%, with an average of 16.2% (D'Aoust, 1995).

Importance of *Salmonella* infections of pigs as zoonoses

From the human-pathogenic point of view, *Salmonella* serotypes without species adaptation play the most important role (Blaha, 1993b). Serotypes of particular pathogenicity to human are *Salmonella* Typhimurium, *Salmonella* Typhimurium *var copenhagen*, *Salmonella* Enteritidis and *Salmonella* Agona (Blaha,

1993a). Serotypes that cause sporadic human salmonellosis include: *Salmonella* Heidelberg, *Salmonella* Newport, *Salmonella* Infantis, *Salmonella* Bareilly, *Salmonella* Hadar, *Salmonella* Saintpaul, *Salmonella* Thompson, *Salmonella* Manhattan (Blaha, 1993) as well as *Salmonella* Panama (Petrovic et al, 1974). However, A rare infections of humans with the pig-adapted serotype *Salmonella* Cholerasuis were also described.

All *Salmonella* serotypes generally have to be regarded as potentially human pathogenic, as even infections with non-pathogenic serovors, especially in cases of immuno-compromised humans, can lead to clinical disease. To date, the serotype *Salmonella* Typhimurium and *Salmonella* Enteritidis though are given the greatest attention as major agents of human salmonellosis (Selbitz et al., 1985). Infections with *Salmonella* Enteritidis lead to severe clinical symptoms and result in high case fatalities in older people (Blaha, 1993b).

Most of non-typhoidal *Salmonellae* enter the human body when contaminated food is ingested. Person-to-person spread of *Salmonellae* also occurs. In rare cases, direct transmission of *Salmonella* from animals to man can occur, particularly to professionals with frequent contacts with farm animals, such as farmers, slaughterhouse personnel, animal traders and veterinarians.

OBJECTIVES OF THE STUDY

In the study investigations, the test system of the European Union (bacteriological tests after ISO 6579 and Mix-ELISA) is used on slaughter pigs in northern Thailand.

a. General objectives

-To determine pre-slaughter prevalences of *Salmonella* infections of pigs at farm level.

-To determine the pre-slaughter proportion of *Salmonella* infected pigs at slaughterhouses.

-To determine the primary contamination rate of slaughter carcasses.

-To compare prevalences of *Salmonella* infections of pigs pre-slaughter with contamination rates of carcasses (post-slaughter).

-To identify chains of infection and their risk factors.

-To assess the applicability of the Danish Mix-ELISA for use in Thailand.

b. Specific objectives

-To identify the serotypes of *Salmonella* bacteria isolates.

-To study the distribution of serogroups in fecal and lymph node samples.

-To study the distribution of serogroups between slaughterhouses.

-To identify the serogroups of the isolates.